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African Journal of Agricultural Research

Full Length Research Paper

## Appraisal of farmers' wheat production constraints and breeding priorities in rust prone agro-ecologies of Ethiopia

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Ethiopia is the second largest producer of wheat in sub-Saharan Africa although yields remain considerably below the global average due to several production constraints. The aim of the study was to identify the primary threats to wheat production, farmers' selection criteria for wheat varieties, and disease management practices with emphasis on wheat rusts in the Arsi, Bale and West Shewa administrative zones of Ethiopia. A total of 270 wheat growing households were interviewed in the three administrative zones in 2012. Participatory rural appraisal tools, a semi-structured questionnaire and focus group discussions were used to engage with the farmers. Main wheat production constraints were wheat rust diseases, the high costs of fertilizers, shortage of improved seeds and high seed prices. The most important traits that farmers sought in wheat varieties were disease resistance (27.8%) and high grain yield (24.8%). Owing to the limited availability of rusts resistant varieties, and the emergence of virulent pathotypes, fungicide application was the main disease management practice used by 60% of respondent farmers. To enhance wheat production and productivity in Ethiopia, it is important to develop rust resistant varieties considering farmers' preferences, promote access to wheat production inputs and strengthen seed multiplication and dissemination of improved varieties.

Key words: Ethiopia, participatory rural appraisal, rust, seed source, wheat production constraints, wheat traits.

#### INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the world's leading cereal grains serving as a staple food for more than one-third of the global population. Globally, it is cultivated on approximately 218 million hectares of land (HGCA, 2014). Ethiopia is the largest wheat producer in sub-Saharan Africa (FAOSTAT, 2014). In Ethiopia wheat is

cultivated on over 1.6 million hectares of land, accounting for 13.33% of the total grain crop area, with an annual production of 4.2 million tons, contributing about 15.81% of the total grain production (CSA, 2015). In terms of area of production, wheat ranks fourth after teff (*Eragrostis tef* Zucc.), maize (*Zea mays* L.) and Sorghum (*Sorghum* 

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Author(s) agree that this article remain permanently open access under the terms of the <u>Creative Commons Attribution</u> <u>License 4.0 International License</u> *bicolor* L.). In total grain production, wheat ranks third after teff and maize in the country (CSA, 2015).

Wheat is largely grown in the mid and highland areas of Ethiopia spanning at altitudes of 1500 to 3000 m above sea level (masl). However, it is mainly grown between 1800 to 2500 masl in the country (Winch, 2007). Arsi, Bale and Shewa administrative zones of the Oromia Regional State of Ethiopia are among the major wheat areas with 53.4% of the wheat produced in Ethiopia coming from these zones (CSA, 2015). The Arsi and Bale zones are included among the highest potential agroecologies in Eastern Africa for wheat production with 467337.42 ha under wheat (Jobie, 2007; CSA, 2015).

In Ethiopia wheat is predominantly grown by small scale farmers at a subsistence level, and these farmers experience a wide range of biotic, abiotic and socioeconomic constraints. Wheat rusts, stem rust (*Puccinia graminis* Pers. f.sp. *tritici* Eriks and Hann), leaf rust (*P. triticina* Eriks) and stripe or yellow rust (*P. striiformis* Westend. f. sp. *tritici*) are the major biotic constraints in all wheat growing regions of the country. To combat yield losses due to wheat rusts and other abiotic constraints, the National Wheat Improvement Program has released more than 30 wheat varieties since 2003. However, only a few rust resistant wheat varieties are being planted by farmers in the country (DRRW, 2010).

Different reports are available on the low adoption rate of improved wheat varieties by resource poor farmers in Ethiopia. For instance, Zegeve et al. (2001), DRRW (2010) and Nelson (2013) indicated that most of the released varieties in Ethiopia had been poorly adopted by the small scale farmers because of lack of effective seed production and delivery mechanism, weak integration of variety requirements between breeders and farmers and less adaptation of the breeders developed varieties to the local environments. In Ethiopia, farmers' variety preferences is not only grain yield but also disease resistance, straw yield, seed color, baking quality and other related social values (Bishaw et al., 2010; Tesfaye et al., 2014). Therefore, in order to enhance the adoption rate of new wheat varieties, and improve wheat production and productivity in the country farmers' production constraints and varietal preferences should be well known.

Participatory rural appraisal (PRA) has been widely used to collect information on farmers' varietal preferences, production constraints and traditional knowledge and experiences to mitigate food insecurity and improve their livelihood (Chambers, 1994). Understanding farmers' preferences, attributes of wheat varieties and wheat production constraints enables breeders to set wheat breeding priorities (Weltzien and Christinck, 2009). By integrating farmers' concerns and conditions into agricultural research, research will develop technologies that become widely adopted, resulting in more productive, stable, equitable and sustainable agricultural systems (Martins et al., 2002; Owere et al., 2012). Participatory rural appraisal has been previously conducted on wheat in Ethiopia (Agidie et al., 2000; Bishaw, 2004; Bishaw et al., 2010; Tesfaye et al., 2014), however, information on presently grown varieties, farmers' key production constraints and preferences in wheat cultivars is inadequate. The objective of the current study was, therefore, to identify wheat production threats, farmers' variety selection criteria, and disease management practices with special emphasis on wheat rusts in Arsi, Bale and West Shewa administrative zones of Oromia Regional State of Ethiopia.

#### MATERIALS AND METHODS

#### Description of the study areas

The study was carried out in three selected administrative zones: Arsi, Bale and West Shewa between February and April 2012. The zones are situated in the Oromia Regional State of Ethiopia. All the zones are wheat potential areas but differ in terms of agroecological diversity and in the use of modern wheat production technologies. The Arsi and Bale zones are situated in the Southeastern of Ethiopia while the West Shewa zone is in the Central highlands of Ethiopia. The study zones are dominated by three major agro-ecologies: highlands (2300-3200 masl), midlands (1500-2300 masl) and lowlands (500-1500 masl). The rainfall pattern in the zones exhibits a bimodal nature: short and long rains during February to May and June to September, respectively (CSA, 2014). In all study zones mixed crop-livestock farming is the predominant mode of agricultural production (Tefera et al., 2002). Wheat, tef, barley and maize are the major cereal crops, together with pulses, oil crops and vegetables (CSA, 2015).

#### Data source

Both qualitative and quantitative data were collected from primary and secondary sources. Primary data were collected through semistructured questionnaires and focus group discussions. The secondary data were obtained from the zone and district agricultural offices of the respective districts included in the study.

#### Sampling

A multi-stage sampling procedure was used involving the selection of zones, districts, peasant associations and wheat farmers. A nonrandom purposive sampling was used to select from the zones through the districts, peasant associations and farmers levels. The sampling procedure involved two districts per zone, three peasant associations per district and fifteen respondents per village. This resulted in a total of three districts, 18 peasant associations, and 270 respondents. Individual farmers were selected from each peasant association representing various socio-economic backgrounds (data not shown) and both gender (Table 1). Thus the farmers selected for the study are believed to be the representative of the wheat farmers in the three zones. Zone level agricultural experts and district agricultural development offices assisted with the identification of the sampled districts, peasant associations and respondents.

#### Data collection

Semi-structured questionnaire was designed on topics related to

Zone	District	Male {No (%)}	Female {No (%)}	Total {No (%)}
Aroi	Tiyo	36 (80)	9 (20)	45 (16.7)
AISI	Munisa	39 (86.7)	6 (13.3)	45 (16.7)
Pala	Sinana	42 (93.3)	3 (6.7)	45 (16.7)
Dale	Gasera	41 (91.1)	4 (8.9)	45 (16.7)
West Chaws	Jeldu	39 (86.7)	6 (13.3)	45 (16.7)
west Snewa	Dandi	41 (91.1)	4 (8.9)	45 (16.7)
	Total	238 (88.1)	32 (11.9)	270 (100)

 Table 1. The selected study areas in Arsi, Bale and West Shewa zones of the Oromia Regional

 State in Ethiopia.

the general socio-economic characteristics of the household, wheat varieties grown, production constraints, wheat rust diseases and their management. Enumerators were recruited for data collection who lives in the area, fluent speakers of local language (Oromifa), well acquainted with local and cultural contexts, and working within the selected districts. They were trained on the contents of the interview schedule and data collection techniques. Pre-test on non-sample respondents was also made under supervision of the researcher. Finally, the formal survey was conducted on 270 households after necessary modification and adjustments were accommodated as per the result obtained from the pre-test.

Focus group discussions were held in each district to understand farmers' varietal preferences and the specific traits that influence a farmer's decision to grow a wheat variety, and the major constraints affecting wheat production. Each group was composed of 10-15 wheat growers (both male and female). Checklists were developed and used to guide focused group discussions with farmer groups and individual key informants. The farmers were encouraged to use their local language that they were most familiar with. The development agents most familiar with the local language facilitated the group discussions. During the discussion, the farmers were asked to list wheat varieties they grow and to identify the traits that they used in selection of the varieties, and list the main constraints limiting wheat production.

#### Data analysis

Data (both qualitative and quantitative) obtained from sample respondents were sorted, coded and subjected for statistical analyses using the Statistical Package for Social Sciences computer software (SPSS Inc., 2005). Both descriptive (means and percentages) and inferential statistical procedures were used to analyze the data obtained from households.

#### RESULTS

#### **Demographic characteristics**

The sample population contained 88.2% males and 11.8% females. Almost all the respondents (99%) who participated in the study were farmers in agricultural production. The mean family size of the sampled population was 5 and about 85% of interviewed farmers had family sizes greater than 3 persons per household. In

the study areas, children were contributing to farm labour significantly. Farmers who were illiterate constituted 21%. Farmers educated up to primary and secondary level constituted 62 and 17%, respectively.

#### Farming system

Household total crop land in the study areas ranged from 0.5 to 15 ha, with mean farm size of 2.5 ha (SD 2.43). The majority of the interviewed farmers allocate most of their land for wheat as the number one crop. Of the 2.5 hectares of mean farm size owned by individual farmers, a mean of 1.85 ha were dedicated to wheat production in the study areas. Farmers in the study areas grow different assemblage of crops. These include cereals, pulses and oilseed crops. In addition to wheat, other major crops grown by majority of farmers in the Arsi zone were barley (Hordium vulgare L.) (71%), maize (Zea mays L.) (51%), teff (E. tef (Zucc.) Trotter) (41%), faba bean (Vicia faba L.) (46%) and linseed (Linum usitatissimum L.) (18%). The three major cereal crops widely grown after wheat were barley (58%), maize (40%), and teff (39%) in the Bale zone. In West Shewa most farmers grow maize (67%), teff (63%), barley (41%), faba bean (38%), grass pea (Lathyrus sativus L.) (24%), and noug (Guzotia abysinica Cass.) (15%).

Wheat is grown both in the main and short rainy seasons in the Sinana and Gasera Districts of Bale zone. The main rainy season has long rains which start in June and end in September. It is the period when the largest wheat area is cultivated. In the short rainy season, the rain starts in February and ends in April. Seventy-three percent of the farmers in these districts grow wheat in both the main and the short rainy seasons, while 27% of them only utilize the main season to produce wheat. On the other hand, farmers in the Arsi and West Shewa zones only grow wheat is produced solely under rain fed conditions.

Castar		Seed source in 20	11 cropping season
Sector	Seed source	Frequency	% response
	Own stock	184	68.1
Informal	Other farmers	24	8.9
	Local markets	20	7.4
	Agricultural Offices	33	12.2
Formal	Research centers	3	1.1
	Producer cooperatives	6	2.2
Total		270	100

 Table 2.
 Farmers' sources of wheat seed in the Arsi, Bale and West Shewa zones of Oromia
 Regional State in Ethiopia.

**Table 3.** Wheat varieties grown, year of release and proportion of wheat farmers in the study areas.

Veriety			% response					
variety	rear of release	Arsi	Bale	West Shewa				
Pavon 76	1982	11.10	1.15	-				
Dashen	1984	-	-	31.75				
Kubsa	1995	53.35	10.15	38.90				
Galama	1995	-	3.35	30.55				
Tusie	1997	39.75	77.20	-				
Madawalabu	2000	6.80	46.20	1.15				
Hawi	2000	1.10	-	-				
Sofumer	2000	9.10	20.35	-				
Digelu	2005	88.75	70.75	40.20				
Kakaba	2010	15.85	3.40	1.10				
Danda'a	2010	10.25	-	-				
Local	-	-	6.75	10.70				

#### Wheat seed source

The sources of seed for farmers are presented in Table 2. The informal sector was the source of seed for 84.4% of the farmers in the area, where 68.1% respondents used seeds retained from the previous harvest, and 8.9 and 7.4% of respondents used seeds from other farmers and local markets, respectively. The formal sector provided for only 15.5%, where 12.2% of households sourced their seed from Agricultural Offices (AO) in the respective districts, 1.1% from research centers and 2.2% from producers' cooperatives (Table 2).

## Wheat varieties grown by farmers and genetic diversity

Table 3 shows the different wheat varieties grown by farmers in the study areas. Most farmers grow more than

one variety, making the proportions above 100%. The most commonly grown wheat varieties in the Aris zone were Digelu, Kubsa and Tusie at 88.75, 53.35 and 39.75%, respectively. In the Bale zone, Tusie (77.2%), Digelu (70.75%) and Madawalabu (46.2%) were the dominant wheat varieties grown by the majority of households. Digelu (40.2%) and Kubsa (38.9%) were popular varieties in West Shewa zone.

Variety Digelu was grown by 88.8, 70.8 and 40% respondents in Arsi, Bale and West Shewa zones, respectively. Fifty three percent of respondents in Arsi and 39% in West Shewa grew variety Kubsa on their farms. The new bread wheat varieties, Kakaba and Danda'a that were released in 2010 were grown in Arsi by 15 and 10% of the farmers, respectively. Danda'a was grown only by 3.4% of farmers interviewed in Bale zone. None of the respondents in West Shewa grew these varieties, while 10.7% of household used local wheat varieties. Bread wheat was the principal type of wheat

		A.II						
Farmers'-preferred traits	Arsi		Bale		West Shewa		All survey	
	Freq	%	Freq	%	Freq	%	Freq	%
Grain yield	25	27.8	15	16.7	27	30	67	24.8
Disease resistance	16	17.8	25	27.8	34	37.8	75	27.8
Grain yield and disease resistance	28	31.1	22	24.4	23	25.6	73	27.0
Environmental adaptability	7	7.8	8	8.9	2	2.2	6	6.3
Disease resistance and food quality	4	4.4	3	3.3	0	0	12	2.6
Grain yield and high market value	3	3.3	2	2.2	1	1	10	2.2
Grain yield, food quality and high market value	2	2.2	5	5.6	0	0	9	2.6
Grain yield, early maturity, disease resistance and food quality	1	1.1	4	4.4	2	2.2	7	2.6
Grain yield, disease resistance, high market value and food quality	4	4.4	6	6.7	1	1.1	11	4.1
Total	90	100	90	100	90	100	270	100

Table 4. Farmers'-preferred traits required of improved wheat varieties in the study zones.

<sup>†</sup>Freq=frequency of respondents.

Table 5. Wheat varieties grown by farmers in the Arsi, Bale and West Shewa zones and their outstanding traits.

Wheat varieties	Preferred traits	Non- Preferred traits
Kubsa	High grain yield, high biomass, multiple use at home, white seed, adaptable to environment	Susceptible to disease
Digelu	High grain yield, multiple use at home, white seed, diseases resistant	Late maturity, hard straw
Galama	High biomass, multiple use at home, adaptable to environment	Susceptible to disease, late maturity
Dashen	White seed	Susceptible to disease
Kakaba	High grain yield, disease resistant, early maturity, white seed, tolerant to lodging, soft straw for animal fodder	-
Madawalabu	High grain yield, disease resistant, early maturity	-
Pavon 76	White seed, early maturity	Susceptible to disease
Tusie	White seed, tolerant to rust	-
Sofumer	High grain yield, disease resistant	Purple seed color
Danda'a	High grain yield, disease resistant, white seed, tillering capacity, bread making quality, long spike	Late maturing, less treshability

grown by farmers in all surveyed areas.

#### Farmers' preferred traits

In all the study areas, farmers used a combination of criteria in selecting wheat varieties. The major and common reasons behind varietal preferences are given in Table 4. The most important criteria across the areas were disease resistance (27.8%), high grain yield (24.8%) and a combination of the two (27%). In Arsi 31.1% of respondents preferred a combined high grain yield and disease resistance as the key criteria for selecting wheat varieties. Disease resistance was a key criterion for 27.8 and 37.8% farmers in Bale and West Shewa, respectively.

Environmental adaptability was a criterion for 7.8% of farmers in Arsi and 8.9% in Bale and 2.2% of respondents in West Shewa. High market value in combination with other traits was also a major selection

criterion in the study areas because wheat is a major source of income in the areas.

Farmers in group discussions were also asked to associate a particular wheat variety they grow with its preferred and non-preferred traits. The most commonly grown varieties, along with their preferred traits are summarized in Table 5. Farmers in the study areas selected wheat varieties Madawalabu, Sofumer, Danda'a and Kakaba for their disease resistance. Tusie is tolerant of rust and is preferred for its market value. Kubsa and Galama are disease susceptible varieties but farmers still grew these variteis for their high grain yield and biomass which is used for animal fodder, fuel and house roofing material. White seeded varieties such as Kubsa, Dashen, Digelu, Kakaba, Danda'a, Tusie and Pavon 76 were largely grown by farmers for sale because they are preferred by urban consumers.

Farmers in group discussions stated that Kakaba is tolerant to lodging because of its semi-dwarf nature. It is early maturing variety and was preferred by farmers who practiced double cropping. Kakaba was also preferred for its soft straw which makes it suitable for animal fodder. In contrast, Digelu has hard straw, making it little use for animal fodder. Farmers raised that variety Digelu is late maturing. However, they were convinced that this variety is high yielding and has the best fit in areas that receive extended rainfall. Danda'a was preferred by the farmers for its tillering capacity, resistance to disease and long spike. The farmers who grew Danada'a considered it as a replacement for the old susceptible wheat variety, Galama. The female famers who participated in the group discussions also stated that the variety has good bread making quality. However, the farmers indicated that Denda'a has less threshing ability and difficult for threshing using manual harvesting and threshing methods. Hence, farmers obliged to use combine harvesting.

#### Wheat production constraints

The farmers' perceptions of wheat production constraints and their ranks between locations are summarized in Table 6. There was a marked agreement in the identified constraints in the three survey zones, with some variation in the ranking between the zones. High seed prices were ranked fourth following a lack of access to seeds of varieties in the Arsi and West Shewa zones, whereas farmers in the Bale zone perceived high seed prices as equally important to the lack of access to seeds of improved varieties and both ranked third. Lack of credit access was perceived as an important constraint in West Shewa while it ranked lower in the Arsi and Bale zones.

Almost all sampled farmers (96%) in the three zones considered the wheat rusts as the most important production constraint. The second most important constraint in the surveyed zones was high fertilizer price (93%). Farmers in the study areas use fertilizers; however, the amount applied per unit area is often lower than the recommended rate because of the high price of fertilizer. Lack of access to seeds of improved wheat varieties (85%) was identified as the third most important limiting factor of wheat production followed by high improved seeds prices (81%). Unavailability and high cost of improved seeds were mentioned as the two most important reasons for not adopting wheat technologies by the respondents. Low market prices of wheat were also regarded as major constraints of wheat production by 66% of the farmers. Most farmers sold the produce in the local markets and were discontented with the low prices of wheat. Farmers mentioned that the low prices were due to the fact that middlemen determined the price of the produce.

#### Wheat rusts and farmers' management methods

Farmers in the study areas were also asked to estimate

yield losses from rusts. This was done based on the differences between yields from stem rust free wheat farms and diseased wheat farms sown to the same varieties. Accordingly, yield losses of 70.7, 60.5 and 60.0% were estimated in the Aris, Bale and West Shewa zones, respectively (Table 7).

To reduce losses from rust infestations, fungicides are being used by most producers. More than 60% of interviewed farmers used fungicides for rust management (Table 8). Tilt® (Propiconazol), Bayfidan® (Triadimenol), and Mancozeb were the major fungicides used by the farmers for rust control. Only 15% of the respondents had adopted new varieties for the control of rusts. Varieties Kakaba, Danda'a and Digelu were widely adopted rust resistant wheat varieties during the study period. On the other hand, a few farmers in Bale (6.7%) were planting early to avoid rust damage. In contrast, almost 20% of the farmers did not use any control measure to protect their wheat farms from rust infection.

#### DISCUSSION

In Ethiopia wheat research programs to develop improved wheat varieties were initiated during the 1950s. Despite 60 years of wheat breeding in the country, most of the released cultivars had been poorly adopted by small-scale farmers (Zegeye et al., 2001; DRRW, 2010). Majority of farmers in the study areas continue to grow old varieties such as Kubsa and Galama that are often susceptible to diseases. The reasons for the persistence of old varieties were lack of farmers' preferred traits in the new cultivars, unavailability of sufficient quantity of new seed or its poor distribution in the study areas and the risk avoidance adopted by farmers who grow a mixture of varieties to spread their risks.

The continued planting of rust susceptible varieties poses a serious threat to stable wheat production in the country. The failure to distribute newly released varieties in a timely way exposes the country to an agricultural time bomb, in a scenario ominously similar to the events leading up to the 2010 and 2013 yellow rust and stem rust epidemics, respectively. Another problem with the continued use of susceptible varieties is that it increases the chances of new mutant races of rusts developing to attack presently resistant varieties (CIMMYT, 1989). To address this, the recently released rust resistant wheat varieties with diversity in genetic background, adaptation and good yield potential should be delivered to small scale farmers in time at affordable prices, to ensure increase wheat productivity.

The study also indicated the predominance of informal seed sector in seed distribution. The predominance of the informal seed systems slows down replacement of older varieties and delays the transfer of benefits from breeding research to farmers. Hence, efforts should be made to understand and solve factors in the seed system that Table 6. Major wheat production constraints and their ranks in Arsi, Bale and West Shewa zones of Oromia Regional State in Ethiopia.

					Zones						0		
Constraints	Arsi				Bale			West Shewa			All Surveyed		
	<sup>†</sup> Freq.	%	Rank	Freq.	%	Rank	Freq.	%	Rank	Freq.	%	Rank	
Rusts (yellow rust and stem rust)	87	96.7	1	86	95.6	1	86	95.6	1	259	96	1	
Lack of seed of improved varieties	81	90.0	3	77	85.6	3	71	78.9	3	229	85	3	
High seed price	78	86.7	4	77	85.6	3	65	72.2	4	220	81	4	
High fertilizer price	85	94.4	2	82	91.1	2	84	93.3	2	251	93	2	
Shortage of fertilizer	15	16.7	9	17	18.9	9	21	23.3	9	53	20	9	
Low producer price	61	67.8	5	62	68.9	5	54	60.0	5	177	66	5	
Weeds (grass weeds)	37	41.1	7	32	35.6	6	25	27.8	8	94	35	7	
Poor soil fertility	11	12.2	10	10	11.1	11	12	13.3	11	33	12	11	
Other diseases and pests	42	46.7	6	29	32.2	7	39	43.3	7	110	41	6	
Unpredictable rain	18	20.0	8	13	14.4	10	16	17.8	10	61	17	10	
Lack of access to credit	7	7.8	11	24	26.7	8	54	60.0	5	85	31	8	

<sup>†</sup>Freq=frequency of respondents.

Table 7. Yield losses due to rusts in study zones.

7	Mean wheat productivity						
Zone	Under low/no rust infestation (t ha <sup>-1</sup> )	Under high rust infestation (t ha <sup>-1</sup> )	LOSS (%)				
Arsi	4.1	1.2	70.7				
Bale	3.8	1.5	60.5				
West Shewa	2.0	0.8	60.0				

Table 8. Wheat rusts control measures practiced in the study areas.

Zone								
Control measures	Arsi		Bale		West Shewa		All surveyed zones	
	<sup>†</sup> Freq	%	Freq	%	Freq	%	Freq	%
None	14	15.6	15	16.7	24	26.7	53	19.6
Chemical	62	68.9	60	66.7	57	63.3	179	66.3
Resistant variety	14	15.6	9	10.0	8	8.9	31	11.5
Cultural practice (Early planting)	0	0	6	6.7	1	1.1	7	2.6
Total	90	100	90	100	90	100	270	100

<sup>†</sup>Freq= frequency of respondents.

may impede rapid varietal replacement. This effort will be instrumental in improving the current seed multiplication and dissemination pathway and widening the genetic bases of wheat that will help in buffering the rust incidence and contribute to household food security of smallholder farmers in Ethiopia. Besides, the seeds of newly developed varieties must be produced in sufficient quantities in the study areas to make the research efforts more successful.

Wheat rusts have been major threats to wheat production in Ethiopia. In recent years, novel pathotypes of the rusts fungus have overcome resistant wheat varieties (ICARDA, 2011). The study areas are among the most rusts prone areas of East Africa and the wheat farmers in these areas frequently suffer serious losses from rusts epidemics (Hodson, 2013; Periyannan et al., 2013). The yellow rust outbreak in 2010 significantly reduced the national wheat annual production. The major wheat producing regions including the study zones were seriously affected of the epidemics with losses up to 70% (Hunde et al., 2012; Yami et al., 2013). Hence, farmers in all the study areas were in agreement that wheat rusts are the most important production constraints. High prices of chemical fertilizers and improved seeds were also important production limiting factors in the study areas. An increase in fertilizer prices due to the removal of government subsidies has decreased fertilizer use in the study areas. Consequently, farmers apply chemical fertilizers below the recommended rates. Under such circumstance, it is difficult to increase the wheat yields on small scale farms. Bishaw et al. (2010) reported a serious gap between the recommended rate and the actual amount applied by the farmers.

Farmers in the study areas were well aware of the benefit of resistant varieties for the control of rust diseases. However, majority of the respondents grow old varieties for the reasons described earlier and due to high improved seed prices, and doubts about the level of resistance provided by these new varieties to rust diseases. Hence, farmers use fungicides for the control of the rusts. The producers applied fungicides at early growth stages but the application rates were below the optimum rates to get the desired level of benefits. Early planting is another important rust control measure. It reduces the time of exposure of the crop to the pathogen and hence reduces yield loss (Tolessa et al., 2014). However, early planting is not a widely adopted disease control measure by the farmers in the PRA zones.

Although farmers in the study zones had a range of preferences regarding wheat varieties and specific traits, they were in agreement that disease resistance is the most important trait compared to all other traits. This indicated that farmers were concerned about the susceptibility of the existing varieties to rust diseases. During the study period, variety Digelu which was released in 2005 was still in high demand and was being rapidly multiplied. However, Digelu developed extremely high levels of stem rust epidemic in Bale zone during the 2013 cropping season, which led to 100% yield losses (Hodson, 2013). It was resistant to stem rust at the time of its release, but become susceptible to stem rust even before its cultivation was in substantial areas. The failure of many promising cultivars such as Digelu even before its cultivation was in substantial areas, indicate disease resistance as high breeding priority (Hei et al., 2014). The farmers in the study areas also indicated grain yield as a key criterion for selecting wheat varieties after rust resistance. Breeding for disease resistant and high yielding wheat varieties should also focus on other important traits such as seed colour and early maturity that were perceived as critical by famers.

In the past, durum wheat was the most widely grown wheat type in the major wheat growing areas of Ethiopia. Ethiopia is a center of diversity for durum wheat (Zohary, 1970) and Ethiopian durum wheat land races are valuable sources of resistance to rust diseases (Denbel and Badebo, 2012). To date bread wheat has become predominant in most wheat areas of the country. Farmers in the study areas shifted to bread wheat production owing to its productivity per unit area relative to durum wheat. However, this may seriously threaten the existence of local durum wheat land races in the country if strategic seed conservation is not undertaken on a national scale.

Taking into consideration the range of attributes that farmer's use when choosing varieties for planting, selection of a large breeding population is a prerequisite when developing wheat varieties for small holder farmers. In general, to ensure a high level of variety adoption and therefore the high productivity of the crop, the wheat breeding programme in the country should put more emphasis on solving the problems of wheat farmers, increase the frequency with which it releases new varieties that resist diseases and yield well. Efforts should also be made to conserve the indigenous durum wheat landraces and make use of them in developing modern wheat varieties.

#### **CONFLICT OF INTERESTS**

The authors have not declared any conflict of interests.

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Full Length Research Paper

## **Operational uniformity for a sugarcane planter**

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Sugarcane planters must simultaneously perform furrowing, fertiliser application, seedling metering and furrow covering operations. Thus, the objective of this study was to evaluate the planting operational stages (including furrow opening, size and shape; the uniformity of seedling metering; and furrow closing) as a function of the planting speed and furrow depth. A completely randomised, 2x2 factorial design was adopted, with two planting speeds (5.0 and 6.5 km h<sup>-1</sup>) and two furrow depths (0.35 and 0.45 m), with 20 replicates. The variables analysed were number of billets m<sup>-1</sup>, number of total and viable buds m<sup>-1</sup>, percent of inviable buds, furrow depth, furrow width, disturbed area and seedling cover height. The results were subjected to descriptive analysis, analysis of variance and statistical process control. Increased planting speed caused an increase in disturbed area and a decrease in cover height. The increase in furrow depth caused increases in disturbed area, furrow width, furrow depth and cover height. Furrow opening, seedling metering, cover height, planting depths and operation speed were all uniform within the planting operation.

Key words: Planting speed, furrow depth, statistical process control, Saccharum officinarum.

#### INTRODUCTION

All operations involved in the agricultural production system of sugarcane can currently be mechanised. However, the option of total mechanisation of planting operations only became available a few years ago because those operations were previously mostly performed in a semi-mechanised manner, with manual seedling distribution (Janini, 2008).

To meet the increased demand for ethanol and sugar in the domestic and foreign markets, several areas of the sugarcane industry have been moving towards mechanised field planting to address a labour shortage and higher production costs due to significant increases in cultivated areas (Cebim et al., 2012).

According to Serafim et al. (2013), three basic stages of mechanised planting exist: 1– Mechanised seedling and harvesting; 2– seedling transport and transfer (conducted with tractors or trucks with a trailer, known as transhipment); and 3– planting with a planter. In these mechanised stages, the seedling buds are subjected to further damage. Therefore, many producers use 18 to 22 buds per metre of furrow at planting to ensure that the final quality of the seedlings allows the ratio of 12 buds

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Author(s) agree that this article remain permanently open access under the terms of the <u>Creative Commons Attribution</u> <u>License 4.0 International License</u> per metre of furrow; the remaining buds are rendered inviable during the process (Raveli, 2013).

Sugarcane planting traditionally uses deep furrows, 0.25 to 0.40 m in depth (Marques and Pinto, 2013). The seedling cover height ranges from 0.05 to 0.10 m and can vary depending on the sugarcane variety and planting season. During summer planting, seedlings should receive a thinner cover (0.05 to 0.07 m) to prevent undesirable silting. For late planting (fall and later), seedlings are better protected from drought with a thicker cover (0.08 to 0.10 m) (Coleti and Stupiello, 2006).

Barros and Milan (2010) reported that a nonstandardised farming operation may influence the process quality, compromising its continuity. The authors studied the continuous improvement process in sugarcane planting to identify its critical factors. With the improvements, they observed an increase in numbers within the desired limits. Similar studies using statistical process control in sugarcane have been conducted by Campos et al. (2008), Silva et al. (2008), Noronha et al. (2011), Cassia et al. (2014) and Ramos et al. (2014).

Planting speed can interfere with seedling metering. In addition, when the speed is changed, the machine must maintain planter uniformity and close the furrow. This fact was found by Taghinezhad et al. (2014) and Namjoo et al. (2015), on development of sugarcane prototypes in Iran. Such behaviours also vary when the desired depth is changed because they hinder the joint action of the furrow opener with the depth regulators as well as the efficiency of the furrow covering mechanism.

Thus, this study aimed to evaluate the mechanised planting operational stages (including the opening, size and shape of furrows; quality and uniformity of billetmetering; and furrow closing) while varying the planting speed and furrow depth for a sugarcane planting prototype.

#### MATERIALS AND METHODS

The study was conducted on February 23, 2013 in the municipality of Ibitinga, São Paulo (SP), Brazil, in an area belonging to the Santa Fé Plant, located near the coordinates 21°48'35.05" S latitude and 48°55'30.27" W longitude, with an average altitude of 475 m. The soil of the experimental area was classified as Alic Redyellow Latosol (Oxisol in the USDA soil classification system), with a medium texture, according to the methodology described by Embrapa (2013). The RB 96-6928 sugarcane variety was used in this experiment.

The mechanised sugarcane planting was conducted with a tractor-planter set, consisting of a Valtra BT 210 4x2 TDA tractor, with a 2200-rpm engine with 154.4 kW horsepower (210 cv), and a two-row sugarcane billet planter, Santal PDM 2 (Figure 1), which performs the planting operation (furrow opening, fertiliser application, seedling metering, insecticide application, furrow closing and compaction) in two rows simultaneously.

A completely randomised design (CRD) was adopted in a 2 x 2 factorial arrangement, with the following treatments: two planting speeds (5.0 and 6.5 km  $h^{-1}$ ) and two furrow depths (0.35 and 0.45 m), with 20 replicates per treatment and each replicate composed of samples from both planting rows (right and left), at a 20 m

distance from each other along the rows.

The billets used in the planting were characterised, and 30 units were collected inside the transhipment truck and planter. The billets were 431 mm in length, 27 mm in diameter, and 243 g in weight, with 3.6 buds per billet and 79% viability. The bud damage caused by the harvesting operation was 14.7%, with 6.4% damage in the transport to the planter.

The number of billets was recorded after planting by direct counting of four metres of the evaluation furrows after the furrows were opened with a hoe. For a better experimental control, only one evaluator performed the count for each treatment (20 replicates).

The total number of buds was counted by direct counting using the same billets previously obtained in the four meters of both evaluation furrows (left and right). The number of viable buds was determined by direct counting of the same billets used to assess the number of total buds in the four meters of both evaluation furrows and performed after the mechanised sugarcane planting. A viable bud was one with no damage caused by pests and diseases and also no cuts from the seedling harvesting until the planting in the furrows.

The percent of inviable buds was obtained from the difference between the total buds and viable buds, thus providing the percentage of damaged buds in relation to total buds. For the variables furrow depth, furrow width, disturbed area and cover height, a profilometer was used, consisting of 30 rods 3-cm apart with a maximum height of 70 cm. A grid with horizontal lines 1-cm apart was placed at the bottom of the profilometer for easy reading, and the readings were analysed using photographic images. The position of the upper end of the rods followed the geometric shape of the furrow, thus enabling the readings.

After planting, with the furrows covered, the measurements were conducted with the profilometer, and the furrow width was defined as the distance from the first rod to the last rod, disregarding the lateral soil elevation caused by the furrowing mechanism of the planter. Then, the furrows were manually opened until the compacted layer was found; it was then possible to model the furrows and to then proceed again with the profilometer measurements.

The furrow depth was defined as the average of the three rods with the highest measurement at the furrow bottom. The cover height was defined as the difference between the furrow depth and the average of the three rods with the highest measurement in the initial furrow.

To calculate the total disturbed area, after the photographic readings, the results were processed in a spreadsheet, where the cross-sectional area of the soil disturbed by the furrowing mechanism was obtained, in cm<sup>2</sup>, through the trapezoid rule methodology (Ruggiero and Lopes, 1996).

During the planter operation, the soil water content was measured using the gravimetric method (Embrapa, 1997), which resulted in 11.1% in the 0.00-0.15 m layer, 13.5% in the 0.15-0.30 m layer, and 13.1% in the 0.30-0.45 m layer.

The results were processed by the Minitab<sup>®</sup> 16 software, in which descriptive analysis was conducted to determine the measures of central tendency (mean and median) and the coefficients of variation, skewness and kurtosis to assess the behaviour of the studied variables. The results were subjected to the Anderson-Darling normality test, and the variables with "non-normal" distribution were transformed. The data-fitting models used were  $Y'=\sqrt{Y}$  for the variable damaged buds and  $Y'= \ln Y$  for the variable furrow width.

The transformed results were subjected to analysis of variance by the F-test, at 5% probability. When the F-test was significant, the means were compared by Tukey's test, at 5% probability, using Sisvar 4.3.

The variability analysis was conducted using statistical process control in Minitab<sup>®</sup> 16 with variable control charts, using as indicators the previously described variables with non-normal data.





Figure 1. Sugarcane billet planter (a and b); billet metering system (c and d).

To obtain the charts, the mean values and upper (UCL) and lower (LCL) control limits for each treatment were plotted; the latter was defined as the overall mean of the variable  $\pm$  three times the standard deviation. When the LCL calculation resulted in negative values, the LCL was considered to be null because negative values have no physical significance for the studied variables.

#### **RESULTS AND DISCUSSION**

As shown in Table 1, the variables billets m<sup>-1</sup>, total and viable buds m<sup>-1</sup>, furrow depth, disturbed area and cover height were normally distributed based on theAnderson-Darling test. The mean and median values are similar, which indicates little data dispersion, except for the variable damaged buds, whose median value is less than the mean.

The data dispersion parameter values reveal a high range and standard deviation for the variables billets m<sup>-1</sup>, total and viable buds m<sup>-1</sup>, and damaged buds, with coefficients of variation classified as high or very high (Pimentel-Gomes and Garcia, 2002), indicating high data variability. The cover height, despite having equal mean, median and range, also had a high standard deviation and consequently a high coefficient of variation.

Although, the data range was high for the variables furrow width and disturbed area, their standard deviations

were low, and they therefore had low and medium coefficients of variation, respectively. For furrow depth, both the mean and standard deviation were low, with a medium coefficient of variation.

Filho and Storck (2007) and Freitas et al. (2009) stated that the coefficient of variation is the most used parameter to evaluate experimental quality, and the lower the coefficient of variation, the greater the experimental accuracy, and vice-versa. This parameter allows the comparison between experiments without the need for equal units.

When analysing the coefficients of skewness (Table 1), which quantify the deviation of a distribution relative to a symmetrical distribution, a small skewnessis observed for the disturbed area and moderate skewness for billets m<sup>-1</sup>, total viable buds m<sup>-1</sup>, furrow width, furrow depth and cover height, where the two latter were negative, meaning that the frequency curve of the data distribution has a longer "tail" to the left. Only the percent of damaged buds had a high coefficient of skewness. The coefficients of kurtosis, or degree of "flattening" of a frequency distribution, reveal that the variables billets m<sup>-1</sup>, total buds m<sup>-1</sup>, damaged buds and furrow width exhibited (or more flattened at the top), with data weakly concentrated around its centre. For the variables viable buds m<sup>-1</sup>, furrow depth, disturbed area and cover height,

Mean	Median	R		CV	Cs	Ck	AD
Billets m <sup>-1</sup>							
9.9	9.5	15.5	2.91	29.6	0.40	0.60	0.347 <sup>N</sup>
Total buds m <sup>-1</sup>							
31.5	30.3	50.0	9.68	30.8	0.40	0.30	0.288 <sup>N</sup>
Viable buds m <sup>-1</sup>							
27.7	27.0	45.5	8.81	31.8	0.34	0.18	0.327 <sup>N</sup>
Damaged buds (%	b)						
11.9	10.1	35.7	6.87	58.0	1.12	1.28	1.899 <sup>A</sup>
Furrow depth (m)							
0.37	0.37	0.20	0.04	11.5	-0.30	-0.31	0.495 <sup>N</sup>
Furrow width (m)							
0.69	0.69	0.21	0.04	5.7	0.33	0.40	1.026 <sup>A</sup>
Disturbed area (cr	n²)						
1444.3	1426.5	1179.0	251.2	17.4	0.07	-0.19	0.164 <sup>N</sup>
Cover height (m)							
0.10	0.10	0.10	0.02	20.1	-0.16	-0.03	0.300 <sup>N</sup>

Table 1. Descriptive statistical parameters for the analysed variables.

R: range; σ standard deviation; CV: coefficient of variation (%); Cs: coefficient of skewness; Ck: coefficient of kurtosis; AD: Anderson-Darling normality test value; <sup>A</sup>: skewed distribution; and <sup>N</sup>: Normal distribution.

Table 2. Analysis of variance and means test for the variables billets per metre and total, viable and dama	ged buds
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Factors	Dilloto nor motro	Buds pe	Domograd hudo (%)	
Factors	billets per metre	Total	Viable	Damaged buds (%)
Planting speed (km h <sup>-1</sup> ) (S)				
5.0	9.8 <sup>a</sup>	31.0 <sup>a</sup>	27.5 <sup>a</sup>	11.4 <sup>a</sup>
6.5	10.0 <sup>a</sup>	31.9 <sup>a</sup>	27.9 <sup>a</sup>	12.4 <sup>a</sup>
Furrow depth (D)				
0.35 m	9.7 <sup>a</sup>	31.5 <sup>a</sup>	27.5 <sup>a</sup>	12.0 <sup>a</sup>
0.45 m	10.0 <sup>a</sup>	31.5 <sup>a</sup>	27.9 <sup>a</sup>	11.8 <sup>a</sup>
F-test				
S	0.080 <sup>ns</sup>	0.176 <sup>ns</sup>	0.033 <sup>ns</sup>	0.471 <sup>ns</sup>
D	0.189 <sup>ns</sup>	0.001 <sup>ns</sup>	0.033 <sup>ns</sup>	0.146 <sup>ns</sup>
SxD	0.437 <sup>ns</sup>	0.001 <sup>ns</sup>	0.065 <sup>ns</sup>	1.557 <sup>ns</sup>
CV (%)	30.00	31.33	32.38	30.38

In each column, for each factor, means followed by the same letters do not differ byTukey'stest at 5% probability<sup>ns</sup> indicates not significant; \* indicates significant at 5% probability by the F-test. CV (%): coefficient of variation.

the distribution was leptokurtic, that is, the frequency curve was more narrow than a normal distribution (or more tapered at the top), with the data strongly concentrated around its centre. Although, the skewness and kurtosis data were not normally distributed, only the percent of damaged buds and furrow width had a nonnormal distribution by the Anderson-Darling probability test. Voltarelli et al. (2013) characterised quality indicators in mechanised agricultural operations and found similar results, with high data variation. The analysis of variance data (Table 2) demonstrate that the variables billets m<sup>-1</sup>, total and viable buds m<sup>-1</sup>, and damaged buds did not significantly differ with increasing planting speed or furrow depth, which can be explained by the very high coefficient of variation (Pimentel-Gomes and Garcia, 2002).

The planting density was 9.8 billets  $m^{-1}$ , greater than that found by Garcia (2008), who found 6 billets  $m^{-1}$  in sugarcane mechanised planting with a planter. The bud density was high (31.4  $m^{-1}$ ), this variable is related to the

Factors	Furrow depth (m)	Furrow width (m)	Disturbed area (cm <sup>2</sup> )	Cover height (m)
Planting speed (km h	<sup>1</sup> ) (S)			
5.0	0.37 <sup>a</sup>	0.68 <sup>a</sup>	1401.9 <sup>b</sup>	0.11 <sup>a</sup>
6.5	0.37 <sup>a</sup>	0.70 <sup>a</sup>	1486.7 <sup>a</sup>	0.10 <sup>b</sup>
Furrow depth (D)				
0.35 m	0.34 <sup>b</sup>	0.67 <sup>b</sup>	1271.8 <sup>b</sup>	0.10 <sup>a</sup>
0.45 m	0.40 <sup>a</sup>	0.70 <sup>a</sup>	1616.8 <sup>a</sup>	0.10 <sup>a</sup>
F-test				
S	0.216 <sup>ns</sup>	1.815 <sup>ns</sup>	4.437*	4.884*
D	60.552*	10.704*	73.529*	1.106 <sup>ns</sup>
SxD	3.436 <sup>ns</sup>	0.037 <sup>ns</sup>	0.037 <sup>ns</sup>	1.278 <sup>ns</sup>
CV (%)	8.59	1.37	12.46	19.54

Table 3. Analysis of variance and means test for furrow depth, furrow width, disturbed area and cover height.

In each column, for each factor, means followed by the same letters do not differ by Tukey's test at 5% probability. <sup>ns</sup> indicates not significant; \* indicates significant at 5% probability by theF-test. CV (%): coefficient of variation.

number of billets, and of those, 27.7 m<sup>-1</sup> were viable buds. Raveli (2013) mentioned that the density of viable buds is the most important feature in the planting process, as it is crucial to ensure good operational results.

The damaged buds totalled 11.9%, which is not a high damage rate for mechanised planting. Raveli (2013) reported that when the billets pass through the seedling metering mechanisms of the planter, the buds become damaged due to the abrasiveness of the mechanism. Buds are sensitive plant structures and easily damaged under such conditions, which results in a reduced quantity of viable buds, hindering the sugarcane sprouting, the plant stand, and consequently the final crop yield.

Raveli (2013) found that 9.48% of the buds were rendered inviable by the mechanised planting operation. Garcia (2008) found 35% inviable buds after planting but did not state the percentage of buds that were rendered inviable by the planting operation.

The furrow depth (Table 3) was not affected by the planting speed, reaching a mean of 0.37 m. For the 0.35 m depth treatment, the furrow depth was similar to the pre-established depth (0.34 m), confirming that the planter has the ability to meet this setting, regardless of planting speed. Furrowing failed to meet the 0.45 m setting, digging 0.05 m lower than desired; however, uniformity was maintained, as indicated by the low coefficient of variation (8.59%), which justifies a new calibration of the depth-limiting wheel.

Anjos and Figueiredo (2008) stated that, in general, the depth should be between 0.25 and 0.30 m and not exceed the depth reached by the tillage system so that the root system reaches the aerated, uncompacted soil in conditions that favour root development. However, with the use of subsoilers in soil tillage, the furrow depth can be increased because the equipment decompacts the soil

at greater depths.

In general, if the soil moisture conditions are favourable, good sprouting rates will occur, regardless of depth. However, in unfavourable climate conditions, a deeper furrow provides better moisture conditions for the billet. In addition, the deep furrow contributes to less erosion by decreasing surface runoff (Cebim, 2008).

For the furrow width, the results showed the same means for the higher planting speed (Table 3). Furrow width increased with greater furrow depth, but very little difference (0.03 m) was observed, which was expected due to the furrow opener shape, as the furrow opener reached higher soil depths under these conditions (0.45 cm).

Because it was directly correlated with the depth and width analyses, the area disturbed by the furrow openers exhibited a similar pattern of results as the furrow depth treatment (Table 3). The disturbed area increased by 6% when the planting speed increased, which was also observed by Silveira et al. (2011, 2013), and a larger disturbed area was observed with increasing furrow depth.

For the cover height of the furrows, although a difference was observed with treatment planting speed, it was small (0.01 m), and the remaining results were similar, averaging 0.10 m. These results indicate that this is an efficient covering mechanism, regardless of the planting conditions. The results can be considered adequate for crop sprouting; according to Coleti and Stupiello (2006), the billet cover height ranges from 0.05 to 0.10 m and can vary depending on the sugarcane variety and planting season.

Regarding the billet metering mechanism (Figure 2a), the right and left furrows were uniform for all treatments (speed x depth). Low variability was observed in the billet metering operation, which indicates operational uniformity,



**Figure 2.** Variable control charts: billets m<sup>-1</sup> (a) and total buds m<sup>-1</sup> (b). LF: left furrow; RF: right furrow; UCL: upper control limit; LCL: lower control limit; X: mean of individual values; and MR: mobile range.

as the results did not go outside the statistical control limits at any points. Thus, even if the planting speed and the furrow depth are increased, the planter performs the billet metering operation within the uniformity standards.

For total buds  $m^{-1}$  (Figure 2b), a pattern similar to that obtain for billet metering was observed, with only one point outside the control limits for the left planting furrow, at a 0.45 m depth, for both planting speeds, 5.0 and 6.5

km h<sup>-1</sup>; this pattern may be attributed to factors such as tractor sliding and lack of pressure in the hydraulic system. However, in general, uniformity is again observed between the right and left furrows, and variability was only increased by the high number of buds per billet (measured in the billet characterisation stage).

The number of viable buds m<sup>-1</sup> (Figure 3a) exhibits similar results to the number of billets m<sup>-1</sup> (Figure 2a) and



**Figure 3.** Variable control charts: viable buds m<sup>-1</sup> (a) and damaged buds (%) (b). LF: Left furrow; RF: right furrow; UCL: upper control limit; LCL: lower control limit; X: mean of individual values; and MR: mobile range.

includes bud damage caused by the planter, given that the damage caused during the harvest and transport stages has already been accounted for. The results stayed within the control limits, with no change as a function of the treatment. The metering operation remains uniform during seedling loading in the planter and is not affected as the planter seedling load decreases. For damaged buds (Figure 3b), some points were outside the normal process variation at the 0.45 m depth and planting speed of 6.5 km<sup>-1</sup>. However, this was an isolated occurrence within the sample, suggesting vulnerability of the operation only in these conditions and thus not hindering the operational quality.

The furrow depth results (Figure 4a) are uniform, with



**Figure 4.** Variable control charts: furrow depth (m) (a) and furrow width (m) (b). LF: Left furrow; RF: right furrow; UCL: upper control limit; LCL: lower control limit; X: mean of individual values; and MR: mobile range.

low values for range and variation and the absence of any abnormal events during the operation. Moreover, depth did not decrease along the seedling metering operation, demonstrating that the prototype furrowing system, which follows the ground surface, is able to compensate for the weight loss of the billets inside the planter throughout the operation. When the planting speed increased, there was also no reduction in furrow depth; that is, the machine maintained uniformity with increasing operational capacity.



**Figure 5.** Variable control charts: disturbed area (cm<sup>2</sup>) (a) and cover height (m) (b). LF: Left furrow; RF: right furrow; UCL: upper control limit; LCL: lower control limit; X: mean of individual values; and MR: mobile range.

For furrow width (Figure 4b), the uniformity in samples is again observed, with reduced range and variation values, as well as the absence of any abnormal events throughout the operation. As with the furrow depth, the planter was able to maintain furrow uniformity regardless of planting speed or changes in the seedling load inside

it, which changes its total weight.

Because it is directly correlated with the depth and width analyses, the results for the soil area disturbed by furrow openers were very similar (Figure 5a). The range between the left and right furrows increased, which does not compromise the operation because, as shown in Figures 4a and 4b, despite the variability in the soil surface, the planter is able to maintain uniformity. The variability remained within the control limits, suggesting that it is an effect of the process, which is constantly subject to change.

The cover height results (Figure 5b) were homogeneous despite the occurrence of one point outside the control limits, which may be linked to the lack of uniformity of the aggregates, thus forming lumps, especially at higher depths and speeds.

#### Conclusions

The increase in planting speed caused an increase in disturbed area and a decrease in cover height. Increased furrow depth caused increased disturbed area, furrow width, furrow depth and cover height. The planting operation demonstrated operational uniformity in furrow opening, seedling metering and cover height at the different planting depths and speeds analysed.

#### **CONFLICT OF INTERESTS**

The authors have not declared any conflict of interest

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Full Length Research Paper

## Growth and yield of baby corn as influenced by nitrogen topdressing

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Baby corn maize (*Zea mays* L.) is a highly profitable alternative to farmers due to its short crop duration, being harvested at the juvenile stage. It demands large amounts of nutrients in a short time, especially nitrogen, which is responsible for the rapid development of crop. This study aimed to evaluate the effect of topdressing application of different doses of nitrogen on the vegetative and spikelet productivity features in the baby corn crop in Janaúba - Minas Gerais. The experiment was laid out in randomized block design with three replications and four treatments. The treatments comprised four doses of nitrogen (0, 40, 80 and 120 kg ha<sup>-1</sup> N applied as urea) top dressed at the V6 (sixty leave with visible auricle) growth stage, which occurs 30 days after sowing the seed. Nitrogen topdressing doses did not interfere in vegetative characteristics of baby corn cultivar Al Bandeirante, but the productivity of spikelet responded to the increasing doses of nitrogen, being the highest productivity at a dose of 120 kg ha<sup>-1</sup> N.

Key words: Fertilizer, urea, Zea mays L., fertilization, yield components

#### INTRODUCTION

Maize (*Zea mays* I.) in Brazil is one of the main cereals grown and consumed, being one of the most important crops in the world. The country is the third largest corn producer in the world, with a production of 84,67 million

tons of grains in the 2014/2015 crop (CONAB, 2015), being surpassed only by the United States and China (FAO, 2013). These tree countries represent 70.11% of world production.

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Depth	<sup>1</sup> Ph	MO <sup>2</sup>	P <sup>3</sup>	K <sup>3</sup>	Ca <sup>4</sup>	Mg <sup>4</sup>	Al <sup>4</sup>	H + Al⁵	SB	v	EC	Prem⁵
cm	Dag	y kg⁻¹	mg	dm <sup>-3</sup>	cmol <sub>c</sub> dm <sup>-3</sup>			%	DS m <sup>-1</sup>	mg L⁻¹		
0-20	6.5	2.1	17	123	3.6	1.7	0	1.4	5.7	81	0.4	43.9

Table 1. Results of the chemical analysis of 0-20 cm layer of Fluvisoil, before the crop establishment.

<sup>1</sup>pH in water; <sup>2</sup> Colorimetry; <sup>3</sup>: Mehlich Extractor-1; <sup>4</sup> Extractor: 1 mol KCI L<sup>-1</sup>; <sup>5</sup>SMP, pH <sup>5</sup>Solution equilibrium of Fr. Prof., sample depth; SB, sum of Bases; V, saturation by bases; Prem, remaining Phosphorus; EC electrical conductivity.Dag kg<sup>-1</sup>=%; mg dm<sup>-3</sup> = ppm; cmol<sub>c</sub> dm<sup>-3</sup> = meq 100 cm<sup>-3</sup>.

Corn offers a wide range of colors and formats. Among them, there is the baby corn, which is the "young corn cob" It is a small young corn ear harvested at the stage of silk emergence (Pereira Filho et al., 1998; Almeida et al., 2005). According to Hardoim et al. (2002) the baby corn is a highly profitable alternative for farmers and can generate a net profit of up to 400% of the amount invested, mainly for those framed as family farming.

Studies carried out by Sahoo and Panda (1999), Verma et al. (1998), Thakur et al. (1997), Faiguenbaum and Olivares (1999) and Kotch et al. (1995) showed good yields of commercial baby corn, which depends on the cultivar used and soil fertility conditions.

The factor "mineral nutrition" is important for obtaining high rate of productivity. Despite the higher plant population density of the of baby corn, usually the fertilization is carried out according to the recommendations to regular corn (Fancelli and Dourado Neto, 2007). The maize demand for nitrogen (N) is a result of its structural function, because it is part of molecules of organic compounds, such as amino acids and proteins (Malavolta, 2006).

In a study conducted by Santos et al. (2014), in southern Brazil conditions, the maximum baby corn yield in the summer crop was obtained with the application of 64.35 kg ha<sup>-1</sup> of nitrogen. In India, Golada et al. (2013) observed that the application of 90 kg ha<sup>-1</sup> promoted the highest baby corn yields.

As the efficiency of the use of nutrients varies according to the culture and environmental factors, it is necessary to determine the nitrogen doses that promote the maximum baby corn yield in different regions.

This work aimed to evaluate the effects of topdressing application of different doses of nitrogen on the vegetative characteristics and yield of spikelet baby corn crops in Janaúba-MG.

#### MATERIALS AND METHODS

The experiment was conducted at the Department of Agricultural Sciences, of the State University of Montes Claros, Janaúba, which is situated a 15°49'48,05" S and 43° 16 ' 7.48". According to Köppen (1948), the climate in the region is semiarid is tropical with dry winter, classified as AW. The mean annual precipitation is 900 mm and the mean temperature is 25°C. The soil of the experimental area is classified as a Fluvisoil (EMBRAPA, 2013). The results of the chemical analysis of soil samples conducted at EPAMIG in Nova Porteirinha prior to the installation of the experiments can be

found in Table 1.

The corn cultivar used was AI Bandeirante, originated from random crossing of cultivars of normal cycle. The experiment was conducted during the period March/June 2014, being the lines arranged every two and four meters between microsprinklers with flow rate of 100 Lh<sup>-1</sup>.

The experiment was laid out in randomized block design with three replications and four treatments. The treatments comprised four doses of nitrogen (0, 40, 80 and 120 kg N ha<sup>-1</sup> applied as urea, with 48% of N) top dressed at the V6 growth stage, which occurs 30 days after sowing the seed.

The experimental plots were composed of three lines of planting spaced 0.8 m among themselves and 3.0 m in length, totaling 7.2  $m^2$ . In data collection were discarded both sidelines and 1.0 m from each side in the direction of its length, totaling 0.8  $m^2$  of useful area. The seeding was done manually at a density of 15 plants  $m^{-1}$  totaling 186567 plants ha<sup>-1</sup>.

Synthetic fertilizer potassium topdressing application was performed at the V6 growth stage, calculated in accordance with the chemical characteristics of the soil and taking into account the recommendations of Ribeiro et al. (1999) for corn growing. It was applied 13 g  $m^{-1}$  of potassium chloride (KCI) in all different treatments. During the crop development, the mechanical control of weeds was performed with hoe between rows, an manually between plants.

The harvests were carried out in the late afternoon, to avoid possible moisture loss of the spikelet. The first one was held seven days after the appearance of the tassels, defaulting as the harvesting point when the spikelets present between 4 to 10 cm long and the diameter of 1.0 to 1.5 cm, cylindrical shape and coloration ranging from pearly white to cream, according to Ritchie et al. (2003) and Hardoim et al. (2002). Generally, this occurs two to three days after exposure of styles-stigmata that have around 2.5 cm long. Thus, the harvest began 63 days after sowing, totaling five harvests every three days. Once harvested, the spikelet were placed in plastic bags, and subsequently forwarded to the Laboratory of Hydraulics and Hydrology of the State University of Montes Claros *Campus* Janaúba- Minas Gerais, where corn mass with and without straw straw was measured. The vegetative and reproductive variables evaluated were:

**Height of plants:** verified on the occasion of the full flowering, when 90% of the plants have issued tassel, measured through the culm length (the surface of the ground to the base of the male inflorescence-"tassel").

**Culm Diameter:** the determination was done on the occasion of full flowering. It was determined at 10 cm height, being the soil as a baseline, from the five plants, which were measured through the use of an electronic caliper.

**Number of sheets per plant:** obtained in full bloom by the count of all the leaves fully expanded and deployed, being considered five plants per plot.

Internodes number: obtained in full bloom by the count of all

**Table 2.** Summary of the analysis of variance for the effect of doses of nitrogen topdressing on the plants height variables (AP), culm diameter (DC), number of leaves (NC), internodes number (NIT), transverse diameter (DT), SPAD reading (SPAD), straw (PP) productivity, productivity without straw (PS), baby corn yield (RD).

Features	Medium squares of treatments		Average	CV <sub>and</sub> (%)	Accuracy	Accuracy (%)
AP	439.73	NS	167.92	9.89	0.61	37.27
DC	0.90	NS	12.91	7.15	0.23	5.44
NF	1.59	*	12.68	4.74	0.88	77.32
NIT	1.59	*	12.68	4.74	0.88	77.32
DT	21.14	NS	92.87	4.66	0.34	11.29
SPAD	156.50	**	37.51	9.35	0.96	92.14
PP	12660020.80	**	5792.27	20.16	0.94	89.23
PS	2841682.06	**	2050.67	22.22	0.96	92.70
RD	76.40	**	34.70	8.56	0.94	88.44

(\*Significant 5%), (\*\*) 1% and significant (<sup>ns</sup>) is not significant, for the F-test; AP (cm), DC (mm), NF (und), NIT (und), DT (cm), SPAD (g.cm  $\mu^{-2}$ ), PP (kg/ha), PS (kg/ha), RD (%).

internodes or stalk present in a plant, being considered five plants per plot.

**Cross-section:** verified on the full flowering, measured by the ends of the plant towards the rows, using measuring tape, being considered five plants per plot.

**SPAD reading:** when the plants were in full bloom, readings were performed using the portable meter of chlorophyll SPAD-502 (Soil-Plant Analysis Development (SPAD) Section, Minolta Camera Co., Ltd, Japan). Measurements with the SPAD were performed at the fifth corn leaf downwards. Evaluations were carried out in five plants per plot, being three readings by leaves.

**Mass of spikelet with straw:** obtained by weighing on digital scale semi analytical of all commercial spikelet with diameter ranging between 0.8 and 1.8 cm and length between 4 and 12 cm, cylindrical shape and spikelet non-fertilized in the parcel. Every spikelet harvested in useful areas was weighted with straw. The data of commercial spikelet weight were transformed into kg ha<sup>-1</sup>.

**Mass of spikelet commercials without straw:** The same process used for mass of spikelet with straw was performed. Every spikelet harvested in useful areas was weighted without straw. The data of commercial spikelet weight were transformed into kg ha<sup>-1</sup>.

Baby corn yield: Obtained by the equation: (PS \* 100)/PP

PS = productivity without straw

PP = productivity with straw

Experimental accuracy was evaluated by estimation of the accuracy by Resende and Duarte (2007), given by the estimator:



Where,  $\hat{r}_{\hat{g}g}$  is the experimente accuracy;  $CVe^2$  is the square of the experimental variation coefficient;  $CVg^2$  is the square of the coefficient of genetic variation e r corresponds to the number of repetitions.

The data were subjected to analysis of variance using the

statistical program GENES B (Cruz, 2013) and, when the F was significant to the level of 5%. For the variables that presented significance, a regression analysis was performed according to the nitrogen doses used.

#### **RESULTS AND DISCUSSION**

The quality of an experiment is usually measured by experimental Coefficient of Variation ( $CV_{and}$ ). The values of  $CV_{and}$  in all the variables (Table 2) were within the limits reported in the literature (Pizolato Neto et al., 2016; Silva et al., 2013, 2016; Souza et al., 2016), what guarantees a good accuracy in the experiment.

However, the CV<sub>and</sub> is not best suited to assess the accuracy of a study, since its estimates considers only the residual variance as a proportion of the average of the experiment (Resende and Duarte, 2007). The measurement of accuracy  $(\hat{r}_{\hat{g}g})$  is best suited to determine the accuracy of an experiment, since the accuracy depends on the magnitude of the residual variation in the number of repetitions, the proportion between the variations of residual nature and the treatments associated with the character in evaluation (Resende and Duarte, 2007). The accuracy is estimated to be between 0 to 1, the closer to 1 the more accurate is the experiment.

In the present work, except for the variables height, diameter and transverse diameter culm, the accuracies estimated ranged from high (> 0.70) to very high (> 0.90) (Table 2) showing that there is a good experimental precision, according to the values recommended by Raj and Duarte (2007). The good accuracy presented in this work leads to the conclusion that the casualization of the blocks and the number of repetitions allowed the reduction of environmental heterogeneity in the study, and verify the influence of doses of nitrogen topdressing on the agronomic performance of baby corn.

There was no significant difference (P > 0.05) for the



Figure 1. SPAD index in baby corn submitted to different doses of nitrogen topdressing. \*\*significant at the 1% level, by t.

variables, height of plants, culm diameter and crosssection for the different doses of nitrogen (N) (Table 2). The plant height considered great for easy harvest of baby corn varies between 1.9 and 2.5 m (Rodrigues et al., 2004). In the culture of baby corn, the largest height of plants combined with the use of high densities is considered a negative factor on productivity, because it favors plant bedding The average height of the plants in this experiment was 1.68 m. This low height of plants can be related to the density of the experiment (186567 ha<sup>-1</sup> plants) and/or cultivar Al Bandeirante for being a rustic breed from random breeding of cultivars of normal cycle after several generations of mass selection and recombination between families of half-siblings.

This work corroborates with Pereira Filho et al. (2005), which did not observe significant differences in culm diameter with increasing nitrogen doses. The culm diameter is a very important variable, as it is directly related to plant bedding, an undesirable process in baby corn crops.

There was significant effects between doses of N topdressing for SPAD index (Table 2), and with quadratic effect according to the doses of N (Figure 1). 82 dose, by ha<sup>-1</sup> N provided the highest SPAD which corresponded to  $42.17 \ \mu \ g \ cm^{-2}$  sheet.

The chlorophyllmeter does not detect the luxury consumption of N (Blackmer and Schepers, 1994), that is, when working with high doses of N, the index SPAD on the sheet tends to increase up to a point, called a photosynthetic maturity point (Costa et al., 2001), from which remains invariant, while the N content continues to increase with increasing doses of this nutrient (Schepers et al., 1992). This can be attributed to the fact the device detects, indirectly, increasing N only when being incorporated into molecules of chlorophyll and not free, nor incorporated (N in<sub>3</sub><sup>-</sup>), in which the N accumulates

when there is luxury consumption (Larcher, 2005).

In full bloom, the chlorophyllmeter reading values were lower than those considered appropriate  $\mu$  g 58.0 cm<sup>-2</sup>. In all treatments, results reflect the insufficient level of N in plants. However, the application of N in this growth stages very efficient, because the greatest demand for this nutrient occurs about two to three weeks before flowering, that is, usually 95% of period total N of the plant had already been absorbed (Plénet and Cruz, 1997).

Argenta et al. (2002) highlighted the possibility of inferring on the nutritional status of N on corn from chlorophyllmeter readings. The feasibility of using this equipment for diagnosing nutrient deficiency and need for nitrogen fertilization is effective, especially when there is greater variation in the supply of N, whether in dose, installments and nitrogenous sources. Thus, with the use of the SPAD in the present study it was possible to verify the variation of the doses tested. In the Figure 1, one can verify that in the dose above 82, by ha<sup>-1</sup> N, there was a decrease of N in foliage.

The productivity of baby corn with and without fallwinter crop straw was influenced by the application of N. There was increased productivity with high doses (Figure 2), being the highest productivity achieved at a dose of 120 kg N ha<sup>-1</sup>, with 7815.06 and 3244.06 kg ha<sup>-1</sup> of baby corn with straw and no straw, respectively. These results corroborate with Thakur et al. (1997) who, evaluating the effect of 4 doses (0, 40, 80 and 120 kg ha<sup>-1</sup> of Nitrogen) in the culture of baby corn, observed that there was an yield increase at doses above 100 kg ha<sup>-1</sup> N.

However, to decide the dose to be used it is interesting to note the economic feasibility, since the same varies throughout the year, depending on the market prices of baby corn and nitrogen fertilizer.

Significant yields of baby corn in India were obtained by Thakur and Sharma (1999) with the dose of 200 kg ha<sup>-1</sup> of



Figure 2. Productivity with straw (PP) and productivity without straw (PS) subjected to different doses of nitrogen topdressing.



**Figure 3.** Baby corn yield, percentage of productivity without straw versus productivity with straw, subjected to different doses of nitrogen topdressing.

N applied in three equal installments, being one part when planting and the other two at 25 and 40 days after sowing. According to these authors, the increase of 100 to 200 kg ha<sup>-1</sup> N promoted increase in yield of 10.26%, and between 100 and 150 kg ha<sup>-1</sup>, there was no significant difference. Sahoo and Panda (1999), working with N doses 80, 120 and 160 kg ha<sup>-1</sup> and three spaces, verified higher income at a dose of 120 kg ha<sup>-1</sup> and density 125,000 plants per hectare.

High doses of N in baby corn crops, according to Miles and Zens (1997), are not necessary, since the baby corn is harvested before the processes of growth and grain filling. Such authors stress that doses of 30 to 50 kg ha<sup>-1</sup> N plus phosphorus and potassium (K) are sufficient to obtain a good production, and that N plus K must not exceed 90 kg ha<sup>-1</sup> in the planting furrows. Unlike that work, it was possible to observe a better yield with higher doses, because the plants, when grown in high densities, promote greater competition for nutrients in function of its rapid growth.

The baby corn yield response (percentage ratio of spikelet without straw production/spikelet with straw production) can be verified in Figure 3. The dose corresponding to the lowest yield was 50.89 kg ha<sup>-1</sup> of N. From that dose there was yield increase, allied to yield without straw (Figure 2), and the highest proportion of spikelet without straw, 40.26% was obtained with the application of 120 kg ha<sup>-1</sup> of N.

Silva et al. (2013) studying the effects of the application of doses of N on baby corn yield, obtained the best



**Figure 3.** Baby corn yield, percentage of productivity without straw versus productivity with straw, subjected to different doses of nitrogen topdressing.

productive response and economic yield with the maximum dose of nitrogen (160 kg ha<sup>-1</sup> N).

#### Conclusions

Nitrogen topdressing doses do not interfere in vegetative characteristics of baby corn cultivar Al Bandeirante, aside from the number of leaves and internodes, in Janaúba-MG.

The spikelet productivity baby corn crops responded to the increasing doses of nitrogen, being the highest productivity and yield of baby corn found at the dose of 120 kg ha<sup>-1</sup> N.

#### **CONFLICT OF INTERESTS**

The authors have not declared any conflict of interests.

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Full Length Research Paper

# Correlation and cluster analysis of white seeded sesame (Sesamum indicum L.) genotypes oil yield in northern Ethiopia

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The experiment was carried out to access the inter relationship of genotypes in their oil yield with seed yield, oil content and other yield components and to determine cluster of white seeded sesame genotypes and environments based on their oil yield. Seventeen sesame genotypes were tested at six environments in northern Ethiopia during 2015 main season. The experiment was laid out in Randomized Complete Block Designs (RCBD) with three replications across all the environments. Seed yield had a significant and positive correlation ( $P \le 0.01$ ) with number of branches (r = 0.414) and number of capsules (r =0.468). Positive correlation seed yield with number of capsules and number of capsules indicated that better yield can obtain from highly branched plants and high number of capsules because the number of capsules increases with the degree of branching. Seed yield was negative significant correlated with days to maturity (r = -0.500) and flowering time (r = -0.626), significant at ( $p \le 0.05$ ) and ( $p \le 0.01$ ), respectively. The negative association between seed yield and days to flowering and maturity indicated that moisture stress after flowering might have caused relatively a yield reduction in the late maturing genotypes. Whereas, the early flowering and early maturing genotypes can escape the moister stress conditions. Based on the cluster analysis of 17 white seeded sesame genotypes classified in to different four distinct clusters. Cluster II, III and IV had high mean yield, oil content and oil yield than the other clusters. Genotypes grouped in those clusters had also greater genetic divergence important for farther yield, oil yield and oil content improvement program breeding in northern Ethiopia. Sesame growing environments also clustered in to four groups. Environments grouped in Cluster I, II and IV had high seed yield, oil content and oil yield. Hence, environments grouped in those clusters are important for seed yield, oil content and oil yield production improvement program in the study areas.

Key words: Cluster analysis, correlation, dendrogram.

#### INTRODUCTION

Sesame (Sesamum indicum L.) belongings to Pedaliaceae family, is an important and ancient oilyielding crop. It has an edible seed and has high quality oil (Pathak et al., 2014). It is a diploid species with 2x = 2n = 26 chromosomes. It is a self-pollinated crop, containing 60 species organized into 16 genera (Zhang et al., 2013). Ethiopian sesame is among the highest quality in the world, as seeds are naturally produced at near-

organic levels. It is 2<sup>nd</sup> in terms of area coverage of 420,494.87 ha and total production of 288770 ton/ha next to noug (Guizotia abyssinica Cass.) (CSA, 2015). Western (Humera) and northwestern (Sheraro) parts of Tigray are the main sesame producers with large commercial farms and small scale farmers and good source of income using as a cash crop and local oil extraction (Fiseha et al., 2015). Cluster analysis is the most widely used technique for classifying environments or genotypes into homogeneous groups. It operates on a matrix of dissimilarity (similarity) indexes for all possible pairs of genotypes or pairs of environments, depending on which is being clustered (Ghaderri et al., 1980). Cluster analysis aims to classify a sample of subjects (Objects) on the basis of a set of measured variables into a number of different groups such that similar subjects are placed in the same group (Cornish, 2007). Ward's method is distinct from all other methods because it uses an analysis of variance approach to evaluate the distances between clusters and it attempts to minimize the Sum of Squares (SS) of any two clusters that can be formed at each step. Observations in a specific cluster share many characteristics in common, but are very dissimilar to observations not belonging to that cluster (Ward, 1963).

Among the different types of clustering hierarchical clustering is by far the most widely used clustering method. In cluster analysis performing Mahalanobis's (D<sup>2</sup>) statistic analysis. Mahalanobis (1936) is important which is use for assessing the genetic divergence among the test entries in different clusters. If there is a large jump in the distance between clusters from one stage to another then this suggests that at one stage clusters that are relatively close together were joined whereas, at the following stage, the clusters that were joined were relatively far apart. In any plant breeding program the final objective is to increase quality and quantity of a required crop. Knowing the association between the required traits and other related traits is a prerequisite for breeding programs. Hence, correlation coefficient can measure such simple linear relationship between different vield components. Correlation coefficients range between -1 and +1 and measure the degree and direction of relationships between various traits. Yield is a dependable complex inherited character as a result of interaction of several contributing factors that may be related or unrelated (Subramanian and Subramanian, 1994). Even though the current sesame production in Ethiopia has many opportunities, large area with suitable environments for production (North western and South Western Ethiopia) (Figure 1), growing in low moisture areas, presence of genetic diversity and export demand and very competitive world wide (Wijnands et al., 2007).

Sesame yield, oil content and oil yield are highly variable depending upon the growing environment, cultural practices and cultivars. Even early released varieties showed variable oil content across environments and years.

Therefore, sesame production for its oil content requires due attention and exhaustive researches to identify the right genotype for different production areas. Sesame production is becoming difficult and frustrating for the sesame producers from time to time due to climate variability and it is sensitive crop due to weather changes. It is location specific oil crop and it is difficult to test at individual sesame growing areas in terms of cost and other resources. Therefore, classifying genotypes and environments into similar groups is important to test at one of the clusters. GEI studies to evaluate sesame genotypes were not practicing for many years ago in Ethiopia. Therefore, inaccessibility of improved, stable and high vielder genotypes. But some of the few studies are (Yebio et al., 1993) reported that year and site had significant influence on oil content. There were also significant differences among lines for oil content. The line-site interaction was highly significant difference suggesting that the ranking of the eight lines for oil content across three locations were inconsistent. Hagos and Fetien (2011) showed that there was significantly among genotypes, environments and GEI and the mean yield of genotypes differed from environment to environment in thirteen sesame genotypes tested across three years and locations from 2006 to 2008 in Western and North Western lowlands of Tigray. Zenebe and Hussien (2010) revealed that significant different of GEI among 20 sesame genotypes tested across six environments of Southern Ethiopia. They showed the GEI had significant values for all characters, indicating unstable in their expression with change in environment. The oil content of these genotypes also had highly significant difference across locations and seasons. This difference is due to the difference of soil type, rain fall and temperature in the test locations.

Mekonnen et al. (2015) Reported that there was significant variations among environments and genotypes across all environments in two cropping seasons of twelve sesame genotypes at eight environments in five locations of eastern Amhara Region (Figure 1). The environments had different impacts on the seed yield and oil yield potential of the genotypes. Mohammed et al. (2015) and Fiseha et al. (2015) reported that significance variation among genotypes, environments and GEI components showed highly significant variation for all traits. Thirteen sesame genotypes were evaluated in three locations in North western and Western Tigray from 2011 to 2013

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Figure 1. Map of test sites in northern Ethiopia.

cropping seasons. Environments were the main sources of variation for most of the traits. While, genotypes the main sources of variation for seed yield. Sufficient GEI studies have been undertaken elsewhere in the world to meet either or all of the objectives, among which Yebio et al. (1993), Kumaresani and Nadarajan (2010), Adebisi (2009), Zenebe and Hussien (2010), Hagos and Fetien (2011), Mirza et al. (2013), Fiseha et al. (2015), Mekonnen et al. (2015) and Mohammed et al. (2015). Even all the above studies had undertaken GEI studies on sesame in Ethiopia and the rest of the world, there was no specifically work did on globally preferred white seeded sesame genotypes in Ethiopia and on clustering and correlation of white seeded sesame oil yield. Therefore, this experiment was done to access (i) the inter relationship of genotypes in their oil yield with seed yield, oil content and other yield components (ii) to determine cluster of white seeded sesame genotypes and environments based on their oil yield.

#### MATERIALS AND METHODS

#### Description of the study areas

#### Experimental plant materials used in the study

Experimental design and management: The experiment was laid out in randomized complete block design (RCBD) with three replications in all testing sites and  $10 \text{ m}^2$  total plot area and  $6 \text{ m}^2$  net plot area with 1m between plots and 1.5 m between blocks keeping inter and intra row spacing of 40 and 10 cm, respectively. Each experimental plot received the same rate of DAP (100 kg/ha) and

Urea (50 kg/ha).

#### **Data collection**

From the harvestable three rows ten plants were selected randomly and tagged to collect data of plant height, length of capsule bearing zone, number of branches, number of capsules and seeds per capsule. The three experimental rows were harvested, tied in sheaves and were made to stand separately until the capsules opened. After the sheaves have dried out fully and all of the capsules opened, seeds were tipped out onto sturdy cloths or canvases and threshing was accomplished by knocking the sheaves.

#### Days to 50% flowering (DF)

The number of days from emergence to which 50% of the population in each plot had flowered.

#### Days to 75% maturity (DM)

The number of days from emergence to when 75% of the plants in each plot had fully matured.

#### Plant height (PH) (cm)

This growth parameter was measured from ten randomly selected and tagged plants from the harvestable rows of each plot with the help of meter tape from ground surface to the top of the plant.

#### Length of capsule bearing zone (LCBZ) (cm)

A height from the first capsule to tip of the plant, measured using

meter tape.

#### Number of primary branches per plant (NBPP):

Branches producing productive capsules were done recorded for randomly selected plants.

#### Number of capsules (NCPP)

The total number of capsules was counted from ten randomly selected plants at maturity.

#### Seed yield (kg/ha)

The total seed yield harvested from the net plot area was weighed using a sensitive balance.

#### Thousand seed weight (TSW)

Weighed in grams of 1000 seed.

#### Number of seeds per capsule (NSPC)

Number of seeds per capsule.

#### Oil contents (OC) (%)

Oil content was determined by wide line nuclear magnetic resonance (NMR). Seeds were taken from each plot and oven dried at 130°C for 2 h and cooled for 1 h. A sample of 22 g of oven dried clean seed was used for analysis of oil content by NMR (Newport analyzer) (Newport Pagnell, Bucks, and UK) (Robbelen et al., 1989).

#### Oil yield

The product of oil content (%) and seed yield (kg/ha).

#### Data analysis

Statistical estimations and computations were performed using different statistical softwares. Homogeneity of residual variances was tested prior to a combined analysis over locations in each year as well as over locations using Bartlett's test (Steel and Torrie, 1998). Analysis of variance for each environment, combined analysis of variance over environments, correlation between different stability parameters were computed using GenStat 16<sup>th</sup> edition (2009).

#### **RESULTS AND DISCUSION**

#### Combined analysis of variance

Variances of homogeneity from results of the Bartlett test revealed that the mean squares of individual environments were homogenous for seed yield, oil contents and oil yield. So, combined analysis of variance could be done. The main effects of environments and genotype and GEI were highly significant at ( $p \le 0.001$ ) for seed yield, oil content, oil yield and yield components. The mean performance of genotypes for oil yield across the tested environments displayed highly Significant variation at (p < 0.001). This significance variation gives opportunity for selecting better performing genotypes for oil yield. The grand mean oil yield over six environments was 296.6 kg/ha and the mean oil yield of genotypes across six environments ranged between 125.48 kg/ha in Humera to 531.21 kg/ha in Sheraro respectively (Table 1). High oil yield variation was detected among genotypes ranged between 193.6 kg/ha in G8 to 409.4 kg/ha in G1 respectively (Table 2).

Therefore, G1 had highest oil yield followed by G7, G16, G14, G5 and G12 while G8 was poorly oil yielding. Environments, Sheraro, Dansha and Gendawuha had received optimum rainfall and gave high oil yield. Conversely, the low yielding environments, Humera, Wargiba and Maykadra affected by moisture stress in 2015 cropping season. Oil yield in (kg/ ha) was the product of seed yield (kg/ ha) and oil content (%). The oil yield of genotypes varied to different environments and years with rank changed, this variation among genotypes indicating that selection should be based on mean performances of the genotypes to their respective environments. High seed yield and high oil content genotypes gave highest oil yield whereas low seed yield and oil content genotypes gave low oil yield. The mean performance of genotypes changed across environments. Hence, the breeding strategy should be based on high yielder genotypes. This result is in line with El-Bramawy and Shaban (2007), Mekonnen et al., (2015), Mahasi et al., (2005) on sesame.

## Correlation of seed yield, oil content, oil yield and yield components

Correlation coefficient analysis was done for yield and other traits for each environments. Seed yield had a significant and positive correlation (P≤0.01) with number of branches (r = 0.414) and number of capsules (r =0.468). Positive correlation seed yield with number of capsules and number of capsules indicated that the best yield was obtained with highly branched plants and high number of capsules because the number of capsules increases with the degree of branching. Similar report by Pham (2011), Fiseha et al. (2015) and Abdou et al. (2015). Seed yield was negative significant correlated with days to maturity (r = -0.500) and flowering time (r = -0.626), significant at ( $p \le 0.05$ ) and ( $p \le 0.01$ ), respectively. The negative association between seed yield and days to flowering and maturity indicated that moisture stress after flowering might have caused relatively a yield reduction in the late maturing genotypes. Whereas, the early flowering and early maturing genotypes helps them to escape the moister stress conditions. This result is similar
	Table 1.	Agro-climatic and	soil characteristics	of six exper	rimental locations i	in northern Ethiopia
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Description	Locations							
Description	Dansha	Maykadra	Humera	Sheraro	Wargiba	Gendawuha		
Altitude(m.a.s.l)	696	646	609	1028	-	760		
Latitude (°N)	13°36'	14°02'	14°15'	14°24'	12° 41'	12°		
Longitude (°E)	36°41'	36°35'	36°37'	37°45'	39° 42'	36°		
R.F. (mm)	888.4	NA	576.4	1000	750	850-1100		
Temp. (°C)	28	NA	18.8-37.6	18.8-34.9	18-25	19.5-35.7		
Soil Characteristics	Vertisol	Chromic vertisol	Chromic Vertisol	Vertisols	NA	Vertisol		

Source: Tigray meteorology data for Dansha, Sheraro, Wargiba, Humera and Maykadra (2015): IPMS Ethiopia, for Gendawuha (2005). NA=Not Available.

Table 2. Description of genotypes used in the study.

Genotype (G)	Code	Status	Sources	Seed color	Remark
HuRC-4	G1	Advance line	HuARC	White	Collection
ACC202514	G 2	Advance line	HuARC	White	Collection
Land race Gumero	G 3	Advance line	HuARC	White	Collection
Abuseffa	G 4	Advance line	HuARC	White	Collection
HuRC-1	G 5	Advance line	HuARC	White	Collection
Rawyan -2	G 6	Advance line	HuARC	White	Collection
HuRC-3	G 7	Advance line	HuARC	White	Collection
Acc 202300	G 8	Advance line	HuARC	White	Collection
Kefif	G 9	Advance line	HuARC	White	Collection
Acc111824	G 10	Advance line	HuARC	White	Collection
Acc 111518	G 11	Advance line	HuARC	White	Collection
Acc 27913	G 12	Advance line	HuARC	White	Collection
Gumero	G 13	Advance line	HuARC	White	Collection
HuRC-2	G 14	Advance line	HuARC	White	Collection
Acc 227880	G 15	Advance line	HuARC	White	Collection
Setit -1(Standard check)	G 16	Standard check	HuARC	White	Collection
Hirhir( Local check)	G 17	Local check	HuARC	White	Collection

Source: Humera Agricultural Research Center (HuARC) (2014)

with Yol (2010) and Fiseha et al. (2015). Days to maturity was negatively correlated with all traits except with SPC (r = 0.194). Number of capsules was positively correlated with number of branches which is in line with several studies (Yirgalem et al., 2013; Fiseha et al., 2015) in sesame. OY was positive and highly significantly correlated with SY (r=0.895) and OC(r=0.512). OY also negatively and significantly correlated with DF(r=-0.725), DM(r=-0.697) and SPC (r=-0.439) (Table 3).

#### Clustering of genotypes based on mean oil yield

Cluster analysis based on Ward's method (Ward, 1963) using squared Euclidean distance of the distance metric and standardized variables was performed using Minitab release 16 (Minitab, 1998) to cluster the genotypes and sesame growing environments

based on their oil yield in different environments (Figure 2). Moreover, Genetic divergence among the seventeen genotypes was estimated using the Mahalanobis  $(D^2)$ statistics (Mahalanobis, 1936). Seventeen sesame genotypes were clustered in to four distinct groups based on their oil yield across six environments (Table 1). Cluster I containing seven genotypes: HuRC-4, HuRC-1, HuRC-2, HuRC-3, Rawyan-2, Kefif and Hirhir (local check) with mean seed yield of (594.26 kg/ha), oil content (42.5%) and oil yield (271.29 kg/ha) (Figure 3). This cluster recorded the lowest seed yield and oil content than all clusters. Cluster II containing only one genotype, Abuseffa (668.6 kg/ha), oil content (50.26%) and oil vield (290.7 kg/ha) contained high seed yield, oil content and oil yield. Cluster III containing seven genotypes viz. ACC202514, Acc111824, Gumero, Land race Gumero, Acc 202300, Acc 111518 and Setit -1 with average seed yield of (600.7kg/ha), oil content (49.83%) and oil yield

	DF	DM	LCBZ	NBP	NCP	PH	SPC	TSW	SY	00	ΟΥ
DF	1										
DM	0.882**	1									
LCBZ	-0.724**	-0.569**	1								
NBP	-0.221	-0.022	0.490*	1							
NCP	-0.364	-0.241	0.683**	0.460*	1						
PH	-0.464*	-0.346	0.581**	0.571**	0.232	1					
SPC	0.36	0.194	-0.4	-0.414*	-0.483*	-0.049	1				
TSW	-0.037	-0.109	0.115	-0.323	-0.231	-0.097	0.006	1			
SY	-0.626**	-0.500*	0.855**	0.414*	0.468*	0.566**	-0.539*	0.194	1		
OC	-0.315	-0.193	0.368	0.377	0.282	-0.108	-0.669**	0.314	0.395	1	
OY	-0 725**	-0 697**	0 873**	0 279	0 494*	0.387	-0 439*	0 239	0 895**	0.5*	1

Table 3. Correlation coefficients between seed yield, oil content, oil yield and yield components.

Ns=non-significant, \*, significant at ( $P\leq0.05$ ), \*\*=highly significant at ( $P\leq0.01$ ). NBPP =number of branches per plant, NCPP= number of capsules per plant, DF =days to flowering, DM=days to maturity, LCBZ= length of capsule bearing zone, PH= plant height, TSW= thousand seed weight, SY= seed yield, OC=oil content, OY=oil yield.



Figure 2. Dendrogram showing of sesame growing environments and degree of relationship based on oil yield.

(227.16 kg/ha). this cluster had average yield and included the released variety standard check(Setit-1).

Cluster IV included two genotypes, Acc 27913 and Acc 227880 with mean seed yield of (641kg/ha), oil content (50.69%) and oil yield (313.75 kg/ha). This cluster had the highest oil yield and oil content and high seed yield. Parsaeian et al. (2011) used cluster analysis and grouped different genotypes of sesame in to five similar clusters based on their Agro-morphological traits. Likewise, Tripathi et al. (2013) also clustered different

sesame genotypes based on morphological and quality traits, Gadisa et al. (2014) clustered different genotypes based on phenotypic traits and Fiseha et al. (2015) based on agronomic traits. The mean value of the 17 quantitative characters in each cluster is presented in Table 2. Cluster I consisted of seven genotypes having the characteristic of early flowering, early maturing, high number of seeds /capsule, longest plant stature, longest length of capsule bearing zone, but low seed yield, oil content (Table 4). Whereas Cluster IV consists of two genotypes. This



Figure 3. Dendrogram showing of sesame genotypes and degree of their relationship based on oil yield.

	Humera	Dansha	Sheraro	Wargiba	Maykadra	Gendawuha
Humera	1					
Dansha	0.357	1				
Sheraro	0.353	0.176	1			
Wargiba	0.611**	-0.14	0.555*	1		
Maykadra	0.606**	0.37	0.245	0.430*	1	
Gendawuha	0.440*	0.326	-0.059	0.228	0.382	1

Table	4.	Correlation	coefficients	between	environments.
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Ns=non-significant, \*, significant at (P≤0.05), \*\*=highly significant at (P≤0.01).

cluster characterized by shortest plant stature, earlier flowering, earlier maturity, high number of branches, high number of capsules, high seed yield, highest oil yield and oil content. Similar work was reported by Gadisa et al. (2014) and Fiseha et al. (2015).

# Clustering of sesame growing environments based on oil yield

Six sesame genotypes environments were clustered in to four distinct groups based on their oil yield (Table 2). Cluster I containing three environments: Humera, Wargiba, Sheraro with mean seed yield of (603.35kg/ha), oil content (49.24%) and oil yield (291.1 kg/ha). This cluster of environments recorded average seed yield. Cluster II containing only one environment, Dansha with mean seed yield of (668.6 kg/ha), oil content (50.26%) and oil yield (261.2 kg/ha). Cluster III containing one environment viz. Maykadra and recorded seed yield (386.76 kg/ha), oil content (48.61%) and oil yield (197.01 kg/ha). This cluster recorded the lowest seed yield, oil content and oil yield. Whereas Cluster IV contained one environment, Gendawuha which recorded the highest seed yield (853.04 kg/ha), oil content (52.54%) and oil yield (447.95 kg/ha) than all clusters. Variation exist among genotypes indicated that oil content was highly affected by environments (rain fall, altitude, soil type, temperature etc.). Gendawuha (52.5%) gave the highest mean oil content followed by Sheraro (50.99%) and Dansha (50.6%) which received high rainfall in the 2015 cropping season (Table 5).

Lower altitude locations were faced to moisture stress and gave low oil content: Humera (48.2%) followed by Wargiba (48.5%) and Maykadra (48.61%).This result is in agreement with Zenebe and Hussein (2010) and Mekonnen et al. (2015). To improve the oil yield and oil content of sesame genotypes, selection should be based

Clustere	Constructor	Mean				
Clusters	Genotypes	OY	SY	OC		
Ι	HuRC-4, HuRC-1, HuRC-2, HuRC-3, Rawyan-2, Kefif, Hirhir	271.29	594.26	42.5		
11	Abuseffa	290.7	668.6	50.26		
Ш	ACC202514, Acc111824, Gumero, Land race Gumero Acc 202300, Acc 111518, Setit -1	227.16	600.7	49.83		
IV	Acc 27913, Acc 227880	313.75	641	50.69		

Table 5. Clustering of genotypes based oil yield and mean seed yield, oil content.

OY= oil yield, SY = seed yield, OC =oil content

on high seed yield with relatively high oil content or cross breeding of high seed yielder with high oil content genotype.

#### Conclusion

White seeded sesame genotypes showed varied response to environmental changes. Thus, the influence of environment was highly prominent in the manifestation of oil yield variation along northern Ethiopia sesame growing environments (Figure 1). The positive correlation yield with number of capsules and number of branches indicated that best yield was obtained with highly branched plants and high number capsules. The negative association between yield and flowering and maturity indicated that moisture stress after flowering might have caused relatively a yield reduction in the late maturing genotypes. Whereas, the early flowering and maturing genotypes can escape the moister stress conditions. Therefore, early maturing genotypes will be recommended for moisture stress areas and late maturing for high rain fall areas. Genotype clusters had high mean yield, oil content and oil yield with greater genetic divergence will be used for oil yield improvement program. Environmental clusters had high yield, oil content and oil yield will be used for oil yield production in the study areas.

#### **CONFLICT OF INTERESTS**

The author has not declared any conflict of interests.

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Full Length Research Paper

# Evaluating the impact of 2010 Rift Valley fever outbreaks on sheep numbers in three provinces of South Africa

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Whilst livestock production plays an increasingly important role in the economies of most nations, it remains vulnerable to diseases. Between 2008 and 2011 South Africa experienced episodes of Rift Valley fever (RVF) outbreaks. Losses of animals especially, sheep were widespread in the Free State, Northern Cape and Eastern Cape provinces. Despite the knowledge that the RVF outbreaks in 2010 affected farmers negatively, no previous quantitative assessment has been conducted to establish the extent of this impact on farmers' herds. This paper evaluated the influence of 2010 RVF outbreaks on sheep numbers using data that were obtained from a 2014 field survey of 150 farms in the most severely affected districts of the Northern Cape, Eastern Cape and Free State provinces. Farmers were asked to indicate their herd size, offspring rate, mortality rate and weaning rate before, during and after the outbreaks. The average performance parameters were checked against the provincial livestock numbers reported by the Department of Agriculture, Forestry and Fisheries in 2013. Sheep numbers declined from 7.5 million in 2008 to 7.3 million in 2010, 6.2 million to 6.1 million, and from 4.9 million to 4.8 million in the Eastern Cape, Northern Cape and Free State, respectively. The total financial value of sheep losses incurred by farmers in the three provinces during the 2010 outbreaks was estimated at R203.4 m.

Key words: Animal losses, financial losses, Rift Valley fever, sheep numbers, South Africa.

#### INTRODUCTION

Globally, the livestock sector plays an important role in the economies of many developing countries (Blench, Chapman and Slaymaker, 2003). About 800 million to 1 billion of the world's poor and landless derive their livelihoods from livestock activities (Livestock in Development [LID] 1999; Birol et al., 2011). Morgan and Tallard (2007) estimated that food derived from items such as meat, milk and eggs in Africa contributes, on average, 30% to agricultural gross domestic product (GDP). About 70% of the rural poor in Africa own

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Author(s) agree that this article remain permanently open access under the terms of the <u>Creative Commons Attribution</u> <u>License 4.0 International License</u> livestock and over 200 million of these livestock owners rely on their livestock for income as well as draught power and fertiliser for crop growing (Morgan and Tallard, 2007).

Although livestock production plays an important role in the economies of most nations, livestock remains vulnerable to diseases. These diseases sometimes result in outbreaks that vary in severity and magnitude of economic impact. Immediate impacts of a disease outbreak include a reduction in the productive capacity of the animals and a subsequent reduction in the supply of meat and meat products (Pritchett et al., 2005).

According to Perry and Randolph (1999) animal disease outbreaks pose substantial threats to livestock sectors in terms of the economic effects of the diseases themselves as well as the actions taken to alleviate the risk of disease introduction or spread. The multidimensional nature of these effects tends to complicate effective policy responses (Rich et al., 2005). The first response to policy-related appraisal of animal diseases control often requires analyses of the economic impacts of a disease outbreak (Rich et al., 2005). The impact of animal diseases on agriculture is usually assessed in quantitative terms; lost revenues, cost of eradication, decontamination, vaccination and restocking, and the numbers of affected farms, animals and humans.

Rich and Wanyioke (2010) assessed the regional and national socio-economic impacts of the 2007 RVF outbreak in Kenya. RVF is a zoonotic disease that is spread by infected mosquitoes. It is characterised by high rates of abortion and neonatal mortality, primarily in sheep, goats and cattle, but also in exotic and wild animals. The total economic losses from livestock mortality were estimated to be over US\$9.3 million, and US\$77.00 for potential milk production, while RVFinduced abortions in sheep resulted in a 22% reduction in potential flock size. However, 'conventional estimates of the cost of disease have no particular significance, because their magnitude carries no indication as to what, if anything, should (or could) be done about the situation' (McInerney et al., 1992).

Velthuis et al. (2010) used an econometric model to estimate the financial consequences of the bluetongue 8 (BTV8) epidemics of 2006 and 2007 in the Netherlands. The costs of the outbreaks were estimated using a deterministic economic model that was compatible with the Dutch livestock production systems for cattle, sheep and goats. The financial consequences of the outbreak included the impact on production, treatment of infected animals, diagnostic costs and costs of control measures applied during the course of the outbreak. The cattle sector incurred the highest costs of all sectors: 88 and 85% of the net costs for the 2006 and 2007 outbreaks. respectively. The study revealed that the introduction and establishment of BTV8 in the Netherlands caused significant losses, due to both the clinical disease and control measures that were constantly required.

Given the vital role played by livestock farming as a source of livelihood for many rural communities and resource poor farmers (Spies, 2011) and its contribution of more than 40% to the total agricultural GDP, it is important understand the true impact of this disease in South Africa. Such information is crucial to justify efficient and effective policies for disease prevention and control.

Between 2008 and 2010, South Africa experienced episodes of RVF outbreaks. The 2010 RVF disease outbreaks were reported to have had a significant economic impact on cattle and sheep production, especially affecting thousands of animals in the Free State, Northern Cape and Eastern Cape provinces. Pienaar and Thompson (2013) documented the temporal and spatial history of RVF in South Africa from 1950 to 2011. According to Pienaar and Thompson (2013) a total of about 484 outbreaks were reported during the 2010 outbreaks with 14 342 animal cases and 8 877 animal deaths. Pienaar and Thompson (2013) also reported that out of 14 342 animal cases that were reported, 13 117(91.45%) cases were those of sheep. Their study did not provide the details on financial losses incurred.

In South Africa, RVF vaccines are manufactured by Onderstepoort Biological Products (OBP). Based on personal communication with Jacob Modumo (2012) who is the head of marketing for OBP, (2012) (Modumo and Venter 2012), for the last fourteen years very few sales of the RVF vaccine were made and most of these were exports. This evidence supports claims that few South African farmers had been vaccinating against the disease until the recent outbreaks. That left livestock producers (and all associated stakeholders) vulnerable to severe losses when RVF occurred: losses that could have been avoided if farmers had implemented continuous vaccination programmes against the disease. Hence, to inform effective policy making in the control and prevention of RVF, this paper evaluates the impact of the 2010 RVF outbreaks on sheep numbers (the most affected animal species during the 2010 outbreaks) in the selected study areas. In the process, the financial value of sheep losses incurred by farmers in the three provinces was estimated. This paper will also test the hypothesis that all farmers including those who applied animal health-care programmes that included vaccination against RVF were equally affected by the outbreak when it occurred.

#### **RESEARCH APPROACH**

#### Sampling design and sample size

The study focused on livestock farmers who kept cattle, goats, and sheep or any combination of the three. The RVF map (Figure 1) obtained from Department of Agriculture, Forestry and Fisheries (DAFF), 2010 indicates that eight provinces; Eastern Cape, Northern Cape, Western Cape, North West, Gauteng, Limpopo, Mpumalanga and Free State were affected by the 2010 outbreaks. This study used a multi-stage sampling method. The first stage



Figure 1. The 2010 Rift Valley fever outbreaks in South Africa. Source: De Klerk and Pienaar, 2010.

involved the purposive selection of the three provinces: The Eastern Cape, Northern Cape and Free State. Pienaar and Thompson (2013) report that livestock farmers in these three provinces were most severely affected by the 2010 outbreaks. Based on the availability of resources (time and personnel), the study targeted two affected districts in each province. A simple random sampling without replacement method was used to select the two districts from each province, Frances Baard and Pixley Ka Seme districts of the Northern Cape Province and Fezile Dabi and Lejweleputswa districts of the Free State Province.

The lists of livestock farmers from the selected six districts of the three provinces were obtained from the respective animal health practitioners. The lists included the name and contact details of the farmer, type and number of livestock kept, land tenure system and the town as the location of the farm. The lists were then compressed into one list of 266 farmers that was used for selecting the sample. To select a representative sample out the 266 population, the study targeted 150 farmers. A systematic random sampling method was used to select 150 sample size of farmers. A systematic random sampling interval ( $\mathbf{k}$ ) using the formula:

$$k = \frac{N}{n}$$

Where, **N** is the population and **n** is the sample size (Black, 2004). The sampling interval (k) was 2; sampling was initiated by randomly selecting farmer number, 3. Applying the sampling interval, the second number was 5 followed by 7, etc. This procedure ensures that each farmer in the population has a known and equal probability of selection. Farmers from the sample were further stratified according to towns and type of livestock. The process ended with 15 towns (stratums – Table 1). Overall the sample included communal farmers, small-holder and commercial farmers.

#### Questionnaire design and data collection methods

A structured questionnaire for farmer interviews was developed and implemented. The questionnaire included open-ended questions to collect qualitative data as well as close-ended question to collect quantitative data. The questionnaire was pre-tested on eight livestock farmers in the Free State Province. Subsequently, the questionnaire was adjusted to incorporate lessons learnt during the pilot survey. Face-to-face interviews were used to collect data from the 150 respondents. The researcher personally conducted the interviews. Farmers whose contact details were available on the list were called to arrange appointment and venue for the interviews. Interviews with both small-holder and commercial farmers were conducted at their respective farms. Some communal farmers were

Drevines	District	Taum		Number	of farmers	
Province	District	Iown	Int	NVRVF	NVaccRVF	VaccRVF
	Casadu	Graaf- Reinet	5	2	0	3
	Cacadu	Aberdeen	6	0	1	5
Eastern Cape		Cradock	19	0	1	18
North and Orea	Chris Hani	Hofmeyr	14	1	5	8
		Middelburg	3	0	1	2
	Pixley Ka Seme	Prieska	10	1	4	5
		Douglas	13	3	3	7
Northern Cape		Britstown	8	1	2	5
	Frances Baard	Kimberley	8	3	0	5
	Fazila Dahi	Steynsrus	13	1	0	12
Free State	Fezile Dabi	Kroonstad	12	2	0	10
		Brandfort	16	0	0	16
	Lejwe leputswa	Bultfontein	12	1	0	11
		Welkom	11	2	0	9

Int = Interviewed, NVRVF = Not vaccinated for RVF, NVaccRVF = Not vaccinated all animals for RVF, VaccRVF = Vaccinated all animals for RVF.

interviewed during the morning and afternoon in the communal kraals after tendering to their animals while others were interviewed at their households. Information gathered with the questionnaire included; demographic details, livestock activities, animal health practices, production and management practices and prevention of RVF as well as the impact of RVF outbreaks on livestock numbers. While some of commercial and smallholder farmers shared filed records of their farming activities including data on production parameters, others consulted with their farm managers to verify the figures. Since communal farmers keep few herds of livestock, they could easily recall animal losses and the trends of their production parameters.

#### Description of questions asked

Questions pertaining to the location (province, district, local municipality and town) of the farmer were included in the questionnaire to permit analysis of the impact on a provincial basis. The questionnaire asked questions related to the demographic characteristics (age and farming experience) of each respondent.

To identify animal activities and animal health care-practices, farmers were asked to provide data on production systems (breeder, feedlot, etc.), land tenure system (communal, leased or private), as well as type and number of livestock kept. Farmers were asked whether they usually vaccinate their animals and against which diseases. They were also asked if they normally applied biosecurity measures as well as the nature of the biosecurity measures applied. On prevention of RVF, farmers were asked about when they started RVF vaccination as well as which type of vaccine they used.

To understand the overall impact of RVF outbreaks on sheep numbers, farmers were asked to indicate their herd size, offspring rate, mortality rate and weaning rate before, during and after the outbreaks. The data collected on production parameters covered the 2008 to 2012 period. The mean performance parameters were then checked against the provincial livestock numbers reported by DAFF 2014. To estimate the financial value of sheep losses, farmers were asked to identify which year they incurred animal losses, number of mortalities and abortions as well as type and nature (pregnancy, gender, age and type of breed) of animals lost.

#### Data management and statistical analysis

The data was captured and stored on a designed excel spread sheet. Validation and exploration of data was performed to check for the inconsistencies in captured data. The coding system was developed and subsequently implemented. The data was also stored in formatted text (space delimited - prn) format to be analysed in statistical package (SAS, 2012). The chi-square test  $(\chi^2)$  for equal proportion technique was used to analyse the data. To analyse the closed-ended questions (quantitative data), frequencies (the actual number of respondents who chose each response) and percentages (the proportion of people who chose each response out of the total number of respondents) were used. In addition, the chi-square test  $(\chi^{\rm z})$  was used to test for independence in a two-way contingency table as well as to compare the achieved sample proportions for the categories of variables of the qualitative data such as demographics data (Holt et al., 1980). Pearson correlation tests between animal losses and selected explanatory variables were also performed (XLSTAT, 2013). The significance of the correlation was tested at 5% significance level (Snedecor, 1967).

To assess the effect of epidemiology of RVF, a multi-factorial ANOVA (Analysis of Variance) with five (5) factors (province, do you usually vaccinate animals, did you vaccinate all animals against RVF, year when they started vaccination against RVF and where RVF vaccine was purchased) was used to compare difference between animal losses (Snedecor, 1967). However, there was a large variation in animal losses. Hence, the animal losses were transformed with a Log10 transformation to stabilize the variance before in order to apply the analysis of variance. Fisher's t-LSD (Least significant difference) were calculated at a 5% level of significance to compare means of significant source effects.

When the trends on production parameters were determined, the

Attributes	Eastern Cape	Free State	Northern Cape
Average age (years)	55	59	55
Average farm experience (years)	18	22	20
Average land size (ha)	972	1.006	1.063
Communal land (%)	45	50	39
Leased land (%)	15	13	15
Private land (%)	40	37	46
Average flock size	1 083	582.8	972.9

Table 2. Attributes of livestock farmers.

Table 3. Correlation between attributes and animal losses.

Variable	AL	Age	FE	TLS	LTS	SN	VaccRVF	DVAC
AL	0	0.018	0.547	0.000	0.034	0.000	0.203	0.056
Age	0.018	0	< 0.0001	0.012	0.393	0.000	0.119	0.069
FE	0.547	< 0.0001	0	0.285	0.009	0.139	0.778	0.829
TLS	< 0.0001	0.012	0.285	0	0.112	< 0.0001	0.000	0.001
LTS	0.034	0.393	0.009	0.112	0	0.000	0.602	< 0.0001
SN	< 0.0001	0.000	0.139	< 0.0001	0.000	0	0.001	0.002
VaccRVF	0.203	0.119	0.778	0.000	0.602	0.001	0	0.268
DVAC	0.056	0.069	0.829	0.001	< 0.0001	0.002	0.268	0

Values in bold are different from 0 with a significance level, alpha=0.05; AL= Animal Loss, FE = Farm Experience, TLS = Total land size, LTS = Land tenure system, SN = Sheep numbers, VaccRVF = Vaccinate all animals for RVF, DVAC = Do you usually vaccinate your animals

study used the deterministic economic model used by Bennett (2003) to estimate the financial cost of the RVF outbreaks on sheep numbers.

The basic model proposed by Bennett (2003) is as follows:

#### for each disease effect (L + R) = p id ie e vl

Where **p** is size of livestock population at risk, **id** is animal incidence of disease as a proportion of population at risk; **ie** is Incidence of disease effects as a proportion of affected population; **e** is magnitude of physical disease effects (R/litres of milk lost);

and **vl** is unit value of lost output (R/abortion occurred)

Data on sheep numbers used to determine *id* and *ie* was requested and obtained from DAFF. However, it is not available in the public domain and should be treated with discretion.

#### **RESULTS AND DISCUSSION**

The attributes of farmers and how they correlate with animal losses are discussed first. The mean age of farmers in Eastern Cape and Northern Cape was 55 while in the Free State, it was 59 (Table 2). There was a significant positive correlation (p = 0.018) between animal losses and age (Table 3). The majority of farmers who kept sheep were younger. Consequently, farmers that were less than 54 years of age in Eastern Cape and Northern Cape were the most affected in terms of revenue losses as they kept large herds of sheep (not shown in the tables). Farming experience was grouped into three categories; < 15 years, 15-24 years and > 25years. The average years of farming experience was highest in Free State at 22 years followed by Northern Cape and Eastern Cape provinces where farming experience averaged 20 and 18 years, respectively (Table 2). A nonsignificant correlation (p = 0.547) between animal losses and farming experience was established (Table 3). However, a statistically significant relationship was found between farming experience and animal losses, with p = 0.0000, p = 0.0147 and p =0.0467 for farmers with less than 15, 15 to 25 years and more than 25 years of farming experience, respectively (not shown in the tables). Farmers with more than 15 years of farming experience were the most affected.

The mean land size in Eastern Cape, Free State and Northern Cape was 972, 1006, 1063, respectively (Table 2). A highly significant correlation (p<0.0001) was found between total land size and animal losses (Table 3). Farmers with more than 3 000 ha were the most affected followed by farmers with less than 500 ha and 500 to 3 000 ha (not shown in the tables). Three forms of land tenure system per province are reported (Table 2). An overall significant correlation (p = 0.034) was established between animal losses and the land tenure system (Table 3).

Year	2008	2009	2010	2011	2012
Provincial sheep numbers*	7 571 170	7 589 870	7 316 381	7 302 429	7 084 656
Herd size	6 610	6 605	6 396	6 036	6 498
Lambing rate (%)	117	110	104	113	122
Mortality rate (%)	4	5	10	6	4
Weaning rate (%)	93	87	82	93	94

Table 4. Impact of RVF on sheep numbers - Eastern Cape.

**Table 5.** Impact of RVF on sheep numbers - Northern Cape.

Year	2008	2009	2010	2011	2012
Provincial sheep numbers*	6 204 217	6 214 617	6 119 201	6 054 733	6 082 972
Herd size	4 207	4 230	4 118	4 018	4 082
Lambing rate (%)	117	115	84	110	117
Mortality rate (%)	8	8	13	9	8
Weaning rate (%)	96	94	82	98	103

Compared to the other two provinces, Eastern Cape farmers had the highest average sheep flock size at 1 083, followed by Northern Cape and Free State farmers at 972.9 and 582.8 sheep, respectively (Table 2). A highly significant correlation (p < 0.0001) between animal losses and sheep numbers was found (Table 3).

There was no significant correlation (p = 0.203) between animal losses and vaccination of all animals against RVF (Table 3). The analysis also shown that there was no significant correlation (p = 0.056) between animal losses and usual vaccination of animals against other prevailing animal diseases. Smallholder farmers indicated that the state provided them with free vaccine and also administered the vaccine to all animals on their behalf. This was to ensure that the right vaccine was administered on time and effectively. Some of the commercial farmers indicated that they vaccinated during the course of the outbreaks using one needle for more than 20 animals while others admitted that they changed the needle only when it broke. This practice has the potential to spread the infection if there are infected animals in the herd. Consequently, 71% of commercial farmers were affected by the outbreak compared to 29% of smallholder farmers whom the state assisted with application of the vaccine (not shown in the tables).

#### Impact of RVF on sheep numbers - Eastern Cape

During the intense episodes of RVF outbreaks (2009-2010), the average lambing rate of sheep declined from 117% in 2008 to 104% in 2010 (Table 4). In 2011 it started to pick up at 113% to 122% in 2012. In addition, the mortality rate increased from 4% in 2008 to 10% in 2010 while the weaning rate declined from 93 to 82%

during the same period. Consequently, the average herd size decreased from 6,610 in 2008 to 6,396 in 2010 and continued to decline to reach 6 036 in 2011 and started picking up in 2012 at 6,498 while the provincial numbers declined from 7.5 million in 2008 to 7.1 million in 2012.

#### Impact of RVF on sheep numbers - Northern Cape

The lambing rate declined from 117% in 2008 to 84% in 2010 (Table 5). It picked up in 2011 at 110% and normalized (117%) again in 2012. The mortality rate increased from 8% in 2008 to 13% in 2010, improved (9%) in 2011 and normalized to 8% in 2012. The weaning rate declined from 96% in 2008 to 82% in 2010 and improved (98%) again in 2011 to reach 103% in 2012. Farmers reported large number of mortalities in suckling lambs. Consequently, the mean herd size declined from 4 207 in 2008 to 4 118 in 2010 and continued to decline to reach 4 082 in 2012. This impact also was felt at the provincial level where numbers declined from 6.2 million in 2008 to reach 6.1 million in 2012 as the farmers were building the replacement stock.

#### Impact of RVF on livestock numbers - Free State

The lambing rate of sheep declined from 125% in 2008 to 120% in 2009 and continued to decline to reach 123% in 2010 and it normalized again in 2011 (Table 6). During the same period, the mortality rate increased from 2% in 2008 to 3% during 2009 up to 2012. The impact on the weaning rate is only observed during the 2009 to 2010 period with a decline from 98% in 2009 to 97% in 2010. While at the farm level, the mean herd size in the sample

Year	2008	2009	2010	2011	2012
Provincial sheep numbers*	4 945 228	4 886 255	4 875 111	4 880 030	4 767 750
Herd size	600	886	883	900	900
Lambing rate (%)	125	120	123	125	125
Mortality rate (%)	2	3	3	3	3
Weaning rate (%)	98	98	97	98	98

Table 6. Impact of RVF on sheep numbers - Free State.

Table 7. Estimated financial losses for sheep numbers in three provinces.

Provir	nce district	Animals	p	id	ie	e	vl	L + R = p id ie e vl
		Pregnant	7316381	0.0011307	6.83398E-06	50	1200	3 392.2
50	СН	Suckling	7316381	0.0361835	0.00134576	1600	300	27 788 959.5
EC		Abortions	7316381	0.0361835	0.00021869	1600	150	13 894 479.7
	CCD	Abortions	7316381	0.0446296	4.10039E-05	300	150	602 499.3
		Pregnant	6119201	0.0301512	1.55E-04	951	1200	32 722 565.0
NO	DKC	Non-pregnant	6119201	0.0002536	1.31E-06	8	800	13.0
NC	PKS	Suckling	6119201	0.0670239	0.0003455	2114	300	89 858 827.0
		Abortions	6119201	0.0634095	0.0003268	2000	150	38 045 718.3
		Pregnant	4875111	0.0007060	4.10247E-07	2	1200	3.4
FS	LLP	Non-pregnant	4875111	0.0028239	1.64099E-06	8	850	153.6
		Abortions	4875111	0.0568302	7.14828E-05	161	150	478 280.5

The basic model proposed by Bennet (2003) is as follows: for each disease effect (L+ R) = p id ie e vl; p: Size of livestock population at risk.

id: Animal incidence of disease as a proportion of population at risk; ie: Incidence of disease effects as a proportion of affected population; e: Magnitude of physical disease effects (R/litres of milk lost); vl: Unit value of lost output (R/abortion occurred)

depicted an increasing trend from 600 in 2008, at the provincial level sheep numbers depicted a decline from 4.9 million in 2008 to 4.8 million in 2010 and continued to decline to 4.7 million in 2012.

#### Financial value of sheep losses

Financial losses of sheep were estimated for the respondents in each province. When these losses were scaled up at provincial level, financial losses incurred by sheep estimated farmers who kept were at R42 289 330.7, R160 627 123.3 and R478 437.0 for Eastern Cape, Northern Cape and Free State provinces, respectively (Table 7). During the survey, some of the farmers in the Northern Cape Province and the Cacadu district of the Eastern Cape Province indicated that due to the geographic attributes (Karoo) of their farming area, which does not pose risk to animal disease, they usually did not follow any vaccination program for their livestock. Hence, it is assumed that this practise might have compromised the immune system of the animals by the time of the outbreaks. Huge amounts of financial losses were incurred as a result of highly reported number of abortions and death of suckling animals. The survey revealed that farmers from the Free State incurred major losses during the 2009 outbreaks. Following the 2009 RVF outbreaks in Free State, farmers intensified their vaccination programme against RVF. In addition, the Provincial Department of Agriculture also provided free vaccines to the communal and small-holder farmers in the perceived high-risk areas.

The study hypothesised that all farmers who applied animal health-care programmes that included vaccination against RVF were equally affected by the outbreak when it occurred. The probability values from the ANOVA results show that all farmers, regardless of vaccination status were affected by the outbreaks (Table 8). However, the Fisher's t-LSD results indicate that farmers were not equally affected. Hence, the hypothesis statement is rejected. Farmers who did not vaccinate all their livestock for RVF and those who bought the vaccine from the cooperatives were the most affected.

#### CONCLUSIONS AND RECOMMENDATIONS

Although the mean performance parameters reported by

Do you usually vaccinate animals		Did you vaccin	ate all animals for RVF	Where did you buy the vaccines		
No	0.66 <sup>a</sup>	No	2.58 <sup>a</sup>	Соор	3.30 <sup>a</sup>	
Yes	1.67 <sup>b</sup>	None	0.33 <sup>b</sup>	None	0.27 <sup>b</sup>	
		Yes	1.24 <sup>b</sup>	State	0.58 <sup>b</sup>	
p-value	0.0015		0.0059		<0.001	

 Table 8. ANOVA and Fisher's t-LSD comparing farmer's financial losses in three provinces.

\*None= Not yet farming during the outbreak; Means with the same letter or letters do not differ significantly at the 5% significance level.

farmers suggest that sheep farmers in these three provinces have recuperated from the outbreaks, provincial sheep numbers as reported by DAFF suggest that sheep numbers in these provinces have not recovered to reach the numbers recorded before the 2010 outbreaks. The mean herd size in the Eastern Cape Province decreased from 6 610 in 2008 to 6 396 in 2010 while the provincial sheep numbers declined from 7.5 to 7.1 million in 2012. In the Northern Cape Province, the mean herd size declined from 4 207 in 2008 to 4 082 in 2012. In the Free State Province, despite the mean herd size showing an increase from 600 in 2008 to 900 in 2012, provincial sheep numbers show a decline from 4.9 million in 2008 to 4.8 million in 2012. The total financial value of sheep losses incurred by farmers in the three provinces during the 2010 outbreaks is estimated at R203.4m. The survey revealed that farmers who did not vaccinate all their livestock against RVF were the most affected by the outbreaks and, in addition, the study has shown the value of effective vaccination. It is therefore recommended that farmers should vaccinate all their livestock following the recommended application guidelines. In addition, farmers who do not see the benefit of vaccinating for sporadic disease outbreaks are urged to make use of available platforms and services to enquire about the possible outbreaks, more especially after heavy rains which are normally associated with outbreaks so that they can vaccinate in time if necessary.

#### **CONFLICT OF INTERESTS**

The authors have not declared any conflict of interest.

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Full Length Research Paper

# Seed yield of onion (*Allium cepa* L.) as affected by bulb size and intra-row spacing

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'Bombay Red' is one of the most widely used onion cultivars in Ethiopia. Seed production of this cultivar is expanding among farmers. However, various constraints are reported for the low seed productivity of this cultivar, out of which inappropriate uses of bulb size and intra-row spacing are the most important ones. Therefore, a field experiment was conducted at Bati Futo watershed. Meskan Wereda, Southern Ethiopia under supplemental irrigation in 2012 to find out optimum bulb size and intra-row spacing for quality and economically feasible seed production of the cultivar. The crop was grown in factorial combinations of four different bulb sizes (3-4.0,4.1-5.0, 5.1-6.0 and 6.1-7.0 cm) and four intra-row spacings (10, 15, 20 and 25 cm) in a randomized complete block design with three replications. The results revealed that significantly highest value of days to 50% bolting (27 and 28 days), days to 50% flowering (75 and 81 days), days to 50% maturity (123 and 124 days), number of leaves per plant (37 and 36), biomass (130 and 146 g/plant), flower stalk height (84 and 74 cm), flower stalk diameter (1.62 and 1.75 cm), umbel diameter (5.52 and 5.82 cm), number of umbels per plant (11 and 10), number of umbels per plot (854 and 1016), 1000-seed weight (4.50 and 4.35 g) and seed germination percentage (92 and 91 %) were obtained from bulbs of 6.1 to 7 cm size and 25 cm intra-row spacing, respectively. However, the maximum (2.05 and 1.80) leaf area index was obtained at 6.1 to 7 cm bulb size and 20 cm spacing, respectively. The interaction effects of bulb size and intra-row spacing on number of seeds per umbel, weight of seeds per umbel, seed yield per plant and seed yield per hectare were significant. Significantly higher seed yield (920 to 995 kg/ha) was obtained at the larger bulb size (5.1 to 7 cm) grown at 20 and 25 cm spacing. The highest net field benefits of 245,678 Ethiopian Birr ha<sup>-1</sup> was obtained from a bulb of 5.1-6.0 cm sizes grown at 20 cm spacing. Hence, for quality and economically feasible seed yield of onion, the treatment combination of 5.1 to 6.0 cm bulb size and 20 cm spacing is recommended to the farmers at Bati Futo watershed.

Key words: Bulb size, intra-row spacing, onion seed production.

#### INTRODUCTION

Onion (*Allium cepa* L.) is a major bulbous vegetable which ranks second only to tomato in terms of total annual world production (Ambulker et al., 1995). Total area under onion cultivation in Ethiopia during *Meher* season was estimated to be about 30,478.35 ha with a

total production of 328, 000 t in the year of 2011/12 (CSA, 2012).

Commonly, the economically important onion group is mostly grown from seeds (Brewster, 1994). However, seed supply from domestic production in Ethiopia is inadequate and therefore, vegetable growers mainly depend on imported seeds that have mostly poor germination percentage, uniformity and susceptibility to diseases (Lemma, 1998).

Onion seed production might be increased by increasing the area with good variety and changing the existing management practices. Khokhar et al. (2001) noted that planting of bulbs of suitable size increased onion seed yield. Larger bulb size (5.5 to 7.0 cm diameter) produced seed yield significantly higher than small sized bulbs (Ali et al., 1998). Asaduzzaman et al. (2012b) also reported that larger sized bulb ( $20\pm1$  g) and wider spacing ( $25 \times 20$  cm) resulted in higher seed yield per plant (3.78 g). Bulb size and plant spacing are the two key factors in producing quality of onion seeds (Mirshekari and Mobasher, 2006).

In Ethiopia, the previous study was conducted in limited bulb categories and spacing and it has been done over 20 years besides the fact that the production have not benefited the seed quality. Farmers in Butajira area simply used this recommendation for Bombay Red cultivar that was made on the rift valley area for Adama Red. The present study was, therefore, undertaken to find out the optimum mother bulb size and intra-row spacing needed to achieve the best quality and economically feasible yield and quality of onion seed for the area.

#### MATERIALS AND METHODS

The study was conducted on the farmers' field at Bati Futo watershed, Meskan Woreda, Guraghie Administrative Zone of the South Nations Nationalities and Peoples Regional State using supplemental irrigation from January to June, 2012. The site is located south west of Addis Ababa in the coordinates of 08°06.422"N latitude and 038°24.909"E longitude with an altitude of 1936 m above sea level. The land was having sandy loam textured soil with pH of 6.5. Four different bulb size (3-4.0, 4.1-5.0, 5.1-6.0 and 6.1-7.0 cm) and four intra-row spacings (10, 15, 20 and 25 cm) were considered as treatments in the experiment. The experiment was laid out in a randomized complete block design with three replications. A uniform plot size of 2 x 5.6 m was used for each plot. In each plot, there were seven double rows (50 x 30 cm) and eight plants per row with a total of 112 plants per plot. Onion cultivar 'Bombay Red' was used as a test crop. It is the most widely grown cultivar under irrigation in the area due to its earliness, high yielding potential of bulb and seeds.

The land was prepared by ploughing properly. The entire quantity of DAP (200 kgha<sup>-1</sup>) were applied at planting, whereas urea (150 kgha<sup>-1</sup>) was applied in two equal splits at planting and 45 days after planting. Cultural practices, such as weeding, irrigation, mulching and chemical application were done as and when necessary. Five plants from each plot were selected randomly at 50% bolting, flowering and maturity for growth, yield, yield components and quality parameters. The parameter under study were days to 50% bolting, days to 50% flowering, days to 50% maturity, number of leaves per plant, leaf area index, biomass, number of umbels per plant, number of flower stalks per plot, flower stalk height (cm), flower stalk diameter (cm), umbel diameter (cm), number of seeds per umbel, weight of seed per umbel (g), seed yield per plant (g), seed yield per ha (kg), 1000-seeds weight and seed germination percentage. Five plants were selected at random from each plot for recording growth attributes whereas the three central rows were considered for yield and quality parameters. The results were analyzed by using analysis of variance techniques (SAS, 2002) and mean separation was made based on LSD at 5% level of significance. Simple partial budget analysis was made for economic analysis of optimum bulb size with appropriate intra-row spacing. The economic analysis was calculated following the formula developed by CIMMYT (1988).

#### **RESULTS AND DISCUSSION**

# Effect of intra-row spacing on growth and quality of onion seed

#### Days to 50% bolting

Different sizes of onion mother bulb showed significant effect on growth and quality of the seeds (Table 1). Days to 50% bolting was significantly earlier (21 days) for bulbs with 3 to 4 cm size while it was significantly late (27 days) for bulbs with 6.1 to 7 cm size. The finding is similar to the results obtained by Gill et al. (1989) who obtained least number of days to complete flower stalk bolting on small bulb sizes as compared to the largest ones. The increase in number of days to 50% bolting with increase in bulb size might be due to the presence of stored food inside the larger bulbs that contributed to the vegetative growth of plants through which bolting was delayed.

#### Days to 50% flowering

Days to 50% flowering were lowest (61 days) for bulbs with 3 to 4 cm sizes, while it was highest (75 days) for 6.1 to 7 cm sizes. This result is in agreement with the work of Jilani (2004) who obtained the lowest and the highest number of days to flowering from small and large sized bulbs, respectively.

#### Days to 50% maturity

The lowest (106 days) days to 50% maturity was achieved at 3 to 4 cm bulb sizes which, however, were statistically similar with 4.1 to 5 cm bulb sizes and the highest (123 days) was obtained at the largest bulb size.

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Table 1. Effect of bulb size on growth and quality of onion seeds

Bulb size (cm)	DB	DF	DM	NLP	LAI	BM (g/plant)	FSH (cm)	FSD (cm)	UD (cm)	NUP	NUPO	TSW (g)	SGP (%)
3-4	21 <sup>d</sup>	61 <sup>d</sup>	106 <sup>c</sup>	29 <sup>d</sup>	0.61 <sup>c</sup>	70 <sup>c</sup>	56 <sup>d</sup>	1.21 <sup>b</sup>	4.25 <sup>d</sup>	6 <sup>d</sup>	495 <sup>°</sup>	3.50 <sup>d</sup>	84 <sup>d</sup>
4.1-5	23 <sup>c</sup>	66 <sup>c</sup>	108 <sup>c</sup>	32 <sup>c</sup>	1 <sup>b</sup>	97 <sup>b</sup>	61 <sup>c</sup>	1.29 <sup>b</sup>	5.10 <sup>c</sup>	7 <sup>c</sup>	536 <sup>b</sup>	3.78 <sup>c</sup>	86 <sup>c</sup>
5.1-6	26 <sup>b</sup>	71 <sup>b</sup>	118 <sup>b</sup>	34 <sup>b</sup>	1.73 <sup>a</sup>	117 <sup>a</sup>	72 <sup>b</sup>	1.53 <sup>a</sup>	5.26 <sup>b</sup>	9 <sup>b</sup>	827 <sup>a</sup>	4.10 <sup>b</sup>	89 <sup>b</sup>
6.1-7	27 <sup>a</sup>	75 <sup>a</sup>	123 <sup>a</sup>	37 <sup>a</sup>	2 <sup>a</sup>	130 <sup>a</sup>	84 <sup>a</sup>	1.62 <sup>a</sup>	5.52 <sup>a</sup>	11 <sup>a</sup>	854 <sup>a</sup>	4.50 <sup>a</sup>	92 <sup>a</sup>
LSD (5%)	0.80	2.59	2.92	1.49	0.33	18.44	1.64	0.25	0.12	0.62	110.62	0.19	1.11
CV (%)	3.93	4.56	3.08	5.40	29.42	21.40	2.89	20.84	2.93	8.74	19.58	5.63	1.51

The letter DB, DF, DM, NLP, LAI, BM, FSH, FSD, UD, NUP, NUPO, TSW and SGP stands for dates of 50% bolting, dates of 50% flowering, dates of 50% maturity, number of leaves per plant, leaf area index, biomass, flowers talk height, flowers talk diameter, umbel diameter, number of umbel per plant, number of umbel per plot, thousand seed weight and seed germination percentage, respectively. Means followed by different letters in the same column are significantly different from each other at 5% level of significance.

The result is in line with the work of Khokhar et al. (2001) who noted that large sized bulbs delayed the maturity of onion seeds.

#### Number of leaves per plant

The lowest (29) and highest (37) mean number of leaves per plant was recorded from bulbs with 3 to 4 and 6.1 to 7.0 cm size, respectively. The finding is similar to the results obtained by Hussain et al. (2001) and Mosleh ud-Deen (2008) who reported that large sized bulbs produced greater number of leaves per plant. This might be due to the fact that larger sized bulbs contain more reserve foods that can support the production of more initial leaves as compared to the small sized bulbs (Asaduzzaman et al., 2012b).

#### Leaf area index

Increasing bulb size from 3 to 6 cm significantly increased leaf area index (LAI) but further increase had no significant effect. Bulbs with 6.1 to 7 cm sizes produced 236% higher LAI than the smallest (3 to 4 cm) bulb size treatments (Table 1).

#### Biomass

The lowest (70 g plant<sup>-1</sup>) plant biomass was obtained with bulbs of 3 to 4 cm sizes and it was highest (130 g plant<sup>-1</sup>) for bulbs with 6.1 to 7 cm sizes which, however, were statistically similar with bulbs of 5.1 to 6 cm sizes. This might be due to the presence of food reserves inside the largest bulb size that contributed to the growing plant and it would have maximum biomass as compared to the smallest bulb sizes.

#### Flower stalk height

The lowest mean flower stalk height (56 cm) was

recorded with the smallest (3 to 4 cm) bulb sizes while the highest (84 cm) was recorded with the largest bulb size (6.1 to 7 cm). This finding is similar to the results obtained by Sidhu et al. (1996) and Mosleh ud-Deen (2008) who showed that flower stalk height of onion was found to vary with bulb size. This might be due to the presence of more stored foods inside large bulbs which contributed for the emergence of more flower stalks.

#### Flower stalk diameter

Significantly lowest (1.21 cm) mean flower stalk diameter was observed at 3 to 4 cm bulb size but the higher ones (1.62 cm) were produced by bulbs with 6.1 to 7 cm, however, it was not significantly different from 5.1 to 6 cm. This is in line with the findings of Shaikh et al. (2002) and Ashrafuzzaman et al. (2009) who obtained maximum flower stalk diameter from large sized bulbs.

#### **Umbel diameter**

The lowest and highest mean umbel diameters of 4.25 and 5.52 cm were obtained from bulbs with 3 to 4 and 6.1 to 7 cm sizes, respectively. These results are in line with the work of Ali et al. (1998) and Ashrafuzzaman et al. (2009), Khodadadi (2012) and Asaduzzaman et al. (2012b) who reported that large sized bulbs have more stored foods that are supplied to the emerged flower stalks and it significantly increased umbel diameter.

#### Number of umbels per plant

The mean number of umbels per plant was lowest (6) for bulbs with 3 to 4 cm sizes, while it was highest (11) for bulbs with 6.1 to 7 cm sizes. The finding is in line with the work of Geetharani and Ponnuswamy (2007) and Ashrafuzzaman et al. (2009) on onion plants. Large bulbs may contain higher food reserves and be responsible for the higher number of flowering stalks per plant.

Table 2. Effect of intra-row spacing on growth and quality of onion seeds

Intra-row spacing (cm)	DB	DF	DM	NLP	LAI	BM (g/plant)	FSH (cm)	FSD (cm)	UD (cm)	NUP	NUPO	TSW (g)	SGP (%)	
10	21 <sup>d</sup>	58 <sup>d</sup>	105 <sup>d</sup>	29 <sup>d</sup>	1.04 <sup>c</sup>	69 <sup>c</sup>	62 <sup>d</sup>	1.20 <sup>c</sup>	4.23 <sup>d</sup>	6.74 <sup>d</sup>	423 <sup>c</sup>	3.58 <sup>d</sup>	85 <sup>d</sup>	
15	23 <sup>c</sup>	64 <sup>c</sup>	111 <sup>c</sup>	32 <sup>c</sup>	1.05 <sup>c</sup>	83 <sup>c</sup>	67 <sup>c</sup>	1.23 <sup>c</sup>	4.47 <sup>c</sup>	7.94 <sup>c</sup>	475 <sup>°</sup>	3.85 <sup>c</sup>	87 <sup>c</sup>	
20	25 <sup>b</sup>	69 <sup>b</sup>	116 <sup>b</sup>	35 <sup>b</sup>	1.80 <sup>a</sup>	115 <sup>b</sup>	70 <sup>b</sup>	1.49 <sup>b</sup>	5.61 <sup>b</sup>	9.06 <sup>b</sup>	795 <sup>b</sup>	4.09 <sup>b</sup>	89 <sup>b</sup>	
25	28 <sup>a</sup>	81 <sup>a</sup>	124 <sup>a</sup>	36 <sup>a</sup>	1.47 <sup>ab</sup>	146 <sup>a</sup>	74 <sup>a</sup>	1.75 <sup>a</sup>	5.82 <sup>a</sup>	10.04 <sup>a</sup>	1016 <sup>a</sup>	4.35 <sup>a</sup>	91 <sup>a</sup>	
LSD (5%)	0.80	2.59	2.92	1.49	0.33	18.44	1.64	0.25	0.12	0.62	110.62	0.19	1.11	
CV (%)	3.93	4.56	3.08	5.40	29.42	21 40	2 89	20.84	2 93	8.74	19.58	5.63	1 51	

The letter DB, DF, DM, NLP, LAI, BM, FSH, FSD, UD, NUP, NUPO, TSW and SGP stands for dates of 50% bolting, dates of 50% flowering, dates of 50% maturity, number of leaves per plant, leaf area index, biomass, flowers talk height, flowers talk diameter, umbel diameter, number of umbel per plant, number of umbel per plot, thousand seed weight and seed germination percentage, respectively. Means followed by different letters in the same column are significantly different from each other at 5% level of significance.

#### Number of umbels per plot

The mean number of umbels per plot was lowest (495) for bulbs with 3 to 4 cm sizes, while it was highest (854) for bulbs with 6.1 to 7 cm sizes. The increase in number of umbels per plot due to increase in bulb size might be due to the increase in number of umbels per plant (Ashrafuzzaman et al., 2009; Mollah et al., 1997).

#### 1000-seed weight

The lowest (3.5 g) and the highest (4.5 g) 1000-seed weights were achieved using bulbs of 3 to 4 and 6.1 to 7 cm sizes, respectively. The finding is in line with the work of Khodadadi (2012) who noted that large size bulbs resulted in maximum weight of 1000-seeds as compared to the smaller ones. High food reserves present in the large bulbs might supply nutrients properly to the seeds, resulting in the highest weight of 1000-seed weight.

#### Seed germination percentage

Mean seed germination percentage was lowest (84%) for bulbs with 3 to 4 cm sizes while it was highest (92%) for bulbs with 6.1 to 7 cm sizes. The finding is similar to the results of Muktadir et al. (2001) and Asaduzzaman et al. (2012b) who obtained the highest seed germination percentage from larger sized bulbs.

# Effect of intra-row spacing on growth and quality of onion seed

#### Days to 50% bolting

The lowest (21 days) and the highest (28 days) number of days to 50% bolting were achieved at intra-row spacing of 10 and 25 cm, respectively. The finding confirms the results obtained by Navab et al. (1998) which showed that wider plant spacing had significantly delayed flowering of onion. The increase in number of days to 50% bolting with increase in intra-row spacing might probably be due to the availability of resources at wider plant spacing and this might increase the vegetative growth of the plant and bolting time was delayed.

#### Days to 50% flowering

The lowest (58 days) and the highest (81 days) number of days to 50% flowering were achieved at 10 and 25 cm intra-row spacing, respectively. It increased with increase in intra-row spacing reaching maximum at 25 cm. This result is similar with the work of Aminpour and Mortzavi (2004) who reported that wider plant spacing delayed flowering of onion due to the absence of competition risk as compared to the closer spacing.

#### Days to 50% maturity

The lowest (105 days) and the highest (124 days) number of days to 50% maturity were recorded at 10 and 25 cm intra-row spacing, respectively. In line with the current result, Mirshekari et al. (2008) also observed that wider intra-row spacing significantly delayed the maturity of onion seeds as compared to narrow plant spacing.

#### Number of leaves per plant

Number of leaves per plant was lowest (29) for 10 cm intra-row spacing, while it was highest (36) for 25 cm intra-row spacing; the other intra-row spacing treatments scored in between the two (Table 2). The finding is similar to the results obtained by Anisuzzaman et al. (2009) who found that increase in the number of leaves per plant was directly related to the number of flower stalks per plant. The result is in accordance with the

findings of Jilani et al. (2010) and Asaduzzaman et al. (2012b) who also reported increased number of leaves per plant with increase in intra-row spacing. Similarly, Hussain et al. (2001) reported that number of green leaves per plant increased with increase in intra-row spacing which might be due to the presence of less competition among plants in wider plant spacing for space, moisture, nutrients and light.

#### Leaf area index

The lowest (1.04) LAI was achieved at 10 cm spacing which, however, was statistically similar with 15 cm spacings. The highest (1.80) LAI was recorded on 20 cm spacing and further increase in intra-row spacing had decreased LAI by 22.45%. The lowest LAI at closer spacing might be due to the fact that at closer spacing, competition among plants and disease severity is very high. Similarly, Lemma and Shimelis (2003) also showed that the lowest plant density in onion crop increased the risk of attack by fungal diseases such as purple bloch caused by *Alternaria porri*.

#### Biomass

The lowest (69 g plant<sup>-1</sup>) plant biomass was achieved at 10 cm spacing which, however, was statistically at par with 15 cm spacing and the highest (146 g plant<sup>-1</sup>) plant biomass were recorded on 25 cm spacing (Table 2). This finding is in accordance with the work of Zamil et al. (2010) who reported increase in plant biomass with widest intra-row spacing due to less competition among plants for nutrients.

#### Flower stalk height

The lowest and highest mean flower stalk heights of 62 and 74 cm were recorded using 10 and 25 cm intra-row spacing, respectively; all the other spacing treatments were performed in between the two (Table 2). This might be due to higher competition at closer spacing resulting in shorter flower stalk height. The result is in accordance with the findings of Khan et al. (2003). Also, as explained by Asaduzzaman et al. (2012a), wider intra-row spacing produces more green leaves due to the free access of soil nutrients which promotes the length of the flowering stalks.

#### Flower stalk diameter

The lowest mean flower stalk diameter (1.20 cm) was achieved using 10 cm intra-row spacing which, however, was statistically similar to 15 cm spacing; the highest

mean flower stalk diameter (1.75 cm) was recorded using 25 cm spacing (Table 2). The finding is similar to the results of Pandey et al. (1994) who obtained larger flower stalk diameter from wider intra-row spacing. The difference in flower stalk diameter might be due to competition associated with closely spaced plants that resulted in lower flower stalk diameter (Mahadeen, 2004).

#### Umbel diameter

The lowest and highest mean umbel diameters of 4.23 and 5.82 cm were recorded at 10 and 25 cm intra-row spacing, respectively; the other spacing treatments were performed in between (Table 2). The finding is in accordance with the work of Pandey et al. (1994) and Asaduzzaman et al. (2012b). This might be due to the greater access of nutrients (especially nitrogen) and water, producing large sized umbels at wider plant spacing.

#### Number of umbels per plant

The lowest (7) and highest (10) mean number of umbels per plant were achieved at 10 and 25 cm intra-row spacing, respectively; the other spacing treatments were performed in between the two (Table 2). The finding is in agreement with the work of Navab et al. (1998) who showed that wider plant spacing resulted in large number of umbels per plant. Plants at the widest spacing produced more number of leaves probably due to less competition for nutrients, light, space, and moisture. Later on, these leaves accumulated photosynthates and ultimately encouraged producing more number of umbels (Asaduzzaman et al., 2012a). Singh and Sachan (1999) also found the highest number of umbels per plant from the widest spacing.

#### Number of umbels per plot

The lowest (423) number of umbels per plot was achieved at 10 cm spacing which, however, was also statistically at par with 15 cm spacing. The highest mean number of umbels per plot (1016) was recorded using 25 cm intra-row spacing (Table 2). The finding is similar to the result obtained by Miccolis et al. (1995) and Navab et al. (1998) which showed that wider plant spacing produced large number of umbels per plot. Bulb size and intra-row spacing were also significant

#### 1000-seed weight

1000-seed weight was lowest (3.58 g) for 10 cm spacing,

Table 3. Interaction	effects	of bulb	size and	d intra-row	spacing	on
number of seeds per	umbel.					

Bulb size (cm)		Intra-ro	w spacin	g (cm)		
Buib Size (cili)	10	15	20	25	Mean	
3-4	331 <sup>f</sup>	376 <sup>f</sup>	420 <sup>f</sup>	461 <sup>ef</sup>	397	
4.1-5	382 <sup>f</sup>	471 <sup>ef</sup>	696 <sup>cd</sup>	720 <sup>cd</sup>	567	
5.1-6	604 <sup>de</sup>	794 <sup>c</sup>	1190 <sup>ab</sup>	1270 <sup>a</sup>	965	
6.1-7	485 <sup>ef</sup>	730 <sup>cd</sup>	1061 <sup>b</sup>	1325 <sup>a</sup>	900	
Mean	450.50	592.75	841.75	944	707.25	
LSD (5%) = 169.47						
CV (%) = 14.37						

Means followed by different letters in the same column are significantly different from each other at 5% level of significance.

while it was highest (4.35 g) for 25 cm spacing; the other spacing treatments was performed in between the two (Table 2). The findings are similar to the results of Daljeet et al. (1990) who obtained highest 1000-seed weight from wider plant spacing. The increase in 1000-seed weight with increased intra-row spacing might be due to the fact that wider spacing supplied more food materials to the growing seeds as compared to the closest spacing.

#### Seed germination percentage

The lowest (85%) and the highest (91%) mean seed germination percentage was recorded at 10 and 25 cm intra-row spacing, respectively (Table 2). This result is in conformity with the work of Asaduzzaman et al. (2012b) who reported that wider intra-row spacing had a significant effect on seed germination percentage.

# Interaction effects of bulb size and intra-row spacing on seed yield of onion

#### Number of seeds per umbel

The interaction between bulb size and intra-row spacing on mean number of seeds per umbel was highly significant ( $p \le 0.001$ ). Mean number of seeds per umbel increased with increase in bulb size and intra-row spacing; however, the magnitude of the increase varied in relation to both bulb size and spacing (Table 3). At 10 cm intra-row spacing, increasing bulb size from 3 to 6 cm increased number of seeds per umbel by 15.26 and 58.32%, whereas further increase in bulb size resulted in a 24.54% decrease. At 25 cm spacing, increasing bulb size from 3-7 cm enhanced the mean number of seeds per umbel by 56.18, 76.39 and 4.33% (Table 3). Similarly, using bulbs with 3-4 cm sizes, increasing intrarow spacing from 10 to 25 cm, enhanced the mean number of seeds per umbel by 13.60, 11.70 and 9.76%. Similar results are evident at the other bulb size and intra-row spacing treatment combinations (Table 3).

The above findings showed that the number of seeds per umbel is determined by bulb size and spacing. The result is in accordance with the findings of Badawi et al. (2009) and Asaduzzaman et al. (2012a) who stated that larger bulb sizes and wider plant spacing resulted in increased number of seeds per umbel.

#### Weight of seeds per umbel

The interaction effects of bulb size and intra-row spacing on average weight of seeds per umbel in onion plant was highly significant ( $p \le 0.001$ ). Mean weight of seeds per umbel increased with increase in bulb size and intra-row spacing; but, the extent of its increase in relation to both bulb size and spacing was different (Figure 1). At 10 cm intra-row spacing, increasing bulb size from 3 to 6 cm increased weight of seeds per umbel by 13.85 and 112.84%, while further increase in bulb size resulted in a 33.47% decrease. At 25 cm spacing, increasing bulb size from 3-7 cm increased the average weight of seed per umbel by 39.25, 105.02 and 1.69% (Figure 1). Likewise, at 3-4 cm bulb size, increasing intra-row spacing from 10 to 25 cm increased mean weight of seeds per umbel by 25.38, 8.77 and 4.91%. Similar results are evident at the other bulb size and intra-row spacing combinations (Figure 1).

It is evident from the data presented above that the weight of seeds per umbel was determined by bulb size and intra-row spacing. The findings are similar to the results of Begum et al. (1998) and Asaduzzaman et al. (2012a) who obtained the highest weight of seeds per umbel from larger sized bulbs grown at wider intra-row spacing. This might be due to the fact that larger bulbs contain higher food reserves which produced higher number of flowers and seeded fruits per umbel; wider spacing had also more available nutrient and less competition risks for the emergence of seeded florets.

#### Seed yield per plant

The mean total seed yield per pant recorded in the present study ranged from 3.36 to 12.84 g (Figure 2). The interaction effects of bulb size and intra-row spacing on mean total seed yield per plant was highly significant ( $p \le 0.001$ ) (Appendix Table 3). Mean total seed yield per plant–increased with increase in bulb size and intra-row spacing; however, the magnitude was different in relation to both bulb size and spacing (Figure 2). At 10 cm spacing, increasing bulb size from 3 to 6 cm increased seed yield per plant of onion by 30.56 and 95.89%, while further increase in bulb size resulted in a 40.20% decrease. At 25 cm spacing, increasing bulb size from 3-7 cm increased the average weight of seeds per umbel



Figure 1. Interaction effects of bulb size and intra-row spacing on weight of seeds per umbel (g umbel<sup>-1</sup>).



Figure 2. Interaction effects of bulb size and intra-row spacing on seed yield per plant (g plant<sup>-1</sup>)

by 13.87, 113.71 and 1.66% (Figure 2). Similarly, at 3-4 cm bulb size, increasing intra-row spacing from 10 to 25 cm, mean seeds per plant was increased by 12.20, 24.93 and 10.19%. Similar results are evident at the other bulb size and intra-row spacing combinations (Figure 2).

The interaction result showed that seed yield per plant of onion is determined by bulb size and intra-row spacing. The results are in line with the findings of Lemma and Shimelis (2003) and Mahadeen (2004) who obtained significantly higher seed yield per plant of onion on large sized bulbs and wider intra-row spacing. Similarly, Amin and Rahim (1995) and Asaduzzaman et al. (2012a) reported that wider intra-row spacing and larger sized bulbs had significantly increased seed yield per plant of onion. The low seed yield per plant under closer spacing is due to the increased level of competition for moisture and nutrients required for plant growth available in the area.

#### Seed yield per hectare

The interaction between bulb size and intra-row spacing on mean seed yield was highly significant ( $p \le 0.001$ ). Mean seed yield per hectare increased with increase in bulb size and intra-row spacing; however, the magnitude was different across the treatment combinations (Table 4). At 10 cm intra-row spacing, increasing bulb size from 3 to 6 cm increased seed yield of onion by 34.74 and 52.22%, whereas further increase resulted in a 53.45% decrease. At 25 cm spacing, increasing bulb size from 3 to 7 cm increased seed yield per ha by 60.86, 152.92 and 6.23% (Table 4). Similar results are evident at the other bulb size and intra-row spacing combinations.

The above finding showed that seed yield of onion is determined by bulb size and spacing. The result is in accordance with the findings of Verma et al. (1994) and Dudhat et al. (2010) who studied the effects of bulb size

Bullh size (am)	Intra-row spacing (cm)								
Buib size (cm)	10	15	20	25	Mean				
3.0-4.0	250 <sup>fg</sup>	350 <sup>defg</sup>	370 <sup>efg</sup>	230 <sup>g</sup>	280				
4.1-5.0	337 <sup>defg</sup>	420 <sup>cde</sup>	472 <sup>cd</sup>	372 <sup>def</sup>	400				
5.1-6.0	516 <sup>°</sup>	753 <sup>b</sup>	988 <sup>a</sup>	937 <sup>a</sup>	799				
6.1-7.0	240 <sup>fg</sup>	725 <sup>b</sup>	920 <sup>a</sup>	995 <sup>a</sup>	720				
Mean	335.75	562	687.50	633.50	549.75				
LSD (5%) = 136.17									
CV (%) = 14.85									

Table 4. Interaction effects of bulb size and intra-row spacing on seed yield per hectare (kg/ha).

Means followed by different letters in the same column are significantly different from each other at 5% level of significance.

Table 5. Partial budget analysis for seed production of onion as affected by bulb size and intra-row spacing.

Treatments	Total variable cost(Birr)	Change in total variable cost (Birr)	Gross field benefit (Birr)	Net field benefit (Birr)	Change in net field benefit(Birr)	Marginal rate of return (%)
B1S4	38459		82919	44460		
B1S3	48088	9629	133330	85241	40781	424
B2S3	80088	32000	169906	89817	4576	14
B3S4	109659	29571	337345	227686	137869	466
B3S3	111088	1429	355766	245678	17992	1259

B1, B2 and B3 stands for bulb size (3-4.0, 4.1-5.0 and 5.1-6.0 cm diameter) whereas S3 and S4 stands for intra-row spacing of 20 and 25 cm, respectively.

and intra-row spacing on seed yield of onion. This may be due to the fact that large sized bulbs had more reserve foods and produce more spouting initial seed stalks; wider plant spacing also avoids disease and competition risks among the emerged flower stalks.

#### **Economic analysis**

The maximum MRR of 1259% was obtained when the treatment combination was shifted from B1S1 to B3S3 and the highest (245,678 ETB ha<sup>-1</sup>) net field benefit was also recorded on this treatment combination of B3S3 (5.1-6 cm and 20 cm spacing). Moreover, treatment combination of B3S3 gave maximum seed yield (988 kg ha<sup>-1</sup>) of onion (Table 5). Further increase of the treatment combination led to incurring additional variable costs without a concomitant increase in benefit and also below the minimum acceptable promising treatment combination (B3S4), maximum variable cost is incurred without gaining income by the farmers. As per the recommendation of CIMMYT (1988), the minimum acceptable MRR for farmer is 100%. Therefore, the treatment combination of 5.1-6 cm bulb size and 20 cm spacing which had the highest seed yield (988 kg ha<sup>-1</sup>) and the highest net benefit (245,678 ETB ha<sup>-1</sup>) together with an acceptable MRR might be taken as profitable option and can be tentatively recommended for the study area.

#### Conclusion

Largest bulb size (6.1 to 7.0 cm) resulted in significantly highest days to 50% bolting (27 days), days to 50% flowering (75 days), days to 50% maturity (123 days), number of leaves per plant (37), LAI (2.05), biomass (130 g), flower stalk height (84 cm), flower stalk diameter (1.62 cm), umbel diameter (5.52 cm), number of umbels per plant (11), number of umbels per plot (854), 1000-seed weight (4.50 g) and seed germination percentage (92%)

The widest intra-row spacing (25 cm) resulted in significantly highest days to 50% bolting (28 days), days to 50% flowering (81 days), days to 50% maturity (124 days), number of leaves per plant (36), biomass (146 g), flower stalk height (74 cm), flower stalk diameter (1.75 cm), umbel diameter (5.82 cm), number of umbels per plant (10), number of umbels per plot (1016), 1000-seed weight (4.35 g) and seed germination percentage (91%) in relation to the closest spacing (10 cm). However, LAI was maximum (1.80) at 20 cm spacing.

The interaction effects of bulb size and intra-row spacing revealed that, the highest number of seeds per umbel (1270 to 1325) and seed yield per plant (12.63 to

12.84 g) was obtained from 5.1 to 7 cm bulb size grown at 25 cm spacing. However, the highest (5.26 to 5.40 and 5.40 g) weight of seeds per umbel was from 5.1-6 cm bulbs on 20 to 25 cm spacing and from 6.1-7 cm bulbs on 25 cm spacing, and seed yield per ha was also maximum (920 to 995 kg ha<sup>-1</sup>) from 5.1-7.0 cm bulb sizes grown at 20 to 25 cm spacing.

Partial budget analysis showed that the combination of 5.1-6.0 cm bulb size and 20 cm intra-row spacing resulted in higher seed yield (988 kg ha<sup>-1</sup>) with a maximum net field benefit of 245,678 Ethiopian birr ha<sup>-1</sup> which was obtained from an acceptable MRR of 1,259%. Based on the result of this study, it can be concluded that bulbs of 5.1 to 6.0 cm and 20 cm intra-row spacing combination are more economically feasible, and recommended for seed yield of Bombay Red onion cultivar in the study area.

#### **Conflict of interest**

The authors have not declared any conflict of interest

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Full Length Research Paper

# User perception towards a motorized thresher ('Kungula') in Uganda: A need finding survey

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Maize which remains the most important food crops in the sub-Saharan Africa is expected to register about a 10% drop in net production globally by 2055. Poor farming techniques, economic diversification in the developing countries due to increased industrialization, could partly explain this trend but addressing food security problem reflected in the world malnourished figures remains a core component of the sustainable development goals. Initiatives such as mechanization intended to boost which are geared towards boosting agricultural production have not registered much success especially in the sub-Saharan African region, largely due to diversities in climates, soils, poverty, culture which often influence the choice of farming techniques. This survey was formulated on the hypothesis that technologies that have worked elsewhere might not necessarily be applicable to other areas without any necessary modifications and/or end-user involvement at the design stage. A human centred design (HCD) approach was adopted; an extended survey using semi-structured interviews and focus group discussions conducted among smallholder maize farmers (randomly sampled) in Nakasongola district (first phase of the bigger sample space) to explore their views and perceptions that would likely influence the uptake of the proposed maize thresher ('Kungula'). Findings show a significant diversity on the crops grown and the amount of land tilled by the smallholder farmers largely due to food insecurity and land tenure system. Post-harvest handling of maize still remains a challenge and rudimentary tools are still popular amongst farmers. There is also a general negativity around the costs of agricultural mechanization but respondents expressed willingness to adopt any technology that would ease their work provided incentives and in a few cases trainings are provided. However, lack of a proper distribution model is one of the hindrances to access of farm inputs which should substantially be addressed. The role of government and other key stakeholder towards economic empowerment of indigenous farmers is still very vital as the success of this user-centred approach hinges largely on the level of economic capability of the final users.

Key words: Maize, farmers, perception, Kungula, thresher.

#### INTRODUCTION

Maize continues to be one of the major food crops in the

sub-Saharan African region covering 25 M ha of

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Author(s) agree that this article remain permanently open access under the terms of the <u>Creative Commons Attribution</u> <u>License 4.0 International License</u> cultivated land between 2005-2008 representing almost one third of cereal area (about 27%) and 34% of total cereal production (Smale et al., 2011) with huge potential for expansion. Conversely though, according to Jones and Thornton's (2003) seminal paper and other projections including Knox et al. (2012), Lobell (2008), Heisey (1998), a decline of about 10% in maize production will most likely be registered mainly in Africa and North America by 2055, and yet substantial agricultural research (especially in Africa) has been directed towards increasing overall crop yield e.g. through improved crop species, development of safeefficient fertilizers and other artificial nutrients, improved farming techniques, etc. It is worth noting that any projected drop in maize and cereal production is in essence attributable to the overall drop envisaged in agricultural production. These declines however could be annotated by changes in climate, reduction in farmland (as a result of population increase and urbanization). poor farming techniques especially in sub-Saharan Africa which also compromise quality of agricultural outputs and increased reduced dependence on agriculture by both developing and underdeveloped economies (due to rapid industrialization). Whereas these factors explain declines in food production, addressing the food security problem reflected in the world's malnourished figures which stands at about 795 million people (mostly in Sub-Saharan Africa and south Asia whose smallholder farmers largely depend on rainfed agriculture) implies a dire need for increasing food production to near double by 2050 (Jones & Thornton, 2003; Knox et al., 2012 and WFP, 2016).

Increasing food production would also mean massive investments in a number of initiatives amongst the agricultural population e.g. increased use of inputs (e.g. fertilizers, pesticides, improved seeds, etc.), diversification and agricultural mechanization. It should also be noted that the basic challenges affecting the agricultural industry are more prominent in the sub-Saharan African countries. Particularly agricultural mechanization (using advanced technologies) through technological advancements which is one of the ways to increase agricultural outputs has not registered much success in sub-Saharan Africa. The reasons for persistent failure of mechanization to register substantial success in the Sub-Saharan Africa resonates with Binswanger and Pingali (1988) major findings that is, diversity in the climates, soils, poverty, cultures and traditions, etc. which have often translated into varying choices of farming techniques. In other words, most technologies do not seem to substantially address these diversities at their conceptual stages, thus the traditional and often inefficient rudimentary tools tend to be preferred by farmers.

In Uganda, maize remains one of the main food crops and the major cereal crop grown covering almost one third of total cultivated land. However, the majority of maize farmers (as with the case of many farmers in sub-Saharan countries) still use rudimentary tools at all stages of production (that is, from ploughing to post harvest handling). These have not only affected quantity produced but also greatly compromised quality. Quantitatively, official estimates showed about 3,000 metric tonnes total weight loss resulting from poor postharvest handling which translated to more about 3.7 million USD by 2014. In principle, poor-harvest handling could primarily increase the risks of incomplete threshing, spillage, physical breaking (making grains grain susceptible to pests) and grain contamination (by soil and stones). Maize post-harvest handling challenges have been exacerbated by the introduction of the draught resistant maize varieties (e.g. WE2101, WE2103, WE2104 and WE2106) in sub-Saharan Africa which have amplified demands for better post-harvest handling technologies due to the alteration of harvest patterns (AATF. 2012).

This survey rather focused on Uganda as a typical country in the sub-Saharan country where food production particularly maize has been seen to fall over the years. The paper presents the findings of an extended survey from semi-structured interviews among smallholder farmers in Nakasongola district on to explore their views regarding a proposed maize thresher 'Kungula'. 'Kungula' is a proposed innovative research with primary intent to improve post-harvest handling practices amongst farming communities in Uganda through increased output efficiency, reduced wastage/ losses and better grain quality at affordable costs. The name 'Kungula' is adopted from one of the widely spoken dialects in Uganda which literally means 'to harvest'. The project aims to design and construct a set of maize threshing machines that is, a motorised thresher for large scale growers and a low-cost manual thresher for the smallholder maize farmers.

In Uganda and a sizeable section of sub-Saharan Africa, machine design, fabrication and to a certain extent technological innovation have been largely dominated by the informal sector mainly manned by local artisans (e.g. 'Jua Kali' as they are referred in Uganda) who train on the job that is, through informal apprenticeship(s). The case for this phenomenon is arguably that agricultural technological improvement (in sub-Saharan Africa) has been largely side-lined by the inevitable investments in agricultural research focused on increasing yield through improved crop species. What cannot go unmentioned though is the role played by this informal sector towards supporting not only agricultural mechanization but also major projects and industries in sub-Saharan Africa such as construction and manufacturing industries. This is largely because locally fabricated equipment and machinery tend to be more readily available and affordable in terms of purchase cost; a case in point is the motorized maize thresher (locally fabricated and assembled) with threshing capacity; 1 tonne/hour/litre of

petrol which costs about \$1000 (USD) in the open market as opposed to \$1500 (USD) for imported thresher of similar capacity.

This prevailing supply chain model coupled with the informal nature of local design and fabrication industry implies that there is minimal end-user involvement at the development stage(s) of these equipment, a gap which the Kungula project seeks to address.

The survey has been formulated based on the hypothesis that diversities in cultures, climatic and topographical variations, nature of labour requirements, size of any given economy, etc. are key in determining the extent to which agricultural mechanization is adopted; As such technologies used elsewhere might not necessarily work if directly employed in other areas. Therefore, either new technologies need to be modified (especially through research and development) to suit local requirements or local conditions can be changed (e.g. investing in infrastructure to allow for ease of implementation, mind set and perception change through massive sensitisation). This paper argues that altering local conditions could be far-fetched often requiring policy formulations, appropriation and monitoring which place huge financial burdens on the institutions of government. On the other hand, a number of benefits such as reduction in equipment development times and end-user trainings, re-doing new versions etc. that come with tailormade designs compare favourably against challenges that arise from trying to alter local conditions (Bevan, 2001). This hypothesis therefore is a strong rationale for attempting to obtain views of targeted end users of a particular technology before actual design commences. This phenomenon could be explained by the Human Centred Design (HCD) approach which tends to ensure product usability and guarantees validity of usability claims by product designers (Earthy et al., 2001). In other words, HCD strives to incorporate end user perspective into the product development process with the aim of achieving high level of product acceptability and userfriendliness. The HCD approach is not exclusive to the product development stage but also encompasses userfeedback based on their use of first phase of products developed. Typical of this is the software development cycle but it can be conceivable that tangible products essentially do go through similar cycles during their development stages. Detailed description of the working and functionality of Human Centred Design however, are not within the scope of this paper though interesting literature could be found in Rouse (1991), Cooley (2000), Norman (2005), and Oviatt (2006).

The findings of this study were used to inform design decision(s) in terms of end-user perceptions and expectations of the proposed innovation. Findings were restricted to the post-harvest handling scenarios (as highlighted below) presented to the respondents but this is not to rule out design modifications as this survey sets out to identify and innovatively address technological gaps regarding post-harvest handling. Preliminary (proposed) information such as feed rates, spiral angle of bar tooth such as feed rates, roller rotation and fan speeds, that is, rotation per minute (rpm), spiral angle of bar tooth, etc. (Wuyun and Kangquan, 2012), which were regarded to be highly technical were deliberately omitted from the different scenarios presented primarily because of the non-expert nature of the targeted respondents.

#### Traditional manual threshing

This operation is arguably the most commonly used among rural farmers (in Uganda) and it involves the confinement of dried maize cobs in nylon bags and thumping them repeatedly with sticks. In addition to the low grain recovery rates, there is also a high risk of physical damage to the grains making them susceptible to moulds and pest attacks.

#### Manual threshing

Almost identical to the traditional manual threshing except that it involves using manual energy to operate a mechanical equipment that extracts dried maize seeds from the cobs. Two people are required during threshing using a manual thresher with one person continually feeding the maize cobs to the machine and the other operating the equipment to control rotational speed. Just like manual threshing, grain recovery rates depend on how much (in terms of physical input) the machine operator can do. These threshers however come in handy in areas where access to energy is a major problem, typical of most rural settlements in sub-Saharan Africa.

#### Motorized (immovable) thresher

This uses the same principle as a manually operated thresher except the machine is driven by a motor using a petrol engine. The equipment is usually kept in a central location within the reach of harvests, hence the name place immovable. Because of the high costs involved in procuring mechanized farm equipment, motorized threshers tend to be owned by clusters of farmers as opposed to individual ownership and in some instances individual farmers buy them to do threshing on a commercial basis. The problems with this model are the associated costs incurred by farmers (e.g. transportation of maize to and from the threshing location, paying for the cleaning, threshing, drying and etc.) and the inconvenience caused by having to travel back and forth.

#### Motorized portable

Motorized threshers can be modified so as to be as

portable as it possibly can be. This not only reduces the capital investment cost thereby making it more affordable than the immovable type but it also addresses the convenience problem which is one of the major challenges with the immovable threshers.

#### METHODOLOGY (SURVEY APPROACH ADOPTED)

Research approach is defined in Creswell (2013, p. 3) as "plans and the procedures for the enquiry that span the steps from assumptions to detailed methods of data collection, analysis and interpretation". These aforementioned plans and procedures often entail having to make numerous decisions at various level, therefore in addressing any given research problem, three nondiscrete approaches could be employed, that is, quantitative, qualitative and mixed methods approach. Details of these research approaches is not within the scope of this paper but important information could be found Golafshani (2003), Rajasekar et al. (2006) and Creswell (2013), etc. Whereas each of these methods could present a number of advantages such as comprehensiveness in understanding the research problem as in mixed methods, delivering precise and conclusive results, that is, quantitative; the nature of this study which is based largely on perception of the respondents using results from semi-structured interviews, implies that a qualitative approach was best suited for this kind of study. The approach encompasses "exploring and understanding the meaning of individuals or groups ascribed to a social or human problem" (Creswell, 2013, p. 3). In other words, no particular attempts were made to have the phenomenon manipulated, instead the situation was analysed from a real life viewpoint. The form of data obtained from qualitative approach comprise mainly information often gathered from questionnaires and interviews (that is, open ended, structured or semi-structured) and focus group discussions, as with the case of this study. This study aims to largely employ an empirical evidence based type approach, usually adopted by various agricultural researchers, policy think tanks, government institutions and international organizations to inform policy formulations. Qualitative primary data obtained during the survey was thus used to inform judgement during the proposed technology design processes.

We conducted semi structured interviews and two focus groups with farmers from two sub-counties (Kakooge and Kalongo) in Nakasongola District between June and July 2015. The respondents were recruited by the Nakasongola District agricultural officials, and these comprised mainly smallholder farmers who had previously participated in a couple of previous government surveys and projects. The decision to delegate the respondents' recruitment was largely resources and logistics based and not scientific as having interviewers recruit the respondents would not only require more time but also more financial resources to facilitate the process (Tables 1 and 2). The overall aim was to assess the farmers' perception that would likely affect the uptake of the proposed maize thresher 'Kungula', thus the interview themes were designed to address the following key aspects:

i) Identifying existing practices related to handling of maize among subsistence and commercial farmers in Nakasongola district.

ii) Investigating existing challenges related to threshing of maize among subsistence and commercial farmers in Nakasongola district.

iii) Assessing existing management practices related to threshing of maize among subsistence and commercial farmers in Nakasongola district.

iv) Exploring distribution models affecting access to agricultural inputs among subsistence and commercial farmers in Nakasongola

district.

v) Establishing attitudes, perceptions and other factors that would affect adopt the proposed maize thresher among subsistence and commercial farmers in Nakasongola district.

#### FINDINGS AND DISCUSSION

The questions in the questionnaire/focus group were generally designed to establish the baseline scenario(s) regarding general maize handling practices (that is, from ploughing to post harvest handling) in the study area, as a means of identifying technological gaps and thus use the said gaps to gauge end-user expectations.

Respondents were asked to elaborate on the existing management practices employed in maize handling from planting to harvesting. All respondents who responded to this question confirmed the fact that re-known traditional farming techniques and procedures (that is, clearing the land using hand hoes, planting and weeding after the shoots sprout) are still popular amongst the farming community. This has been largely attributed to inadequate resources as most farmers do not have the necessary resources to invest in irrigation, herbicides and other machineries. Additionally, it is worth noting that the financial resource constraint is not exclusive to maize farmers but rather the entire agricultural community. This is not to suggest that availing rural based farmers the financial resources would immediately translate to mechanised farming practices since promoting mechanised agriculture requires a rather broad based approach which could encompass both physical infrastructure and government policy. This narrative can be confirmed by a quick assessment of farming communities in urban areas who have easy access to infrastructure. These tend to use more efficient farm equipment (however simple) compared to their ruralbased counterparts, not because of the intensity of their farming but simply due to the accessibility of these equipment. This sentiment was also echoed by a number of respondents claiming the problem is 'everything is in town and Kampala areas' (that is, urban areas and the capital city)

Likewise, a number of challenges specifically related to post-harvest handling of maize/maize threshing were identified and admittedly, all respondents cited labour intensiveness and inefficiency of the traditional maize threshing techniques (e.g. thumping using sticks) as a major threshing challenge. However, as intimated in the previous texts traditional techniques are still very popular amongst maize growers and this has also been majorly due to inadequacy of financial capacity to invest in improved techniques and partly due to ignorance. This cost factor was emphasised by Pingali and Binswanger (1988) as the major impediment to transitioning from traditional tools to highly mechanized tools such as tractors. In other words, if the cost of mechanization outweighs the savings in the cost of manual labour (a very likely scenario in sub-Saharan Africa), farmers are inclined to stick to the traditional approaches. In some instances, especially where topography permits, animals such as oxen and donkeys have remained intermediaries between rudimentary and highly mechanized techniques. Animal power however, is mostly suited for cultivation and perhaps certain aspects of harvesting which implies that improvisations and intermediaries are not plausible options for harvesting and post-harvest handling.

Other more specific threshing challenges identified included weight loss resulting from improper drying, low quality grains due to poor winnowing thereby leaving chaff and other unwanted additions/residues in the threshed maize. One respondent elaborated that up to 3kg of maize is lost to the buyer for every 100 kg to cater for chaff and other unwanted additions. It could be conceivable that these estimates could have no basis or perhaps unrealistic and as such there was no consensus on the actual amount of loss incurred as this seemed to vary from buyer to buyer, however all respondents (including buyers) concurred on the monetary implication attributed to losses due to chaff and 'impurities' which obviously cost farmers a considerable amount of income. From a mechanized threshing view point, the locally available threshing machines apart from their scarcity, operability and maintenance costs, and portability, they also lack some of the end-user desired technical abilities such as winnowing, drying and cleaning alongside threshing. In fact, farmers are more interested in having a robust, potable and diverse threshing equipment that can enable them perform multiple processes concurrently. This was the basis of this research that is, to gauge targeted end-user expectations and use it to inform design decisions of the proposed thresher. A total deviation from the existing thresher designs to meet all farmer expectations might not be realistically practicable but removal of chaff and other impurities from the harvest (to improve quality) is undoubtedly a worthwhile problem that could be addressed at the technical design phase. Additionally, there were mixed responses towards farmers' expectations of the proposed thresher especially regarding its mode of operation (that is, manual or motorized). However most (that is, 25 out of 29 who gave clear answer(s) to the question) respondents specifically suggested that any new technology should at least be incorporated with a winnowing component like fan to be able to clean their products to improve value. Both these technical and logistical challenges were taken into consideration during design. A summary description of the baseline scenario regarding maize handling practices can be found in Table 3.

#### Summary

Like most farming communities in the sub-Saharan Africa

region, more than 70% of the farmers surveyed practice mostly subsistence farming mainly attributed to the land tenure system which makes it hard to practice large scale farming due to the fragmentation. Likewise, land tenure system and food insecurity have also been responsible for the diversification in crop production among the indigenous farming communities not only in the area surveyed but through most parts of Uganda. Rudimentary tools still remain popular amongst the smallholder farming communities mainly due to affordability and accessibility (in terms of distribution models) of modernised equipment, lack of awareness about agricultural mechanisation.

Furthermore, locally available maize threshing equipment lack the end-user desired technical features/ abilities e.g. winnowing, cleaning, drying and above all user friendliness and portability. These were incorporated in the design process of the proposed technology with the aim of producing a user-desired product.

#### Limitations of the study

Very often, qualitative data are seen to be too subjective, inconclusive and over reliant on respondents. Therefore, interpersonal skills that combine empathy and analytical abilities are very vital for qualitative researchers. Other than the 'common' challenges of qualitative research, the findings of this study were also subject to certain specific limitations which have been discussed next.

#### Bias and misrepresentation

Because of limited resources and time, respondents were not directly recruited by the researchers and as such there was no direct contact with the respondents prior to the study. This meant that the district agricultural officers delegated to identify and recruit participants had the sole responsibility of determining who took part in the survey. There is a reasonable possibility that this could have compromised the responses especially if the recruiters had personal biases or if the process itself created a bias amongst the respondents. It is also worth noting that the district officials interact regularly with the local farmers therefore the nature of their relationship can be critical while conducting studies of this nature.

#### Some level of mistrust and discomfort

The interviews were conducted by total strangers as there were no prior correspondences with the respondents. Naturally, there could have been some concerns on the part of the interviewees as to how much information they could disclose to the interviewers. This implies that some respondents could have chosen not to 
 Table 1. Summary extract of questionnaire.

Existing practices relating to maize handling for ploughing to harvesting       Describe the practices involved in maize growing from planting to harvesting.         Challenges relating to maize threshing       Describe the challenges you face related to post harvest handling of maize (e.g. threshing management practices related to post harvest handling (Probe for threshing management strategies)         Management practices related to threshing of maize       Investing management practices related to post harvest handling (Probe for threshing management strategies)         Baseline scenario       Management practices related to threshing of maize         Distribution models affecting access to agricultural inputs among subsistence and commercial farmers in farmers       In your opinion, do you think post-harvest practices of threshing Provide for threshing management strategies)         End User- Perception       Attitudes and perceptions that would affect adoption proposed maize threshing       From the explanation provided; what do you think about the proposed maize threshing How do you pay (that is, finance) for agricultural activities and how can it be improved?         End User- Perception       Attitudes and perceptions that would affect adoption proposed maize thresher       From the explanation provided; what do you think about the proposed maize thresher?         What are the difficulties you find with financing your agricultural activities and how can it be improved?       How way preference in terms of source of power for the thresher?         What are the difficulties you find with financing your agricultural activities and how can it be improved?       From the expl					
End User- Perception         Challenges relating to maize threshing         Describe the challenges you face related to post harvest handling of maize (e.g. threshing management practices related to post harvest handling (Probe for threshing management practices affect quality and quanity of the maize produced?           Management practices related to threshing of maize         Have you done anything to try to improve post-harvest handling reactives Do you use any form of machinery for post-harvest handling? If yes which ones?           Baseline scenario         Have you done anything to try to improve post-harvest handling? If yes which ones?           Do you use any form of machinery for post-harvest handling? If yes which ones?         Have you consider when buying agricultural supplies e.g. fertilizers, seedlings, machinery, etc.           Distribution models affecting access to agricultural inputs?         What do you get to know about agricultural supplies e.g. fertilizers, seedlings, machinery, etc.           What do you pay (that is, fnance) for agricultural supplies? Unbat are the difficulties you find with financing your agricultural supplies? Unbat are the difficulties you find with financing your agricultural activities and how can it be improved?           From the explanation provided; what do you think about the proposed maize thresher         From the explanation provided; what do you think about the proposed maize thresher?           What are the difficulties you find with financing your agricultural activities and how can it be improved?         How big (in terms of volume of maize thresher) designed to thresh maize thresher?           What abou to cost of the machine, how much would you be will		Existing practices relating to maize handling from ploughing to harvesting	Describe the practices involved in maize growing from planting to harvesting		
Baseline scenario       Management practices related to threshing of maize       In your opinion, do you think post-harvest practices affect quality and quantity of the maize produced?         Baseline scenario       Have you done anything to try to improve post-harvest handling practices Do you use any form of machinery for post-harvest handling? If yes which ones?         Do you have any preference, in terms of any innovations you use for maize threshing? If yes, what are some of these innovations you use for maize threshing? If yes, what are some of these innovations (methods)         How do you get to know about agricultural supplies e.g. fertilizers, seedlings, machinery, etc.         What do you consider when buying agricultural inputs? Are you helped in anyway while deciding what to buy?         How do you pay (that is, finance) for agricultural supplies? Individually or groups?         What are the difficulties you find with financing your agricultural activities and how can it be improved?         How do you pay (that is, finance) for agricultural supplies? Individually or groups?         What are the difficulties you find with financing your agricultural activities and how can it be improved?         From the explanation provided; what do you think about the proposed maize thresher         Perception         Attitudes and perceptions that would affect adoption for propsed maize thresher would affect adoption for by ing in thresher?         Would you be willing to take up a maize threshed would you want this thresher?         Would you be willing to take up aremaize threshed) would you want this thresher?		Challenges relating to maize threshing	Describe the challenges you face related to post harvest handling of maize (e.g. threshing methods, their challenges, etc.)		
Baseline scenario       In your opinion, do you think post-harvest practices affect quality and quarity of the maize produced?         Have you done anything to try to improve post-harvest handling practices.       Do you use any form of machinery for post-harvest handling? If yes which ones?         Baseline scenario       Do you use any preference, in terms of any innovations you use for maize threshing? If yes, what are some of these innovations (methods)         How would you rate your satisfaction with the current means of threshing? If yes, what are some of these innovations (methods)         How would you consider when buying agricultural inputs? Are you helped in anyway while deciding what to buy?         Distribution models affecting access to agricultural inputs? Are you helped in anyway while deciding what to buy?         How and where do you buy your supplies? What are the difficulties you find with financing your agricultural activities and how can it be improved?         How do you gay (that is, finance) for agricultural activities and how can it be improved?         How do you pay (that is, finance) for agricultural supplies? Individually or groups?         What are the difficulties you find with financing your agricultural activities and how can it be improved?         How do you pay (that is, finance) for agricultural activities and how can it be improved?         How do you pay (that is, finance) for agricultural activities and how can it be improved?         How do you baw any preference, in terms of source of power for the thresher?         Would you be willing to take up a maize thresher designed to th			Describe existing management practices related to post harvest handling ( <b>Probe</b> for threshing management strategies)		
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Baseline scenario       Do you have any preference, in terms of any innovations you use for maize threshing? If yes, what are some of these innovations (methods)         How would you rate your satisfaction with the current means of threshing       How would you rate your satisfaction with the current means of threshing         How would you rate your satisfaction with the current means of threshing       How would you rate your satisfaction with the current means of threshing         How would you rate your satisfaction with the current means of threshing       How would you cansider when buying agricultural supplies e.g. fertilizers, seedlings, machinery, etc.         What do you consider when buying agricultural inputs? Are you helped in anyway while deciding what to buy?       What are when would your agricultural activities and how can it be improved?         How do you pay (that is, finance) for agricultural supplies? Individually or groups?       What are the difficulties you find with financing your agricultural activities and how can it be improved?         How do you be willing to take up a maize thresher designed to thresh other crops? If so, which ones?       From the explanation provided; what do you think about the proposed maize thresher         Perception       Attitudes and perceptions that would affect adoption proposed maize thresher designed to thresh other crops? If so, which ones?       How big (in terms of volume of maize threshed) would you want this thresher (Kungula) to be         Do you have any preference in terms of source of power for the thresher?       What about cost of the machine, how much would you be willing to pay for the thresher? </td <th></th> <td>Management practices related to threshing of maize</td> <td>Do you use any form of machinery for post-harvest handling? If yes which ones?</td>		Management practices related to threshing of maize	Do you use any form of machinery for post-harvest handling? If yes which ones?		
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			What about cost of the machine, how much would you be willing to pay for the thresher?		
Would you love any special trainings on how to use the thresher			Would you love any special trainings on how to use the thresher		

Format: Author.

Table 2.	Respondents'	Information.
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Education Level	Number of respondents	Acres of Maize cultivated	Number of respondents	
Above A level	3	0-5	20	
High School (A Levels)	1	5-10	3	
O Level	1	10-15	0	
Below O Level	12	Above 15	1	
Not provided	14	Not provided	7	
∑ sum	31	∑ sum	31	

Format adapted from: Jenkinson et al., 1993, p.2.

disclose certain information they consider sensitive, thereby impacting negatively on the data collected

especially if the information could have been valuable for the study.

Operation	Description
Ploughing	Farmers use mainly hand hoes, ox-ploughs and in very rare cases tractors for primary ploughing. In some cases, (rough areas) secondary tillage is necessary which further prepares the land for planting
Planting	No special equipment other than hand tools employed during planting. Planting is done mainly at the beginning of rainy season(s)
Mechanical weed control	Usually done within the first three weeks of planting (first weeding), then one month after first weeding
Harvesting	Starts after 3-5 months of planting (depending on the breed). Done manually (with hands) and no specialized tools
Drying/Transportation	Harvested maize transported to the drying yard, sunshine is the main mode of drying
Threshing	Manual threshing (that is, thumping with sticks) and mechanical threshing using motorized equipment
Cleaning/winnowing	Also done manually with no specialised tools
Bagging and storage	Maize seeds stored in bags and silos

Table 3. Baseline scenario of existing maize handling practices (Format adapted from: Bacenetti et al., 2016).

#### Exaggeration of responses

Naturally, there is a tendency of interview respondents speculating the intention and objective(s) of interviews and this often tend to influence how participants respond to certain questions. It was noticeable that a sizeable number of individuals (about 6), and mostly those who had attained at least high school qualifications attempted to provide exaggerated figures (that is, over estimating) especially relating to volumes of output and size of land. It is worth mentioning that the government of Uganda has over the years tried to put in place initiatives e.g. agricultural grants, advisory services, subsidies to farm inputs, etc. to boost agricultural outputs. Benefiting from such initiatives however, are always through a prescribed criteria developed by government experts often targeting medium and large scale subsistence farmers. This survey could have easily been mistaken for a way of identifying beneficiaries of one of those government programs, and fact that more calculated, the rehearsed and premeditated answers came from respondents who had slightly better education qualifications confirms this argument. As a mitigation measure, the survey questions were designed and customised to suit almost every respondent and the team tried as much as they possibly could to clearly explain the objectives of the study. It is likely though that this could have not been sufficient to counter all well thought answers.

#### intended to inform design decisions of the proposed maize thresher. Major findings of the study were adopted and eventually incorporated in the final product design. We recommend the following for future research as well as improve farming practices:

i) A robust equipment that performs multiple operations (that is, threshing, cleaning, drying, etc.) concurrently and threshes different crops is plausible for future research. Likewise, handling of agricultural wastes was not factored in at conception stage of the proposed technology. The question of what happens to the residue (maize cobs, chaff, dust, etc.) after the threshing activity

ii) The lack of a clear distribution model for farm inputs and equipment still remains one of the major inhibitions to mechanised and large scale farming. We recommend and emphasise a model where farmers work in clusters and local groups so they can collectively afford mechanized equipment and also easily voice their needs. Farmer groups and clusters have proven to work in low income countries and all respondents agreed that farmers who mobilised themselves in groups often have better access to aid initiatives, government programs, mechanised equipment and a higher bargaining power in the market. This can only be achieved through massive and continuous sensitization on the benefits of 'coming together', mechanisation and large scale farming.

#### Conclusion

#### RECOMMENDATIONS

This was an empirical need finding study primarily

Rudimentary and traditional practices will most likely remain popular amongst rural smallholder farming communities in Uganda and sub-Saharan Africa. It is evident that economic levels, attitudes and perception do play an impeccable role in determining the farming practices amongst indigenous and smallholder farmers. Agricultural mechanisation through innovation and technological advancement is one of the practical ways to minimise and/or mitigate losses attributed to post harvest handling and to boost not only agricultural productivity but also improve quality of agricultural output. User-centred design approach adopted by the 'Kungula' design team is a realistic way to develop tailored local solutions to agricultural mechanization challenges, and above all achieving maximum adoption of any new innovations amongst smallholder farmers. However, the success of this strategy will largely hinge on the level of economic empowerment within the farming communities thus the role of government and other stakeholders cannot be trivialised.

#### ACKNOWLEDGEMENT

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#### **CONFLICT OF INTERESTS**

The authors have not declared any conflict of interests.

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African Journal of Agricultural Research

Full Length Research Paper

# Seed quality evaluation by tetrazolium staining during a desiccation study of the recalcitrant seeds of *Carapa guianensis* Aubl. and *Carapa surinamensis* Miq. - Meliaceae

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Crabwood, a popular name of several pantropical timber species, has become increasingly important for its seed oil of pharmaceutical and cosmetic use. Due to the recalcitrant character of the seeds, plantations are limited. The aim of this study was to develop a tetrazolium (TZ) staining protocol and validate viability staining with germination tests. Seed preparation was standardized in order to localize and cut the tiny embryonic axis longitudinally, which is inserted in the fused cotyledonal seed mass. Staining intensity was determined by testing different concentrations of TZ solution (0.05, 0.10, 0.25 and 0.50%) at three temperatures (25, 30 and 35°C) during a period of up to 6 h. Taking into account the large seed size, costs and working time, a solution of 0.10% TZ at 30°C for 3 h was considered appropriate for both species. The method was validated with seeds of different qualities (between 0 and 90% germination capacity), obtained by controlled drying over a fan. The desiccation revealed initial damage near the seed surface close to the radicle meristem. Images of the stained seeds were classified in four viability classes and were re-evaluated with the germination results (radicle  $\ge 0.5$  cm and normal seedlings). The proposed method for tetrazolium staining was effective in assessing seed viability of both species.

Key words: Andiroba, Carapa procera D.C., water content, embryonic axis, germination, crabwood.

#### INTRODUCTION

Amazonia is known for a high diversity of species with commercial importance, some widely exploited due to their multiple uses are submitted to deforestation and non-sustainable exploitation (Souza et al., 2008). Among these are the species of the genus *Carapa* (Meliaceae), known popularly as crabwood, andiroba, roba-mahogany,

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Author(s) agree that this article remain permanently open access under the terms of the <u>Creative Commons Attribution</u> <u>License 4.0 International License</u> among others. These species occur in the Neotropics, Africa and India (Kenfack, 2011), with similar use and extraction of the seed oil on all continents (Weber et al., 2010). A recent taxonomic revision restricted the occurrence of *Carapa procera* D.C. to the African continent, and according to the same study, three species, *Carapa guianensis* Aubl., *Carapa surinamensis* Miq. and *Carapa vasquezii* Kenfack (Kenfack, 2011) exist in the Brazilian Amazon region. In this way, the results published up to 2011 on *C. procera* seeds collected in the Amazon were attributed to *C. surinamensis*.

Andiroba seeds are large and individual seed weight averages 25 g for *C. guianensis* and 16 g for *C. surinamensis* (Ferraz et al., 2002). The seeds are recalcitrant (Connor et al., 1998) and this characteristic is one of the causes of poor seed trade. Recalcitrant seeds are generally considered to germinate immediately after dispersal (Daws et al., 2005). Under nursery conditions, *C. guianensis* seeds needed between 26 and 180 days for emergence, and *C. surinamensis* between 11 and 38 days, both showed fast subsequent development, and in about ten days the shoot length until the first expanded leaves reached 40 and 25 cm, respectively (Ferraz et al., 2002). Seed testing rules (Brasil, 2009; ISTA, 2015) evaluate germination until the development of a normal seedling. Thus, seed testing in the laboratory is difficult.

The internal seed morphology reveals a uniform seed reserve tissue, formed by the conferruminate, that is, fused cotyledons. A visual distinction between the two cotyledons is not possible (Harshberger, 1902). The small embryonic axis (length ca. 2 mm) is inserted in the cotyledonal tissue near the micropyle on the tetrahedron face of the seed (Ferraz et al., 2002). Some seeds of *C. surinamensis* may have several embryonic axes (Ferreira et al., 2017).

Seed quality assessment of recalcitrant seeds, or those with slow germination (>60 days), can be done with viability tests (Brasil, 2009). The best known is topographic staining with tetrazolium (2,3,5-triphenyl tetrazolium chloride - TZ) (Lakon, 1949). The respiratory activity of seeds in living tissue, specifically hydrogen ions ( $H^+$ ) released by dehydrogenases, reduce soluble and uncoloured tetrazolium to a red insoluble compound, known as triphenylformazan. Thus metabolically active tissues present red staining while dead or damaged tissues remain unstained.

Viability tests generally overestimate germination results and should be validated with germination tests. Species-specific protocols may be necessary. The embryonic axis has to be evaluated and, depending on seed morphology, the vital parts have to be visible. Seeds may need conditioning in water, and the concentration of the TZ solution, immersion time and temperature has to be known, as well as tissue consistency and the location and size of possible lesions in relation to seed morphology (França-Neto et al., 1998; Brasil, 2009; Fogaça et al., 2011). Instructions for TZ concentrations for 154 species, including agricultural and forestry species, were published in the Brazilian Seed Testing Rules, and solutions of 0.5 and 1.0% were recommended in most cases (Brasil, 2009). The International Seed Testing Association (ISTA) recommends solutions of 1% TZ and 30°C as immersion temperature during 18 to 48 h; for the 120 forestry species studied, emphasis is given to seed preparation, to permit the evaluation of the embryonic axis (Leist and Krämer, 2011). *Carapa* spp. have several seed characteristics which would need fast quality assessment, such as large recalcitrant seeds, slow and not uniform germination, and tall seedlings, however no information on TZ staining was found.

In this sense, the aim of this study was (a) to elaborate a protocol for seed preparation and staining of *C. guianensis and C. surinamensis* with tetrazolium solution, and (b) to validate the TZ staining with germination tests.

#### MATERIALS AND METHODS

#### **Collection area**

The seeds of *C. guianensis* and *C. surinamensis* were collected in March 2014 during the natural seed dispersal in a 40-year-old plantation at the Experimental Forestry Station of the Brazilian National Institute for Amazonian Research (Instituto Nacional de Pesquisas da Amazônia - INPA), located at km 45 on the BR-174 highway north of Manaus (02° 35' 55.5" S and 60° 02' 14.8" W), Amazonas State. Due to the desiccation intolerance of the seeds, fruits and seeds were transported immediately after collection in semi-permeable plastic bags to the seed laboratory of INPA.

#### Processing

The capsule-type fruits were opened manually by removing the fruit valves. Seeds with visible damage were eliminated. After submersion in water for 24 h to drown potential larvae of *Hypsipyla* sp., a lepidopteran known as a seed borer, the seeds were superficially dried above wires at room temperature for about 2 h.

#### Adequacy of the tetrazolium test

Preliminary tests aimed to determine the tetrazolium concentration, temperature and immersion time. After longitudinal sections, the seeds were preconditioned in distilled water for 24 h at 25°C (Brasil, 2009). Due to the size and morphology of the seed, it was possible to cut them a second time, in order to observe the staining at four TZ concentrations (0.05, 0.10, 0.25 and 0.50%) on the same seed. Each section was totally immersed in the TZ solution in a 50 mL plastic cup, covered with plastic film to reduce evaporation, and maintained in the dark. Three immersion temperatures were compared: 25, 30 and 35°C (± 2°C), using germination chambers (Fanem<sup>®</sup>). In each of the twelve conditions (four concentrations × three temperatures) 24 seeds were observed, totalling 288 samples. The staining intensity of the seeds was assessed hourly. The colours were classified in intensity levels according to the plant tissue colour chart (Munsell Color Charts, 1977) without staining (natural colour of tissue 2.5Y ± 8/2), very weak (2.5R 8/4), weak (2.5R 7/4), adequate (2.5R 7/6 and 7/8) and excessive staining (2.5R 5/8 and 5/10).

#### Seed preparation and exposure of embryonic axis

Seeds were transversely sectioned into two parts (Figure 1A and B). The distal part was used for moisture content determination, while the part with the embryonic axis was destined for tetrazolium staining. The woody seed coat has to be removed carefully with a metal spatula. A circular light brown area is differentiated from the brown seed surface. The centre of the circle has a small elevation and papyraceous tissues, slightly darker brown than the circle area, covering the axis. These tissues must be carefully removed to reveal the basal part of the embryonic axis (Figure 1C). A longitudinal cut through the axis permits its exposure to the TZ solution (Figure 1D).

#### Viability validation

Seeds of different germination capacities were obtained by controlled drying during 0, 1, 3 and 7 days at room temperature ( $25 \pm 2^{\circ}$ C,  $\pm 60\%$  RH), at 70 cm above an air circulator (Arno WWB3/5). The seeds were maintained in nylon mesh nets for fruits, which allowed circulation of air between the seeds. Each net contained 120 seeds, with four repetitions of 15 seeds for viability staining and the same amount for germination tests. For TZ staining, seeds were prepared as described previously and, based on results of the preliminary tests, immersed in 0.10% TZ for 3 h at 30°C and rinsed afterwards with distilled water.

TZ staining was observed under a stereo microscope (Leica S8 APO). Images of each seed were recorded (Leica DFC295), however they were assessed only when the germination results were available. Staining evaluation was based on França-Neto (1999), where bright red or pink colours were living tissue, and milky white or yellowish colours, dead tissue. These colours were identified with the plant tissue colour chart (Munsell Color Charts, 1977) as 2.5R 7/6 and 7/8 (living tissue), 2.5Y 7/6, 7/8 and 7/10 (dead tissue). Seeds with no red or pink colouration were easily recognized as dead, nevertheless the ones which still showed some reddish colours were difficult to evaluate. Thus, the images of all seeds were organized according to their colouration patterns, considering primarily the embryonic axis and secondly the tissue of the fused cotyledons. Four classes with decreasing red colouration could be established. Afterwards, the results of the germination tests were matched with the TZ evaluation, considering class 1, 2 or 3 as viable seeds. The relation between germinability and viability was evaluated with a Pearson correlation coefficient (r).

#### Seed moisture content

Determined gravimetrically after drying at 105°C (Brasil, 2009); the samples were weighted every 24 h until mass stabilization (0.001 g) and the moisture content was expressed as percentage of fresh weight. Moisture content was assessed for each seed of the viability test, using the distal portion (about 10 g), sectioned twice for faster drying. The results were evaluated in four repetitions of 15 seeds, for each treatment.

#### Germination test

The seeds were sown in plastic trays  $(30 \times 22 \times 7 \text{ cm})$  in moistened vermiculite of medium granulation (Brasil Minérios®) (2 g water / 1 g vermiculite). The trays were covered with transparent polyethylene bags (40 × 60 cm and 8 mm) to reduce evaporation. The test was performed at 25°C (± 2°C) with 12 h photoperiod (cool white fluorescent light, PAR 015 × 10 µmol<sup>-</sup>m<sup>-2</sup>·s<sup>-1</sup>).

The first germination criterion, protrusion of the radicle (≥ 0.5 cm), was assessed daily. Germinated seeds were transferred to the



Figure 1. Seed preparation of crabwood seeds, exemplified with C. surinamensis, to expose the embryonic axis to the tetrazolium solution. (A) Seed with seed coat and hilum view. (B) Seed without seed coat and same view as above, the dotted line indicates the first cross section (C). After removal of the papyraceous tissues, the basal end of embryonic axis is visible in the centre of the light brown circle; the horizontal dotted line indicates the second section. (D) The embryonic axis with about 2 mm length after TZ staining.

nursery in larger trays ( $57 \times 25 \times 17$  cm) and development monitored until the expansion of the first pair of leaves (second germination criterion). A normal seedling, according to seed testing standards, had radicle and shoot with perfect development and showed high probability of establishment under favourable conditions (Brasil, 2009). The results were expressed as percentage of germinated seeds for each criterion.

Temperature (°C)	TZ (%)	Immersion time (hour)						
		1	2	3	4	5	6	
25	0.05	None	Very weak	Very weak	Very weak	Weak	Adequate	
	0.10	None	Very weak	Very weak	Weak	Adequate	Adequate	
	0.25	Very weak	Very weak	Weak	Adequate	Adequate	Excessive	
	0.50	Very weak	Weak	Adequate	Excessive	Excessive	Excessive	
30	0.05	None	Very weak	Weak	Weak	Adequate	Adequate	
	0.10	Very weak	Weak	Adequate	Adequate	Excessive	Excessive	
	0.25	Very low	Weak	Adequate	Adequate	Excessive	Excessive	
	0.50	Very low	Weak	Adequate	Excessive	Excessive	Excessive	
35	0.05	None	Very weak	Weak	Adequate*	Adequate*	Excessive*	
	0.10	Very low	Weak	Adequate*	Adequate*	Excessive*	Excessive*	
	0.25	Very low	Weak	Adequate*	Adequate*	Excessive*	Excessive*	
	0.50	Weak	Adequate*	Excessive*	Excessive*	Excessive*	Excessive*	

**Table 1.** Immersion time at three temperatures and four concentrations of tetrazolium solution (TZ) to achieve four qualities of tetrazolium staining in *Carapa* sp. seeds; no-uniform staining is marked with (\*).

None (natural tissue colour 2.5Y ± 8/2); very weak (2.5R 8/4); weak (2.5R 7/4); adequate (2.5R 7/6 and 7/8) and excessive (2.5R 5/8 and 5/10) colour indication in accordance to Munsell Color Charts (1977).

#### Statistical analyses

The experimental design was completely randomized. Normality was tested with the Shapiro-Wilk test for germinability and viability, and the homogeneity of variances with Levene's test at 0.01% significance. Results were compared by analysis of variance (ANOVA) followed by comparison of the means through Tukey test at 5% probability, using SISVAR (system for variance analysis) (Ferreira, 2011). Pearson correlation coefficients (r) between tetrazolium and germination tests (radicle protrusion and normal seedling) were calculated. The significance of r values was determined by the *t*-test at 1% probability (Pimentel-Gomes, 2000).

#### **RESULTS AND DISCUSSION**

The immersion time to achieve an adequate staining with TZ depended on the immersion temperature, the TZ concentration and using less TZ being more economical. Adequate staining was achieved at 25°C after 3 h with 0.50% TZ, while less-concentrated solutions needed 4 h (0.25%), 5 h (0.10%), and 6 h (0.05%). At 30°C, adequate staining was achieved after 3 h in concentrations of 0.10, 0.25 and 0.50%, the staining remained adequate with 0.10 and 0.25% up to the fourth hour before becoming excessive (Table 1). At 35°C, staining at all concentrations was not uniform and not appropriate for viability evaluation, as staining uniformity and tone are important for reliable interpretation of the results (Moore, 1985; Bhering et al., 2005). Intense staining may also result in misinterpretation, as shown for Enterolobium contortisiliquum (Vell.) Morong seeds (Nogueira et al., 2014).

The individual assessment of seed viability, with a solution of 0.10% TZ, needs about 20 ml of solution per

seed, totalling 2 L for 100 seeds, corresponding to 2 g of TZ. The assessment after 3 h at 30°C allows running the test in a working day, taking into account the time consumed in seed preparation and staining evaluation with a magnifying glass.

The instructions of ISTA indicate 1.0% TZ at 30°C for forestry species with variation only in the immersion time, which was predominantly 18 h for 90% of the 121 species studied (Leist and Krämer, 2011). The instructions for Seed Testing in Brazil (Brasil, 2009) recommend concentrations between 0.5 and 1.0% TZ, immersion periods from 6 to 24 h at 30°C in average, and according to the species, recommended temperature ranges between 20 and 40°C.

The importance of considering the amount of TZ used and the duration of the test was reported in other studies (França-Neto et al., 1998; Dias and Alves, 2008; Corte et al., 2010), which revealed the possibility of using lower concentration without reducing the quality of the results. A concentration of 0.07% TZ was recommended, e.g., for Enterolobium contortisiliquum (Vell.) Morong (Nogueira et al., 2014), Poecilanthe parviflora B. (Pinto et al., 2008) and Mouriri elliptica Mart. (De Lima et al., 2016). For other species, 0.50% TZ was reported, e.g., for Ceiba speciosa (A.St.-Hil.) Ravenna (Lazarotto et al., 2011) and Acrocomia aculeata (Jacq.) Lodd. ex. Mart. (Ribeiro et al., 2010) and 0.075% for Glycine max (L.) Merril (Zuffo et al., 2015). A concentration equal to this study (0.10%) TZ) was used for seeds of Schizolobium parahyba Vell. Blake (Fogaça et al., 2011) and Peltophorum dubium (Sprengel) Taubert (Oliveira et al., 2005).

The immersion time varied by tree species. Some required a very short time, such as 90 min for



**Figure 2.** Germinability (radicle protrusion and normal seedling) and Viability (TZ staining) in relation to seed moisture of the recalcitrant seeds of *C. guianensis* and *C. surinamensis*.

Poecilanthe parviflora Benth. (Pinto et al., 2008), others a similar time to this study, from 3 to 4 h of immersion, e.g., *Enterolobium contortisiliquum* (Vell.) Morong (Nogueira et al., 2014), *Schizolobium parahyba* Vell. Blake, *Copaifera langsdorffii* Desf. (Fogaça et al., 2011), *Acrocomia aculeata* (Jacq.) Lodd. ex. Mart. (Ribeiro et al., 2010) and *Glycine max* (L.) Merril (Zuffo et al., 2015). For *Ricinus communis* L. a staining period of 6 h is recommended (Oliveira et al., 2014a). However, most require a longer period with the results assessed the next working day (Leist and Krämer, 2011; Abbade and Takaki, 2014).

Different seed qualities of both Carapa species were obtained by a controlled drying of the recalcitrant seeds. A similar approach to obtain different seed qualities were done recently for Acrocomea aculeata (Jacq.) Lood. ex Mart. (Rubio Neto et. al., 2015). Initial moisture content was 47.1% (C. guianensis) and 43.6% (C. surinamensis) and both species showed high percentages of radicle protrusion and normal seedlings (C. guianensis 88.3 and 80.0% and surinamensis 90.0 and 75.0%, С. respectively). Drying for seven days reduced the moisture content to 28.6% (C. guianensis) and 16.1% (C. surinamensis) and also the percentage of radicle protrusion (to 18.3%) and normal seedlings (to 16.7%) in C. guianensis, and caused the death of C. surinamensis seeds (Figure 2).

With the germination results, the viability classes for TZ staining were validated and consolidated for both species (Figures 3 and 4). The staining pattern was similar between the species; however, the staining of *C. guianensis* seed reserves was more homogenous than of *C. surinamensis*. Evaluating strictly the embryonic axis, a uniform colouring in red (2.5R 7/6 and 7/8 in accordance

with Munsell Color Charts, 1977) can be considered as indicating viability for both species, in congruence with this, classes 1, 2 and 3 were defined as viable seeds, while class 4 was considered unviable (Figures 3 and 4).

Topographic staining revealed that the first area to show reduced staining caused by seed desiccation was close to the seed surface at the basal end of the radicle meristem (Viability class 3 in Figures 3 and 4). However, the seeds of class 3 showed radicle protrusion, of 70.0% (*C. guianensis*) and 31.7% (*C. surinamensis*) and developed normal seedlings at 66.7% (*C. guianensis*) and 11.7% (*C. surinamensis*) (Table 2), thus class 3 seeds were still viable, maybe with reduced vigour. Other comparative studies between TZ and germination mentioned the possibility of assessing efficiently viability and seed vigour (Bhering et al., 2005; França Neto et al., 1998; Nakagawa, 1994).

The results obtained by TZ staining showed no significant difference to the germination test for both species; normal seedling development however was more sensitive to drying than radicle protrusion as shown after 3 and 5 days of drying (Table 2). Data analysis, based on the coefficient of the linear correlation between TZ staining and both germination criteria was positive and significant (Table 3). For *C. guianensis*, the correlation between tetrazolium and radicle protrusion was 0.848, and normal seedling development, 0.792. Similar strong correlations were obtained for *C. surinamensis* between TZ staining and radicle protrusion (0.910) and normal seedling development (0.918).

A positive and significant correlation between TZ staining and field emergence or germination was earlier reported for the seeds of soybean (Barros and Marcos
Class 1: Viable seeds with uniform red brilhant colour (2.5R 7/6 and 7/8) of the embryonic axis and the seed reserves, tissues of normal aspect and firm.



Class 2: Viable seeds, red colouration similar to class 1 (2.5R 7/6 and 7/8) however with reduced colour in the centre of the embryonic axis and some spots without colour in the cotyledon mass.



Class 3: Viable seeds, red colouration similar to class 1 (2.5R 7/6 and 7/8) in ca. 50% of the cotyledonal seed reserves and reduced staining in some parts of the embryonic axis; central part of the embryonic axis whitish and radical meristem intense red or yellowish, indication tissue deterioration.



Class 4: Unviable seeds: embryonic axis and cotyledons yellowish (2.5Y 7/6, 7/8 and 7/10) with some red spots; seed reserve tissues with flaccid consistency.



Figure 3. C. guianensis seeds organized according to the staining patterns in four vigour classes with three examples for each class.

Filho, 1997), zucchini (Barros et al., 2005), watermelon (Bhering et al., 2005), leucena (Costa and Santos, 2010), silk floss tree (Lazarotto et al., 2011) and palm heart (Oliveira et al., 2014b).

#### Conclusions

The tetrazolium test can be used to assess seed quality in the recalcitrant seeds of crabwood. The seed axis has to be exposed to the solution with only a part of the seed reserves, the majority of the seed mass can be discarded or used for other measurements, e.g., moisture content. Considering the large seed size, and the time for seed preparation and later staining evaluation, a 0.10% TZ solution with an immersion of 3 h at 30°C was appropriate for both species and the results could be correlated with radicle protrusion and normal seedling development. The desiccation study revealed initial damage near the seed surface close to the radicle meristem. The recommendations for TZ staining of crabwood seeds differed from other tree species in the Class 1: Viable seeds with uniform red brilhant colour (2.5R 7/6 and 7/8) of the embryonic axis and the seed reserves, tissues of normal aspect and firm.



Class 2: Viable seeds, red colouration similar to class 1 (2.5R 7/6 and 7/8) however with reduced colour in the center of the embryonic axis and some spots without colour in the cotyledon mass.



Class 3: Viable seeds, red colouration similar to class 1 (2.5R 7/6 and 7/8) in ca. 50% of the cotyledonal seed reserves and reduced staining in some parts of the embryonic axis; central part of the embryonic axis whitish and radical meristem intense red or yellowish, indication tissue deterioration.



Class 4: Unviable seeds: embryonic axis and cotyledons yellowish (2.5Y 7/6, 7/8 and 7/10) with some red spots; seed reserve tissues with flaccid consistency.



Figure 4. C. surinamensis seeds organized according to the staining patterns in four vigour classes with three examples for each class.

seed preparation, immersion time and temperature, and TZ concentration.

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#### **CONFLICT OF INTERESTS**

The authors have not declared any conflict of interests.

Species	Criterion	0 day	3 days	5 days	7 days
	Radicle	88.3 <sup>a</sup>	85.0 <sup>a</sup>	70.0 <sup>a</sup>	18.3 <sup>a</sup>
	Seedling	80.0 <sup>a</sup>	78.3 <sup>ab</sup>	66.7 <sup>a</sup>	16.7 <sup>a</sup>
	Viability	95.0 <sup>a</sup>	91.7 <sup>a</sup>	58.3 <sup>a</sup>	38.3 <sup>a</sup>
C. guianensis	CV	13.28	6.24	27.72	57.86
Ū	LSD	23.02	10.97	35.60	27.94
	W	0.755	0.010	0.235	0.342
	F	0.195	0.218	0.215	0.188
	Radicle	90.0 <sup>a</sup>	70.0 <sup>a</sup>	31.7 <sup>a</sup>	0.0 <sup>a</sup>
	Seedling	75.0 <sup>a</sup>	51.6 <sup>a</sup>	11.7 <sup>b</sup>	0.0 <sup>a</sup>
	Viability	91.7 <sup>a</sup>	58.3 <sup>a</sup>	18.3 <sup>ab</sup>	15.0 <sup>a</sup>
	CV	6.62	21.76	37.84	96.87
C. sunnamensis	LSD	11.19	25.79	15.36	9.67
	W	0.301	0.856	0.374	0.000
	F	0.194	0.272	0.380	0.028

**Table 2.** Comparison of two germination criteria (radicle protrusion and seedling development) (%) and viability (%) assessed with tetrazolium staining of the recalcitrant seeds of *C. guianensis* and *C. surinamensis* during a controlled drying process of up to 7 days.

Means followed by different letters in the columns differ by Tukey test at 0.05 of significance; W = Shapiro-Wilk test; F = Levene test. Values in bold indicate residues with normal distribution and homogeneous variances to 0.01 significance level. LSD = least significant difference. CV = coefficient of variation

Table 3. Pearson correlation coefficients between the results of radicle protrusion (R), normal seedling	
development (NS) and the tetrazolium staining (TZ) for <i>C. guianensis</i> seeds and <i>C. surinamensis.</i>	

Species	Test	TZ	R	NS
C. guianensis	TZ	1	0.848**	0.792**
	R		1	0.961**
	NS			1
	ΤZ	1	0.910**	0.918**
C. surinamensis	R		1	0.990**
	NS			1

\*\*Correlation is significant at the 0.01 level (2-tailed).

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Full Length Research Paper

## Soil and water conservation, and soil fertility management effects on rain water productivity of maize hybrid in Burkina Faso

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In the evaluation of Soil and Water Conservation (SWC) techniques, little attention is paid to the rain water productivity of crops. The aim of this research work, is to assess the combined effects of SWC practices and soil fertility management on rain water productivity of maize hybrid. On-farm experiments were carried out in the districts of Houndé and Péni both located in the Hauts-Bassins region in Burkina Faso. The treatments were built as association of two SWC technologies combined with three fertilization options. The SWC combinations were: Stone rows and zaï pits (SR+Zaï), Grass strips and zaï pits (GS+Zaï), Earth bunds and contour ploughing (EB+CP). The fertilization options were: 5 t ha<sup>-1</sup> organic fertilizer (OM), OM + 100 kg ha<sup>-1</sup> urea (46% N), OM + 200 kg ha<sup>-1</sup> NPK (14-23-14) + urea. The treatments were laid out in a randomized block design where each farmer constituted a replication. As result, the combination of GS+ Zaï+ OM+ NPK+ Urea gave 26% additional maize grain yield and maize rain water productivity of 4.51 kg ha<sup>-1</sup> mm<sup>-1</sup> in the South-Sudan agro-ecological zone. While the combination of SR+ Zaï+ OM+ NPK+ Urea increased maize yield by 106 % and water productivity of maize was 6.61 kg ha<sup>-1</sup> mm<sup>-1</sup> in the North-Sudan agro-ecological zone. To improve maize yields and water productivity in rainfed agriculture, the use of the combination of soil and water conservation techniques and optimum organic and mineral fertilizer application is recommended.

Key words: Crop, water use, nutrients, maize yield, harvest index, Burkina Faso.

#### INTRODUCTION

The majority of the population in Burkina Faso is smallscale farmers practicing rainfed agriculture. High interannual variability and erratic rainfall distribution result in water-limiting conditions during the cropping season. Soils have also very low fertility, limiting nutrients availability to crops. Food security is then under threat due to the low water availability and increasing soil degradation. The degradation of the soils has a negative

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Author(s) agree that this article remain permanently open access under the terms of the <u>Creative Commons Attribution</u> <u>License 4.0 International License</u> impact on the incomes of the rural populations as agriculture is their sole means of existence.

Many technologies were developed to control the degradation of the natural environment, such as soil and water conservation practices (SWC); sand dunes stopping techniques, zaï pits, earth bunds, stone rows, reforestation, grass strips (Kaboré/Sawadogo et al., 2012; Serme et al., 2015; Zougmoré et al., 2004). Indeed, Zougmoré et al. (2004) have proved the effectiveness of the stone rows and grass strips on the reduction of runoff and erosion control. In addition, these technologies are effective in keeping rain water in the plots to reduce crop water stress. Improved varieties of sorghum, maize, cowpea and millet with shortened cycles and high yields have been developed by agricultural research to cope with the reduction of rainy season length.

The use of SWC techniques help to prevent the loss of farmlands due to water erosion, increase infiltration of the rainwater, trap rich sediments and organic matter carried away by the water overflow. For instance, in the north-Sudan ecological zone of Burkina Faso, grass strips contribute to reduce runoff by 51% and soil erosion by 34% and constitute fodder for the animals and straw for domestic use. It also replaces the stone rows where the stones are unavailable (Kaboré/Sawadogo et al., 2012; Reij et al., 2005). Soil and water conservation techniques were mainly promoted in the dry semi-arid area, while the more humid zone was considered not appropriate for these technologies as the mean annual rainfall exceeds 900 mm. In the current condition of climate uncertainties and general trend of land degradation, refinement and upscaling of SWC are needed for all the agro-ecological zones to increase crop production and food security at the farmers' level. In the evaluation of the impact of SWC techniques, little attention was paid to the rainwater productivity of crops subjected to these technologies. Water productivity is assessed generally in irrigated systems. The aim of this research work, from 2012 to 2014, was to assess the combined effects of SWC practices and soil fertility management on rainwater productivity of maize hybrid. It will contribute to the refinement and adaptation of the SWC practices to the different agro-ecological zones and to food security.

#### MATERIALS AND METHODS

#### Sites description

The experiments were carried out on-farm in the districts of Houndé (11°29'0" N, 3°31'13" W; 328 m a.s.l) and Péni (10°57'0" N, 4°28'60" W; 430 m a.s.l), both located in the Hauts-Bassins region in the west of Burkina Faso (Figure 1).

Houndé belong to the North-Sudan savanna agroecological zone, with a mean annual rainfall, during the last ten years, of 926 mm unimodal distributed between April and October. During the rainy season 2014, when the water productivity evaluation was done, the site of Houndé received 981 mm precipitation distributed on 63 rainy days. Monthly mean maximum and minimum temperatures ranged from 30 to 38°C and from 19 to 26°C,

respectively. The soils include lixisols, poorly evolved erosional soils, and hydromorphic mineral to gleysols overlying material of varied texture (CILSS and OMM, 2001). The main characteristics of the soils used are given in Table 1.

The district of Peni is located in South-Sudan savanna zone, with an annual mean rainfall of 1084 mm, during the ten last years, and unimodal distributed between Mars and October. During the rainy season 2014, the site of Péni received 1488 mm precipitation distributed on 60 rainy days. Monthly mean maximum and minimum temperatures ranged from 30 to 37°C and from 19 to 26°C, respectively. The main soil types are lixisols poorly to fully leached, hydromorphic mineral to gleysols and ferralsols; partly desaturated overlying variable textured material (CILSS and OMM, 2001).

The savanna landscape is often park-like with many big trees including *Faidherbia albida* (known for its reverse phenology bearing leaves during the dry season but shedding leaves with the start of the rains), *Adansonia digitata, Butyrospermum paradoxum* subsp. *parkii, Lannea microcarpa* and *Tamarindus indica*. In the shrubby stratum, combretaceae are well represented. The most regular species are: *Acacia dudgeoni, Acacia gourmaensis, Acacia seyal, Bombax costatum, Combretum micranthum, Combretum glutinosum, Combretum nigricans, Grewia bicolore, Guiera senegalensis* and *Sterculia setigera* (Fontes and Guinko, 1995).

#### Experimental design

The selection of the technologies has been based on the soil and water conservation (SWC) technologies evaluation results from different projects and research institutes (Traore and Adama, 2008). For instance, ridging, look liking earth bund, is common in the western part of Burkina Faso and stones are available in the hilly areas. The grass *Adropogon gayanus* is available in most of the Sudanese zone in West Africa. The SWC technologies used were:

i) Stone rows: They are rows of stones fixed on contour lines with rows spacing 30 - 50 m, depending on slope (Figure 2);

ii) Zaï pit: zaï is a micro basin of 30 to 40 cm diameter for 10 to 15 cm depth, dug in quincunx on lines with 80 cm spacing. The earth from the pit is disposed in the form of a crescent towards the upstream in order to capture the runoff water (Figure 2).

iii) Grass strips: They are biological barriers composed of herbaceous (*Andropogon gayanus* or other grass), set in the fields following the contour lines. The strips are 30 - 50 m spacing depending on the slope (Figure 3).

iv) Earth bunds: They are built on contour lines and have 80 cm wide, 30 cm height and 33 m spacing (Figure 4).

The treatments were built as association of two SWC technologies combined with three fertilization options. Three levels of SWC association were used as follows:

i) Stone rows built on contour lines and zaï pits; SR+Zaï.

ii) Grass strips of *Andropogon gayanus* built on contour lines and zaï pits; GS+Zaï.

iii) Earth bunds built on contour lines and contour ploughing; EB+CP.

The three levels of fertilization options were:

i) Organic fertilizer available at farmers level (mixture of cow dung and crop residues);  $\mathsf{OM}$ 

ii) OM + urea (46% N) iii) OM + NPK (14-23-14) + urea

The experiment design was a randomized block design where each farmer constituted a replication. In each district, 15 farmers hosted



Figure 1. Location of the experimentation sites in Burkina Faso.

Table 1. Soils chemical and	physical characteristics at	Péni and Houndé, 0-20 cm de	pth
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		NI 0/	<b>K</b> <sub>total</sub>	K <sub>avail</sub>	<b>P</b> <sub>total</sub>	PBrayl			0:14 0/	Cond 0/
Sites		N <sub>total</sub> %	(g kg⁻¹)		pnwater	Clay %	SIIT %	Sand %		
Péni	1.38	0.074	0.581	0.139	0.349	0.0089	5.43	16.40	25.90	57.70
Houndé	1.46	0.092	1.459	0.080	0.715	0.0036	6.57	19.10	42.40	38.50

Source: Authors' data; OM = organic matter.

the experiments of 1 ha size per farmer, divided into four treatments. The elementary plot size was  $50 \times 100$  m equivalent to 0.25 ha.

In the district of Houndé the treatments tested were:

i) Farmers' practices (oxen ploughing and application of mineral fertilizer); Control

ii) SR + Zaï + OM (5 t ha<sup>-1</sup>)

iii) SR + Zaï + OM + urea (100 kg ha<sup>-1</sup> in two fractions)

iv) SR + Zaï + OM + NPK (200 kg ha<sup>-1</sup>) + urea

v) GS + Zaï + OM (5 t ha<sup>-1</sup>)

vi) GS + Zaï + OM + urea (100 kg ha<sup>-1</sup> in two fractions)

vii) GS + Zaï + OM + NPK (200 kg ha<sup>-1</sup>) + urea

The combination involving stone rows (SR) in addition to the control plot were conducted in 9 farms and the one involving grass strips were in 6 farms.

In the district of Péni; the following treatments were applied:

i) Farmers' practices (oxen ploughing and application of mineral fertilizer); Control

ii) EB + CP + OM (5 t ha<sup>-1</sup> each 2 years)

iii) EB + CP + OM + urea (100 kg ha<sup>-1</sup> in two fractions)

iv) EB + CP + OM + NPK (200 kg ha<sup>-1</sup>) + urea



Figure 2. Stone row combined with zaï pits.



Figure 4. Earth bund built on contour line.



Figure 3. Grass strip built on contour line.

v) GS + Zaï + OM (5 t ha<sup>-1</sup> each two years) vi) GS + Zaï + OM + urea (100 kg ha<sup>-1</sup> in two fractions) vii) GS + Zaï + OM + NPK (200 kg ha<sup>-1</sup>) + urea

The same proportions of combinations were made in Péni like in Houndé with 9 farmers for EB. The crop variety used (maize Bondofa) was a hybrid variety from INERA research stations. It has white grains and a growing cycle of 95 days to maturity, its potential yield is 7 t ha<sup>-1</sup>. The maize was sown at 40 x 80 cm spacing and thinned at two plants per hill.

#### Maize yields evaluation

For maize grain and straw yields evaluation, two subplots of 5 m x 5 m =  $25 \text{ m}^2$  were taken in each elementary plot, in the way to avoid farm trees effect. The weight of the grain and the straw harvested on these plots were extrapolated to 1 ha to get the different yields.

#### Rain water productivity determination

The rainwater productivity (WP) is defined here, as the amount of grain produced per ha and per mm of rain received from the maize plantation to the harvest (maturity). Water productivity was calculated by dividing grain yield by total rainwater received as follows (Abideen et al., 2014; Kambou et al., 2014; Van Halselma and Vincent, 2012):

WP (kg ha<sup>-1</sup> mm<sup>-1</sup>) = 
$$\frac{Yield (kg ha^{-1})}{Rain applied (mm)}$$

This calculation of WP considered the time between sowing and harvesting, the rain received during this time on each farm, and minimize the runoff and the drainage beyond the maize rooting zone.

#### Grain yield

The grain yield from each plot harvested from the harvestable area was calculated and the yield extrapolated to kg ha<sup>-1</sup> using the formula below:

Grain yield (kg ha<sup>-1</sup>) =  $\frac{10000 \text{ m}^2 \text{ x Grain weight (kg)}}{\text{Harvest area } (\text{m}^2)}$ 

#### Harvest index

The harvest index (HI) calculated as the ratio of the grain weight to the above ground dry matter including the grain and the straw weights.

HI = 
$$\frac{\text{Grain yield (kg }ha^{-1})}{\text{Biomasse + Grain yields (kg }ha^{-1})}$$

#### Laboratory analysis

Soil organic carbon was measured using the Walkley and Black method on composite samples from three sampling points per plot, total organic N by the Kjeldahl method, soil total P was measured

SWC Techniques	Grain Yields (kg ha <sup>-1</sup> )	Straw yields (kg ha <sup>-1</sup> )	Harvest index
GS+Zaï	3065 <sup>ab</sup>	2899	0.49
SR+ <i>Zaï</i>	3768 <sup>a</sup>	2979	0.58
Control (ploughing)	2157 <sup>b</sup>	2357	0.47
LSD	1428	1031	0.11
CV%	31.9	24.9	15.1
F Probability	0.02	0.19	0.11
Significance	S	NS	NS

 Table 2. Effects of soil and water conservation techniques on maize yields at Houndé.

NB: Numbers following by the same letter in a column are not statistically different. NS=Not Significant, CV%= Coefficient of Variation, LSD= *Low significant difference*, S= significant; GS= Grass Strip; SR= Stone Rows.

by acid extraction and soluble P by the Olsen–Dabin method (Baize, 1988). Soil particles size distribution was analyzed using Robinson pipette method on air dried soil sieved on 2 mm mesh and following the procedure described by Mathieu and Pieltain (1998). The pH<sub>water</sub> was measured in a 1:2.5 soil:water suspension, using potentiometric method (Baize, 1988).

#### Data analysis

Statistical analyses, ANOVA was performed using Genstat 12 (General Statistic) software. The comparison of means was done based on the test of Newman Kuehl and the least significant difference with the probability of significance at 5%.

#### RESULTS

## Effects of soil and water conservation technologies on maize straw and grain yields at Houndé and Péni

At Péni there were no significant differences between soil and water conservation technologies (SWC) for maize straw and grain yields (results not shown). The highest grain yield was harvested on the combination GS+Zaï (3733 kg ha<sup>-1</sup>) that gave 6475 kg ha<sup>-1</sup> straw yield. The control plot gave 3317 kg ha<sup>-1</sup> and 6630 kg ha<sup>-1</sup> grain yield and straw yield, respectively.

At Houndé, the maize grain yields were significantly different between SWC practice plots, but not different for the straw yields. The highest maize grain yield was harvested on SR+Zaï technologies plot and the lowest on the control. The treatments SR+Zaï and GS+Zaï gave 75% and 42% grain yield increase, respectively, over the control (Table 2). There were no significant differences between SWC techniques for the harvest index. However there was a trend of its improvement with the combination SR+Zaï as compared to the other technologies (Table 2).

## Treatments effects on maize yields at Péni and Houndé

The maize hybrid, Bondofa variety, yielded on the

average 3645.6 kg ha<sup>-1</sup> at Péni and 3236.9 kg ha<sup>-1</sup> at Houndé. Both at Péni and Houndé there were significant differences between treatments (combination of SWC techniques and fertilization option) for maize grain yields but not for the straw yields (Tables 3 and 4).

At Péni, the treatments GS+ Zaï+ OM+ NPK+ Urea and EB+CP+ OM+NPK+ Urea yielded more than 4 kg ha<sup>-1</sup> maize grain and the other treatments gave less than this value. They produced respectively 26 and 23% additional grain yields over the control. The highest straw yield was recorded on the treatment GS+ Zaï+ OM+ NPK+ Urea (8070 kg ha<sup>-1</sup>) and the lowest on the treatment EB+CP+ OM with 4590 kg ha<sup>-1</sup> (Table 3). There were no significant differences between treatments for the harvest index. The lowest harvest index was recorded in the treatment GS+ Zaï+ OM+ NPK+ Urea with the highest grain and straw yields (Table 3).

At Houndé the highest grain yield was harvested on the treatments SR+ Zaï+ OM+ NPK+ Urea (4457 kg ha<sup>-1</sup>) following by the treatment SR+ Zaï+ OM+ Urea (3824 kg ha<sup>-1</sup>) and the lowest was recorded on the control (2157 kg ha<sup>-1</sup>) (Table 4). The same treatments gave the highest and lowest straw yields. The treatments SR+ Zaï+ OM+ NPK+ Urea yields improvements were 106 % and 55 % over the control, respectively for the grain and straw. The harvest index did not differ significantly between treatments. The treatment SR+ Zaï+ OM+ Urea gave the highest maize harvest index (Table 4).

#### Rain water productivity of maize at Péni and Houndé

Both at Péni and Houndé there were significant differences between treatments for rainwater productivity of maize (Table 5). On the average WP of maize was 3.90 kg ha<sup>-1</sup> mm<sup>-1</sup> at Péni and 4.64 kg ha<sup>-1</sup> mm<sup>-1</sup> at Houndé.

At Péni the treatment GS+ Zaï+ OM+ NPK+ Urea had the highest WP (4.51 kg ha<sup>-1</sup> mm<sup>-1</sup>) of maize followed by GS+ Zaï+ OM+ NPK+ Urea (4.31 kg ha<sup>-1</sup> mm<sup>-1</sup>) and the lowest WP (3.28 kg ha<sup>-1</sup> mm<sup>-1</sup>) was recorded on the treatment EB+CP+ OM (Table 5).

At Houndé the highest WP (6.61 kg ha<sup>-1</sup> mm<sup>-1</sup>) of maize

Treatments	Grain yields (kg ha <sup>-1</sup> )	Straw yields (kg ha <sup>-1</sup> )	Harvest index
GS+ Zaï+ OM	3367 <sup>ab</sup>	6095	0.39
GS+ Zaï+ OM+ NPK+ Urea	4192 <sup>a</sup>	8070	0.35
GS+ <i>Zaï</i> + OM+ Urea	3642 <sup>ab</sup>	5260	0.42
EB+CP+ OM	3017 <sup>b</sup>	4590	0.42
EB+CP+ OM+NPK+ Urea	4067 <sup>a</sup>	5960	0.42
EB+CP+ OM+ Urea	3917 <sup>ab</sup>	5730	0.43
Control (Ploughing+NPK+Urea)	3317 <sup>ab</sup>	6630	0.36
LSD	906	2626	0.07
CV%	11.3	19.0	9.05
F Probability	0.03	0.08	0.07
Significance	S	NS	NS

Table 3. Treatments effects on maize yields at Péni.

NB: Numbers following by the same letter in a column are not statistically different. NS=Not Significant, CV%= Coefficient of Variation, LSD= *Low significant difference*, S= significant; GS= Grass Strip, EB= Earth Bund, CP= Contour Ploughing, OM= Organic Matter; NPK= Nitrogen, Phosphorus, Potassium.

Table 4. Treatments effects on maize yields at Houndé.

Treatments	Grain yields (kg ha <sup>-1</sup> )	Straw yields (kg ha <sup>-1</sup> )	Harvest index
GS+ Zaï+ OM	2232 <sup>b</sup>	2432	0.43
GS+ Zaï+ OM+ NPK+ Urea	3507 <sup>ab</sup>	3132	0.52
GS+ <i>Zaï</i> + OM+ Urea	3457 <sup>ab</sup>	3132	0.51
SR+ <i>Zaï</i> + OM	3024 <sup>ab</sup>	2457	0.54
SR+ Zaï+ OM+ NPK+ Urea	4457 <sup>a</sup>	3657	0.56
SR+ <i>Zaï</i> + OM+ Urea	3824 <sup>a</sup>	2824	0.63
Control (Ploughing+NPK+Urea)	2157 <sup>b</sup>	2357	0.47
LSD	1590	1171	0.14
CV%	27.1	21.9	14.5
F Probability	0.01	0.08	0.16
Significance	S	NS	NS

NB: Numbers following by the same letter in a column are not statistically different. NS=Not Significant, S= significant, CV%= Coefficient of Variation, LSD= *Low significant difference*; GS= Grass Strip, SR= Stone Rows, OM= Organic Matter, NPK= Nitrogen, Phosphorus, Potassium.

was recorded on the treatment SR+ Zaï+ OM+ NPK+ Urea, while the lowest (3.10 kg ha<sup>-1</sup> mm<sup>-1</sup>) was recorded on the control. The improvement of WP by this treatment reached twice the WP of the control (Table 5).

#### DISCUSSION

Maize is a crop sensitive to water stress and to soil fertility, particularly to soil nitrogen content and N supply through fertilization (Hammad et al., 2011; Sarr et al., 2011). Because of water requirement for maize crop, it is grown in the South Sudan agro-ecological zone where the mean annual rainfall is more than 900 mm. Likewise, to meet soil fertility requirement for maize cropping, in unsuitable areas for maize, it is farmed around the

houses in villages to benefit from the domestic wastes. Maize high demand of water and nutrients is well known (Ashraf et al., 2016; Hammad et al., 2011; Mansouri-Far et al., 2010). In 2014 at Péni, soil and water conservation (SWC) practices did not have significant differences for maize grain and straw yields. Whereas at Houndé, there were significant differences between SWC practices for maize grain yields. This can be due to the difference in rainfall characteristics between the two sites. During the year 2014, the site of Houndé received less rainfall than Péni. Water was not a limiting factor at Péni, so that SWC practices did not induced significant improvement in maize yields. Probably this was not the case in Houndé where the combination SR+Zaï and GS+Zaï gave 75 and 42% grain yield increase over the control, respectively. Soil and water conservation techniques increase water

Péni		Houndé			
Treatments	WP (kg ha <sup>-1</sup> mm <sup>-1</sup> )	Treatments	WP (kg ha <sup>-1</sup> mm <sup>-1</sup> )		
GS+ <i>Zaï</i> + OM	3.59 <sup>ab</sup>	GS+ <i>Zaï</i> + OM	3.21 <sup>c</sup>		
GS+ Zaï+ OM+ NPK+ Urea	4.51 <sup>a</sup>	GS+ Zaï+ OM+ NPK+ Urea	4.84 <sup>abc</sup>		
GS+ <i>Zaï</i> + OM+ Urea	3.90 <sup>ab</sup>	GS+ <i>Zaï</i> + OM+ Urea	4.79 <sup>abc</sup>		
EB+CP+ OM	3.28 <sup>b</sup>	SR+ <i>Zaï</i> + OM	4.43 <sup>bc</sup>		
EB+CP+ OM +NPK+ Urea	4.31 <sup>a</sup>	SR+ Zaï+ OM+ NPK+ Urea	6.61 <sup>ª</sup>		
EB+CP+ OM + Urea	4.21 <sup>ab</sup>	SR+ <i>Zaï</i> + OM+ Urea	5.49 <sup>ab</sup>		
Control	3.53 <sup>ab</sup>	Control	3.10 <sup>c</sup>		
LSD	0.997		2.17		
CV%	11.69		26.89		
F Probability	0.04		0.02		
Significance	S		S		

Table 5. Rain water productivity of maize at Péni and Houndé.

NB: Numbers following by the same letter in a column are not statistically different. WP= Water Productivity, S= significant, CV%= Coefficient of Variation, LSD= *Low significant difference*; GS= Grass Strip, EB= Earth Bund, CP= Contour ploughing, SR= Stone Rows, OM= Organic Matter, NPK= Nitrogen, Phosphorus, Potassium.

availability to crop by reducing the runoff, increasing rain water infiltration and stock into the soil. Improvement of water availability increase maize nutrition and thereby the grain and biomass yields. This effect seems limited in the areas with high annual mean rainfall ( $\geq$  1000 mm).

The combination of SWC technologies with organic and mineral fertilization, improved maize grain yields both at Péni and Houndé. At Péni, the treatments GS+ Zaï+ OM+ NPK+ Urea and EB+CP+ OM+NPK+ Urea yielded respectively 26 and 23% additional grain yields over the control. At Houndé the treatments SR+ Zaï+ OM+ NPK+ Urea and GS+ Zaï+ OM+ NPK+ Urea grain yields improvement were 106 and 63% respectively over the control. Yet, the yields improvement due to the combination of SWC practices and soil fertility management was better at Houndé than Péni, maybe because of the difference in water stress severity. This means that the efficacy of SWC practices decrease with the increase in the mean annual rainfall. The highest yields were recorded in the combination where organic matter, NPK and urea fertilizer were applied, probably because of the improvement in water nutrition and nutrients uptakes that lead to more maize tissue formation. This is in phase with the finding supporting that water supply is important for crop production as much as nutrients in semi-arid condition (Mansouri-Far et al., 2010; Hammad et al., 2011). Nutrients availability to crop or uptake may be modified by water supply through irrigation or water harvesting technologies in rainfed agriculture (Erkossa et al., 2011; Serme and Ouattara, 2016) and then, conditions crop performance. Zougmoré et al. (2004) indicated the limited effect of SWC technologies on crop yields without nutrients supply in the condition of Sub-Saharan Africa. The combination of SR+Zaï with the application of organic and mineral fertilizer improved the translocation of water and nutrients to maize grain formation instead of biomass production as expressed in better harvest index. At Péni, the lowest HI obtained on GS+ Zaï+ OM+ NPK+ Urea plot is maybe due to other limiting factors of maize growth and development (Maobe et al., 2010; Ion et al., 2015).

The average WP of maize was 3.90 kg ha<sup>-1</sup> mm<sup>-1</sup> at Péni and 4.64 kg ha<sup>-1</sup> mm<sup>-1</sup> at Houndé. Rainwater productivity of maize seems to decrease with high annual rainfall. Water productivity increase with the reduction of water losses. Thus, the risk of runoff and drainage increases with the amount of water supply and the nonapplication of SWC techniques. Similarly, Samila et al. (2009) found in Egypt, in irrigation condition, that maize water productivity increase gradually as less water volume was applied up to 30% of the required irrigation. At Péni, the combination of GS+ Zaï+ OM+ NPK+ Urea corresponding to GS+ Zaï+ (5 t manure + 74N-46P-28K) ha<sup>1</sup> had the highest WP (4.51 kg ha<sup>1</sup> mm<sup>1</sup>) and at Houndé SR+ Zaï+ OM+ NPK+ Urea corresponding to SR+ Zaï+ (5 t manure + 74N-46P-28K) ha<sup>-1</sup> had the best WP (6.61 kg ha<sup>-1</sup> mm<sup>-1</sup>). Water management in combination with nutrients supply improved maize water productivity. The water productivity improvement was 28 and 113% over the control at Péni and Houndé respectively, indicating the effect of the rainfall amount on WP. In Ethiopia on nitisols and with mean annual rainfall of 1451 mm, Erkossa et al. (2011) obtained, in the same range of our results, maize grain water productivity increase of 48 and 54%, with near optimal and nonlimiting soil fertility, respectively. Improvement of water productivity of maize depends on water supply, soil fertility management and also on the potential of the variety used. The maize water productivity, in our study, was low as compared to the water productivity of 8.2 and 8.2 kg ha<sup>-1</sup> mm<sup>-1</sup> of traditional and hybrid maize varieties, respectively, obtained in irrigated conditions by Abideen

et al. (2014) on station, in Pakistan with the application of 160N-80P-0K kg ha<sup>-1</sup> fertilizer. There is room to improve maize productivity, in semi-arid Africa, with higher productive variety and optimizing water and fertilizers use.

#### Conclusion

Soil and water conservation practices (SWC) had limited effect on maize yields in South-Sudan savanna zone where the mean annual rainfall exceed 1000 mm. But in the agro-ecological zone where the mean annual rainfall is less than 950 mm, these practices improved maize grain yields up to 75% over the control (oxen ploughing). The combination of GS+ Zaï+ OM+ NPK+ Urea corresponding to GS+ Zaï+ (5 t manure + 74N-46P-28K) ha<sup>-1</sup> that gave 26% additional maize grain yield and 28% improvement of maize water productivity is adapted to the South- Sudan agro-ecological zone. While the combination of SR+ Zaï+ OM+ NPK+ Urea corresponding to SR+ Zaï+ (5 t manure + 74N-46P-28K) ha<sup>-1</sup> that increased maize yield by 106% and water productivity of maize by 113% is the better practice for the North-Sudan agro-ecological zone. To improve maize yields and water productivity in rainfed agriculture in the semi-arid zone of Africa, the use of the combination GS+ Zaï+ (5 t manure + 74N-46P-28K) ha<sup>-1</sup> and SR+ Zaï+ (5 t manure + 74N-46P-28K) is recommended.

#### CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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African Journal of Agricultural Research

Full Length Research Paper

## Initial growth of African mahogany plants in response to zinc fertilization

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African mahogany (*Khaya senegalensis* A. Juss) has been an important option to supply the increasing demand for noble forest products for the Brazilian furniture industry. In the present study, the objective was to evaluate the initial development of mahogany plants in response to different doses of zinc. The experiment was carried out inside a greenhouse, in the experimental area of the State University of Goiás, Ipameri Campus, in 7 dm<sup>3</sup> plastic containers, and using soil samples, identified as Dystrophic Red-Yellow Latosol (Oxisol), as substrate. The experimental design was completely randomized, with five treatments and five replications, totaling 25 experimental units. The treatments consisted of five Zn concentrations: 0, 2.5, 5, 10 and 20 mg dm<sup>-3</sup>, obtained by zinc sulfate fertilizer. At 150 days, the measurements of the plant height, the root crown diameter, dry matter mass of leaves, stem, root and total were evaluated. The tested increasing zinc doses did not promote increased plant growth. African mahogany presented low zinc requirement in the early stages of development for the studied edaphoclimatic condition, and no application of zinc via fertilizers was required. This fact may resulting from the natural concentration of 0.2 mg dm<sup>-3</sup> of Zn in the used soil, which was enough to supply the nutritional demand of the plants in the first five months of development.

Key words: Khaya senegalensis A. Juss, morphology, micronutrient.

#### INTRODUTION

Planted forests have great economic, social and environmental importance for the country, contributing

significantly to the tax generation, jobs, products and well-being; besides directly favoring the conservation and

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Author(s) agree that this article remain permanently open access under the terms of the <u>Creative Commons Attribution</u> <u>License 4.0 International License</u> preservation of natural resources (Ciriello et al., 2014). Wood from reforestation is a promising future option, and this practice is growing. Among the used species, African mahogany is a great option to supply the growing demand of noble forest products for the furniture industry (Pinheiro et al., 2011).

In Brazil, African mahogany (*Khaya senegalensis* A. Juss) stands out among exotic timber species due to its good development in areas of predominantly humid tropical climate, besides its ability for adapting to subtropical climate regions. For this reason, among noble wood species, it is the exotic tree presenting the best growth in the Cerrado, so the reforestation companies are interested in this species. In origin areas, African mahogany occurs over a wide range of climate, relief, ecological, and edaphic conditions (Danquah et al., 2011).

In order to reach the plant maximum productive potential, a correct supply of nutrients is necessary. In addition to macronutrients (nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, carbon, oxygen, and hydrogen), it is also necessary to consider micronutrients which, although required in small amounts, they are fundamental to the performance of main cellular metabolic functions (Corcioli et al., 2016). Thus, the adequate supply of these nutrients is a key point for plant growth and development, including for forest species.

Among the micronutrients required for plants, zinc along with boron are the most deficient in Brazilian soils (Malavolta, 1980). The most important contribution of zinc in the metabolic processes of plants is participating in the composition of many enzymes, such as proteases, proteinases dehvdrogenases. and phosphohydrogenases. Symptoms of zinc deficiency are the most frequently observed, due to its low concentrations in the lithosphere. Its content in the soil is, on average, is lower than in the rocks, occurring in minerals, adsorbed or forming soluble and insoluble organic complexes. From the total zinc amount in the soil. only a small part is available, such availability is influenced by factors such as its content, pH, and soil organic matter content (Malavolta et al., 1997).

Although, African mahogany has stood out as a good choice in timber production, there remains the necessity of researches related to the species fertilizing. There are few studies indicating their nutritional needs, aiming at production of good quality seedlings, and consequently obtaining greater survival when submitted in the field. Some studies demonstrated nutrient deficiency symptoms and positive responses to fertilizing on African mahogany growth (Vieira et al., 2014; Perez et al., 2016; Smiderle, 2016; Vasconcelos, 2016). However, these results need to be detailed by local surveys for determining the critical levels of nutrients in the soil and the appropriate doses of fertilizers to ensure the productivity of commercial plantings of the species (Nikles et al., 2008). Therefore, the objective of the

present study was to evaluate the initial growth of African mahogany grown under greenhouse conditions, in response to different doses of zinc.

#### MATERIALS AND METHODS

The experiment was conducted in the greenhouse, of the experimental area of the State University of Goiás, Ipameri Campus (17°43'19"S; 48°09'35"W; 764 m height). Ipameri municipality is located in the southeastern region of the Goiás State, where the climate is classified as Aw (Tropical seasonal), and it is characterized by two well-defined seasons, one drought winter and one rainy summer. Annual precipitation is approximately 1600 mm, and the average temperature is about 23°C (Alvares et al., 2013). Inside the greenhouse, the average temperature was of 27°C.

The used substrate was an agricultural soil classified as a dystrophic Red-Yellow Latosol (Oxisol), collected in the subsurface layer (0.20 to 0.40 m). Physicochemical analysis presented the following initial values for the substrate: 300, 80.0 and 620.0 mg dm<sup>-3</sup> of clay, silt and sand, pH (CaCl<sub>2</sub>) = 5.1; H + Al = 2.2 cmol<sub>c</sub> dm<sup>-3</sup>; Ca = 0.8 cmol<sub>c</sub> dm<sup>-3</sup>; Mg = 0.3 cmol<sub>c</sub> dm<sup>-3</sup>; P (melich) = 1.2 mg dm<sup>-3</sup>; K= 0.04 cmol<sub>c</sub> dm<sup>-3</sup>; organic matter = 9.0 g dm<sup>-3</sup>; CTC = 3.36 cmol<sub>c</sub> dm<sup>-3</sup>; V% = 34.57; Cu = 1.9 mg dm<sup>-3</sup>, Fe = 43.9 mg dm<sup>-3</sup>, Mn = 3.4 mg dm<sup>-3</sup>, Zn = 0.2 mg dm<sup>-3</sup> and B= 0.19 mg dm<sup>-3</sup>, respectively.

The used species was *K. senegalensis* A. Juss, whose seedlings were produced from imported African seeds. Germination occurred in tubes of 53 cm<sup>3</sup> capacity, where the seedlings remained until they reached a mean height of 15 cm and a crown root diameter of 8 mm, then they were transplanted to the vessels. During this period, they received no fertilizing.

The experimental design was completely randomized, with five treatments and five replications, totaling 25 experimental units. The treatments consisted of five Zn concentrations: 0, 2.5, 5, 10 and 20 mg dm<sup>-3</sup>, obtained by zinc sulfate fertilizer.

Based on the physical-chemical analysis of the used soil, all treatments received liming to raise the soil base saturation of 60% and fertilization with nitrogen (80 mg dm<sup>3</sup>), phosphor (150 mg dm<sup>3</sup>), potassium (80 mg dm<sup>3</sup>), and micronutrients: manganese (1.5 mg dm<sup>3</sup>), boron (0.5 mg dm<sup>3</sup>), copper (0.5 mg dm<sup>3</sup>) and molybdenum (0.1 mg dm<sup>3</sup>) (Ciriello et al., 2014). The sources used for liming and fertilization were PRNT 92 dolomitic limestone, urea, triple superphosphate, potassium chloride, manganese sulfate, boric acid, copper sulfate and sodium molybdate, respectively. The doses of the established nutrients were applied individually in each vessel.

When the seedlings reached 120 days of age, in October 2014, they were transplanted to opaque black polyethylene vessels, with perforations at the bottom, and filled with 7dm<sup>3</sup> of soil. Soil moisture was maintained throughout the experimental period in approximately 60% of the maximum water retention capacity of the soil. The volume of evapotranspirated water was replenished daily by weighing the vessels.

After 150 days, the growth variables were assessed (height of the plants and root crown diameter) by measuring the height of the plants, from the root crown to the apex of the plants, using a graduated ruler, and root crown diameter, using a digital caliper and counting leaflets number. Then the plants were separated into leaves, stem and roots for the determination of the dry mass matter. The plant parts were washed with distilled water, and then they were placed into a forced air circulation oven for 72 h at 70°C until the obtention of a constant mass. After drying, they were weighed in an analytical scales, with a precision of 0.01 g, to determine the dry matter mass was calculated.

After compliance verification with the assumptions of homogeneity of variances and normality, data were submitted to



**Figure 1.** Results of regression analysis for plant height (A), root crown diameter (B), and the number of leaflets (C) of African mahogany plants fertilized with different doses of zinc. <sup>ns</sup>not significant (p > 0.05), \*significant ( $0.01 ) and **significant(<math>p \le 0.01$ ).

analysis of variance and regression, with  $\alpha = 0.05$ , using SISVAR 5.4 (Ferreira, 2011).

#### **RESULTS AND DISCUSSION**

Increasing doses of zinc did not increase growth in height or root crown diameter, since there was no significant regression adjustment for these variables (Figure 1A and B). The plants that did not receive supplementation with Zn presented no differences when compared with the plants supplied with the maximum Zn dose (20 mg dm $^{-3}$ ). However, plants subjected to the maximum dose (20 mg dm<sup>-3</sup>) showed no symptoms of toxicity, and Zn excess also did not affect plant growth. It is important to stress that, although Zn is an essential micronutrient, this element can affect the normal growth and metabolism of plant species when present in toxic levels in the environment (Marschner, 1995). The levels considered toxic to the eucalyptus (E. maculata) crop for example, were found using a concentration of 1200 mM Zn (Soares et al., 2001).

This result may be related to the low requirement of the micronutrient by the African mahogany in its initial phase of development. A similar result was observed by Corcioli et al. (2016) in another African mahogany species (*Khaya ivorensis* A. Chev) where the omission of zinc in fertilization did not influence the plant growth in the early stages. Evaluating the growth of Australian cedar under boron and zinc supplementation, Carmo et al. (2010) concluded that Zn doses did not influence plant height and diameter during the 3-month experimental period. Additionally, Mendonça et al. (1999) demonstrated that seedlings of aroeira-do-sertão (*Myracrodruon urundeuva* Fr. All) cultivated in soil with a concentration of 0.7 mg de Zn dm<sup>-3</sup> without supplementation with Zn, did not have their growth affected.

Leaflet production was not significantly affected by the application of Zn (Figure 1C). The plants submitted to the dose 0 mg dm<sup>-3</sup> of Zn had an average production of 100 leaflets and a standard deviation of 29.6, while the plants submitted to the maximum dose (20 mg dm<sup>-3</sup>) presented a statistically similar leaflet production, corresponding to 139 leaflets and standard deviation of 39.7.



**Figure 2.** Results of the regression analysis for leaf dry matter mass (a), stem dry matter mass (b), root dry matter mass (c), total dry matter mass (d) of African mahogany plants fertilized with different doses of zinc. <sup>ns</sup>not significant ( $p \le 0.01$ ), \*significant (0.01< $p \le 0.05$ ) and \*\*significant ( $p \le 0.01$ ).

For the plant dry mass, none of the evaluated parts or the sum of them (leaves, stem, root and total) presented response to the different zinc doses. As with the other analyzed variables, there was no significant regression adjustment (Figure 2A, B, C and D). Evaluating the initial growth of pink cedar, Locatelli et al. (2007) observed that the Zn was the micronutrient that less limited the production of dry matter mass.

Other works concerning the production of eucalyptus seedlings have shown that there is no need for generalized application of Zn (Barros et al., 1985; Couto et al., 1985; Sgarbiet al., 1999); in some cases, even this element has brought to growth detriment of these plants when subjected to soils with high zinc content and or micronutrient high doses (Soares et al., 2001).

It is also important to emphasize that the zinc content found in the substrate 0.2 mg dm<sup>-3</sup> is considered low for the initial development of the plant (<0.7 mg dm<sup>-3</sup>), soil zinc content may have been enough for the initial development of African mahogany plants. Although, the zinc is considered an essential nutrient for the development of plants, it is required only in small quantities (Hooda, 2010) since the plants submitted to the dose 0 mg dm<sup>-3</sup> of Zn presented the same growth rates in all analyzed variables. However, according to Raij et al. (1996), the zinc content found in the substrate 0.2 mg dm<sup>-3</sup> is considered low for the initial development of the plant (<0.7 mg dm<sup>-3</sup>). In this case, monitoring the species development in larger experimental periods, and even when submitted in the field, would be possible to show plants eventually presenting symptoms of Zn absence.

These data corroborate the results of the present study, which did not demonstrate the need to correct the Zn contents for the initial development of the plants (up to five months), similar to that observed in the studies conducted by Camargos et al. (2002), with nutrient solution for Brazil nut, showing that the omission of zinc was not limiting to the growth of the plants.

Likewise, Silva et al. (2007), evaluated the micronutrient

effect on the growth of mahogany seedlings (*Swietenia macrophilla* King) which concluded that zinc omissions did not affect the growth characteristics of plants grown in a soil with concentration of 0.6 mg and Zn dm<sup>-3</sup>. This fact could be explained by assuming that the available zinc levels in the soil have been mobilized for all the organs of the plant, satisfying their needs, as in the present work.

African mahogany plants subjected to the 0 mg dm<sup>-3</sup> dose of zinc did not present commonly observed deficiency symptoms such as shortening of internodes, size reduction and narrowing of new leaves, becoming lanceolate and chlorotic (Parker et al., 1992). Zinc deficiency symptoms are associated with disorders of auxin metabolism, mainly indolacetic acid (IAA), the phytormonium responsible for plant growth, a condition that was not observed in the present study. Therefore, the obtained results in the present study shows that the Zn available in the soil (0.2 mg dm<sup>-3</sup>) was enough for the development of the African mahogany plants in the initial phase, and no additional fertilization of this nutrient is required.

#### Conclusions

The African mahogany (*K. senegalensis* A. Juss) presented low zinc requirement in the development early stages, for the edaphoclimatic condition studied, and the application of zinc via fertilizers was not necessary when the used soil had contents of 0.2 mg dm<sup>-3</sup> Zn. The African mahogany (*K. senegalensis* A. Juss) plants are tolerant to the application of zinc up to the 20 mg dm<sup>-3</sup>.

#### **CONFLICT OF INTERESTS**

The authors have not declared any conflict of interests.

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African Journal of Agricultural Research

Full Length Research Paper

## Harvest moisture content affecting yield and quality of wheat grains in Brazil

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The wheat quality and yield may be limited or compromised by several factors in the field, being the climatic main factor at the end of the crop cycle. The aim of the study was to determine whether different harvest times affect crop yield and wheat grain quality; four experiments were conducted, differing by cultivars type and sowing time, at Ponta Grossa city, PR, Brazil. The experimental design used for both cultivars (BRS-Pardela and Quartzo) were randomized complete block with five grain moisture content at harvest time (30, 25, 20, 15 and 13%) being 4 replications. For the first sowing time, the different harvesting times had no effect in yield, falling number (FN) or hectoliter weight (HW) for both cultivars. In the second sowing time, the yield components and yield for both cultivars were not affected by the different harvest times. A linear and quadratic decrease on the pH with the delaying of harvest time was noticed for BRS-Pardela and Quartzo, respectively, for the FN only the BRS-Pardela showed quadratic reduction delaying the harvest time.

Key words: Triticum aestivum, no-tillage, harvest time, industrial quality.

#### INTRODUCTION

Wheat crop (*Triticum aestivum* L.) is important cereal crop because it is increasing the demand of the population for these cereal products, making it important to the economies (Seghezzo and Cuniberti, 2010; USDA, 2011). As a result, there are research institutions in various regions with breeding programs and management aiming higher yield. In addition to yield, wheat grain must also possess desired technological quality by the industry, thus avoiding the use of additives for reasons of cost and food security (Franceschi, 2009).

Yield and quality of wheat grains can be defined as a

result of interaction between what the culture suffers in the field, the effect of ground conditions, the management of culture, cultivate, harvest and weather conditions (Edwards, 2004; Shewry et al., 2003). The weather has a profound impact on production and helps explain why different parts of the world produce wheat with better quality (Bozzini, 1988). In Southern Brazil, the main pr oblems are the high moisture content of the air in September and October, the high temperatures in the grain filling stage, the possibility of hail and frosts in silking and rains at harvest (Franceschi, 2009; Noda et

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#### al., 1994).

The high rainfall after wheat physiological maturity influences negatively the hectolitre weight, thousand grain weight, number of fall, the P/L and grain yield due to increased severity of disease and enzyme activity (Guarienti et al., 2003, 2005).

The grain moisture content at harvest is directly related to the resistance of grain to mechanical damage and the humidity range from 13 to 15% is considered ideal for the harvest, depending on the cultivar. The late harvest, after physiological maturity contributes to undesirable changes in the technological quality of wheat (Carneiro et al., 2005). One option to improve grain quality is an early harvest. To this end, the producer must take into account the need and availability of drying, the risk of deterioration and energy spent on drying (Carneiro et al., 2005; Embrapa, 2010; Quick and Buchele, 1978).

The early harvesting process after physiological maturation can be an alternative to obtain a product with appropriate industrial quality, when there are high levels of starch, protein and water, preventing the grain to be exposed longer to diseases such as fungi producing mycotoxin (Carneiro et al., 2005).

The early harvesting can affect the number of fall (Falling Number) and hectolitre weight, among other characteristics of wheat grain, being necessary to assess whether the moisture content at harvest interfere in these characteristics of grain.

#### MATERIALS AND METHODS

Four experiments were conducted in the city of Ponta Grossa – PR, Brazil. The experimental design used in the four experiments, which differ by sowing date and cultivar was randomized blocks, with 5 moisture content at harvest (30, 25, 20, 15 and 13%) and 4 repetitions. In all experiments, the plots showed 8.0 m long and 6.0 m wide and useful production area of  $6.0 \times 4.0$  m. The moisture content at harvest was obtained within the range of physiological maturity (30%) and the moisture content for wheat storage (13%). The cultivars used were Quartzo and BRS-Pardela.

The harvest dates for each treatment, for both varieties are shown in Table 1. Wheat was grown at no tillage system with soybean as the previous crop. The first sowing was done on June 11th 2013 and the second was done on July 3th of the same year. As fertilization, 300 kg ha<sup>-1</sup> of 14-34-00 was carried out at the sowing time. At the beginning of the tilling stage, 40.5 kg ha<sup>-1</sup> of nitrogen and 60 kg ha<sup>-1</sup> of K<sub>2</sub>O was applied as coverage fertilization. The seeding rate used in two seasons and in both cultivars was 75 viable seeds per meter, 0.17 m spacing between rows and average depth of seeding 0.04 m.

When the grains have reached the mature stage (Table 1), hand sickles were used to harvest the plants, and one meter of plant row was sampled for the assessment of yield components. The meter of plant row per plot was evaluated on the number of ears; the number of spikelets per spike, grain per spikelet and also the weight of a thousand grains. The yield was determined by the useful production area of each plot and its moisture was corrected to 13% being the value converted into kilograms per hectare.

Of harvested wheat, grain was also evaluated with industrial quality: hectolitre weight (HW) and falling number (FN). The HW was measured in the Laboratory of Plant Science at the State University of Ponta Grossa through the DALLEMOLLE balances

and the FN in CONAB company by the apparatus of brand Perten model FN 1700.

Data were subjected to analysis of variance by F test and the differences among the averages of the different moisture contents were analyzed by polynomial regression.

Correlation analysis was performed to verify a possible relationship between the meteorological variables and industrial quality variables. For this, they were calculated in average maximum and minimum temperature and precipitation for harvest period, the sum of rainfall, the amount of days of rainfall and days when the plant was in the field after physiological maturity. For the interpretation of correlation coefficients (r), the table proposed by Shikamura (2014) was used.

#### **RESULTS AND DISCUSSION**

The moisture content at harvest did not affect the weight of a thousand grains (WTG) and yield of both cultivars in the two sowing dates (Table 1), as the harvest seasons were very close, with no weather changes (rainfall and temperature) substantially between treatments (Figure 1). This indicated that probably are needed sharp changes in temperature and rainfall to affect WTG.

Guarienti et al. (2003) observed reduced WTG and yield due to high temperature (above 35 °C) in the grain filling period. They also indicated that the grain fillers stage there is influence of temperature, precipitation and solar radiation in WTG and grain yield, but the harvest time in this work were carried out after physiological maturity, that is, low temperature variation between treatments in the grain filling period and thus the WTG and yield were not affected.

The hectolitre weight (HW) was not influenced by harvest times in both cultivars in the first sowing time and the second sowing time the cultivars showed different results for HW (Table 1). To BRS-Pardela the answer was negative linear, with lower HW with the delayed harvest, i.e. for crops with lower moisture contents. To Quartzo response was quadratic, with maximum point in humidity of 22% where the HW was 78.1 and then decrease as the crop was more time in the field, confirming the downward trend HW with delayed harvest.

According to Hirano (1976), there is a deterioration mechanism of wheat industrial quality due to rainfall which occurred over periods of more than 20 days before harvest. Who also argued that due to the rain the grains start to get chochos and the HW decreases. Guarienti et al. (2003), also showed the negative influence of rainfall on the HW when precipitation occurs in periods of more than twenty days prior to harvest.

Comparing the results of the second sowing with wheat quality table (IAPAR, 2013), BRS-Pardela in harvest times with 30%, 25% and 20% humidity would be classified as wheat type 2, HW value between 75-78 and crops with 15% and 13% moisture content wheat classified as type 3, HW value between 70-75. To cultivate Quartz the time of 30% wheat crop had type 2, the harvest with 25% and 20% had type 1 wheat and the harvest with 15% and 13% moisture content wheat was

Malatuma kamunat	First time	sowing	Second tin	ne sowing
Moisture narvest	BRS-Pardela	Quartzo	BRS-Pardela	Quartzo
		Thousand	grain weight (g)	
13	37.84	37.38	40.5	39.4
15	36.46	39.09	40.4	40.6
20	36.52	38.62	39.9	38.9
25	35.93	40.00	38.5	38.4
30	34.53	37.74	38.8	39.3
Regression	NS	NS	NS	NS
		Yiel	ld (kg/ha)	
13	2.022	2.752	1.549	2.194
15	1.948	2.822	1.699	2.016
20	1.936	2.677	1.647	2.107
25	2.355	2.800	1.152	2.002
30	2.284	2.934	1.413	2.104
Regression	NS	NS	NS	NS
		Hecto	litre weight	
13	77.04	76.91	77.62	77.06
15	78.79	78.51	77.63	78.47
20	77.39	76.57	75.98	78.80
25	79.53	77.46	74.01	76.16
30	75.95	77.23	73.86	75.27
Regression	NS	NS	L	Q
		Fallir	ng number	
13	313.3	337.2	269.5	367.2
15	296.5	318.6	232.0	369.0
20	290.0	346.7	288.0	355.2
25	294.5	348.5	325.5	367.0
30	322.7	373.5	187.0	308.2
Regression	NS	NS	Q	NS

**Table 1.** Thousand Grain Weight (TGW), Yield, Hectoliter Weight (HW) and Falling Number (FN) to the wheat cultivars BRS-Pardela and Quartzo in two sowing dates at different levels of moisture harvest (Ponta Grossa, PR, 2013).

NS = Not significant; Q = Quadratic; L = Linear; Hectolitre weight: BRS-Pardela: Y= 71.37 + 0.223x (R<sup>2</sup>= 90.83); Quartzo: Y=  $67.23 + 0.98x - 0.022x^2$  (R<sup>2</sup>=84.12); Falling Number: BRS-Pardela: Y=  $-330.44 + 53.27x - 1.103x^2$  (R<sup>2</sup>=95.79).

type 3, with the clear advantage the anticipated harvest to prevent the reduction of HW in both cultivars.

The correlation between HW and days which precipitation occurred was performed where the correlation was very weak, but negative, confirming that rainfall before harvesting reduce the HW of wheat. However, as in this work the weather conditions were little rain before the harvest, the HW was not affected by harvest time.

According Guarienti et al. (2003, 2005), precipitation, relative humidity and the water surplus negatively influence the HW, the WTG, the Falling Number (FN) and the grain yield. The decrease in HW can be attributed to the successive changes in grain moisture due to rainfall

(Mellado et al.,1985), suggesting that it is more damaging to the quality of wheat when there are several days of rain during the crop maturation period than just a rainy day with a high volume of water.

Finney and Yamazaki (1967) observed that the intermittent wetting and drying the wheat grains reduce the WH, as a result of decreasing grain density. The wheat grain density may decrease because the grain have started the twinning process, may cause decreased yield, WTG and HW due to the high rate of respiration, which consumes the accumulated carbohydrates in grains (Bhat et al., 1981; Fleurat-Lessard, 2002; Bhattacharya and Raha, 2002).

These theories can explain the results of this study, in



Figure 1. Temperature Analysis, maximum and minimum, and rainfall in the municipality of Ponta Grossa, PR. Brazil, 2013 (Source: IAPAR).

which the HW values showed strong negative correlations on BRS-Pardela and moderate negative to Quartzo, with the sum of rainfall, the number of days the sum they occurred and precipitation summation days when plants were in the field. These results demonstrated that the longer the crop is in the field after physiological maturity, may be prone to adverse weather conditions, reducing the industrial quality of wheat grain. To minimize these losses quality is recommended anticipate the harvest.

The different moisture content at harvest did not affect the FN of both cultivars in the first sowing. In the second sowing date there were no significant differences between the moisture content at harvest to cultivate Quartz and for BRS-Pardela the response was quadratic with maximum point with 24% grain moisture (Table 1). This difference between the cultivars may be related to susceptibility to germination in the ear. The cultivar Quartz is moderately resistant to germination in the ear (Sementes, 2014) and despite the BRS-Pardela be moderately susceptible (Embrapa, 2014), both cultivars showed high FN, an answer that may be related to climate, without precipitation excesses before the point of harvest, which comprises the harvest times that composed the treatments.

Barnard and Smith (2012) conducted a study with eleven cultivars to determine the influence of climate on

FN and observed that the rains during the latter stages of the filling stage and grain maturity showed negative effect on the FN in seven of the eleven cultivars, while that high temperatures in these periods were positively correlated with the FN in eight of the eleven cultivars, suggesting that higher maximum temperatures after physiological maturity can lead to higher values of FN.

To BRS-Pardela in the first sowing the correlation between the FN and minimum temperatures was weak but negative and the total days the plants were in the field was moderate negative, indicating that there is a downward trend when FN culture is for longer exposed to climate variations. Correlations were moderate negative to the precipitation, for the amount of days that occurred rainfall and the days when the treatments were in the field, i.e. the longer the culture is exposed to bad weather, are more damaging to the FN. In the second sowing time for BRS-Pardela, the minimum temperature, the sum of rainfall and rainy days summation may have affected the FN because there was a negative correlation of these factors with the FN. To Quartzo that was not observed.

These results showed that the FN decreases due to rainfall after their physiological maturity and the longer the period in which the crop in the field is, the lower the NF. Barnard and Smith (2012) observed similar results regarding correlations where frequent rainfall immediately after physiological maturity were more important in determining FN values lower than a large amount of rain in a single period.

According Guarienti et al. (2004) in field germination before the harvest is induced when grains absorb water at low temperature and also the soaking water at low temperature promotes dormancy breaking and germination results in pre-harvest, reducing the FN due activation of the synthesis of alpha-amylase enzyme (Indrani and Rao, 2007).

The results of this study showed that the harvest season exerted very marked influence on the quality of wheat grain, but the correlations with climate variables indicate that weather conditions affect the industrial quality but are necessary temperature variations and frequent rainfall to decrease values TGW, HW and FN. THG, HW and FN are negatively influenced by high temperatures that occur during the grain filling stage, but higher temperatures favor physiological maturity after these characteristics due to rapid drying of the grains.

Frequent rainfall, or several days after the rains with physiological maturity are more detrimental to the TGW, the HW and the FN to high rainfall in fewer days, as a result of intermittent wetting and drying reduces the density of grains and activate the enzyme alpha-amylase, which reduces NF. To minimize these effects, the anticipation of the harvest becomes an interesting option because it reduces the time that culture is exposed to bad weather, but it is necessary to evaluate its feasibility due to the need for drying grain, or even the use of desiccants.

Both wheat sowing dates were within the recommended by climatic zoning for the region, but noted that for the sowing of the second season yield was lower (27% on the average of treatments), and this can be attributed to tillering phase, where for the first time, the average temperature was lower compared with the second time, resulting in increased number of tillers and larger number of ears per meter, which may have resulted in greater yield.

In the second, sowing the FN was lower (14.2% on average) compared to the first time to BRS-Pardela, which can be attributed to more frequent rainy days during the harvest second season. To Quartzo the difference was a little, so to the FN, the cultivars show a difference response, due to BRS-Pardela have greater susceptibility to germination in Quartzo spike and cultivating be moderately resistant to resistant to this characteristic.

#### Conclusions

Frequent rainfall after their physiological maturity is more detrimental to the THG, the HW and the FN to high rainfall in fewer days. The larger is the period that the culture is in the field exposed to climate variations, is more damaging to industrial grain quality and to minimize these effects, the anticipation of the harvest becomes an interesting option, it does not affect yield since is performed after physiological maturity.

#### **CONFLICTS OF INTERESTS**

The authors have not declared any conflict of interests.

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Full Length Research Paper

# Germination and vigor of *Macrotyloma axillare* cv Java seeds under different methods for overcoming dormancy

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The aim of this study was to evaluate the germination and vigor of seeds *Macrotyloma axillare* cv Java (archer) under different methods to overcome dormancy. The seeds were submitted to the following treatments to overcome dormancy: (a) control, (b) manual scarification with sandpaper, (c) immersion in sulfuric acid 98% for five minutes, (d) immersion in sodium hypochlorite solution 2% for 15 min. The experimental design was completely randomized with four treatments and four replications with 50 seeds per replication. The sandpaper method was the one that provided the highest percentage of germination (54.5) and speed of germination index (4.56) and did not differ (p = 0.17) from immersion in hypochlorite for 15 min (4.18). The scarification with sandpaper presented higher seedlings length (8.37 cm), while immersion in sulfuric acid and in sodium hypochlorite (5.66 and 5.15 cm) was not statistically different. Therefore, the manual scarification with sandpaper, which promoted the highest percentage of germination, speed of germination index and seedlings length, can be recommended as the best method to overcome dormancy of archer seeds.

Key words: Archer, Fabaceae, seed quality physiology.

#### INTRODUCTION

The *Macrotyloma axillare* (E. Meyer) Verdc. (Archer) is a perennial legume, that has voluble growth and average to low demand of soil fertility, high grazing tolerance, and good ability to consorting with *Brachiaria* and *Panicun* 

grasses (Paiva et al., 2008). However, the establishment phase is one difficulty in its implantation in the pasture, which may be linked to many factors, such as the low germination percentage of its seeds. Despite all the

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Author(s) agree that this article remain permanently open access under the terms of the <u>Creative Commons Attribution</u> <u>License 4.0 International License</u> benefits such as improvements in soil chemistry and nutritional quality of forage intercropped, the difficulty of implementation of legumes in pastures established by grasses is a determinant of low utilization of grasslegume consortium (Almeida et al., 2015).

Some legume seeds, even though in favorable environmental conditions, usually do not germinate due to impermeable seed coat to water, which characterizes the seeds as hard or with integumentary dormancy. Thus, the study of methods to overcome dormancy in macrotiloma seeds is essential, because it diminishes the costs for pasture implementation. Moreover, it is important to identify the vigor of the lots with greater or lesser probability of presenting better performance in the field or during storage, so that, vigor tests become important tools, as auxiliary instrument to germination test in research on physiological seed quality (Guedes et al., 2009)

Dormancy is a characteristic that increases the survival of the species over time in the environment, but it is a problem when spread for cultivation and production of plants is desired among others, because these seeds do not germinate (Leal et al., 2008). In Fabaceae, it is mainly related to the presence of impermeable seed coat to water, which characterizes the seeds as hard or with tegumentary dormancy (Costa et al., 2010). Thus, the dormancy in species of Fabaceae must be overcome for an effective method to increase the rates of germination and allow a greater quantity of plants in the field (Silva et al., 2014).

The germination and vigor tests are essential components of the quality control process of seed companies (Torres et al., 2014). Among the vigor tests, the seedling length test has potential to provide additional information to those obtained in the germination test and enable to estimate the potential for seedling emergence in the field (Guedes et al., 2009)

Due to the above, the objective on this study was to evaluate the germination and vigor of *Macrotyloma axillare* cv Java (archer) seeds under different methods of overcoming dormancy.

#### MATERIALS AND METHODS

The study was conducted at the Instituto de Agronomia of the Universidade Federal Rural do Rio de Janeiro (UFRRJ) Seropédica – RJ (Brasil), during November of 2015. The archer seeds were submitted to different treatments for overcoming dormancy: (a) control, (b) manual scarification with sandpaper, (c) immersion in sulfuric acid ( $H_2SO_4$ ) 98% for five minutes, and (d) immersion in sodium hypochlorite (NaCIO) solution at 2%, for 15 min.

The samples used in the seed germination test were randomly picked from the "pure seeds" portion after the purity test. After homogenization, 200 seeds were counted per treatment in 4 replications of 50 seeds each, in a germination chamber BOD type (biochemical oxygen demand) with 12 h of light, where the seeds were placed in "gerbox" boxes on germitest paper sterilized and imbibed with distilled water in a ratio of 2.5 times the weight of the paper, in order to proceed with the germination test and the speed of germination index.

The seedling length was obtained by measuring parts of normal seedlings with a ruler, and the average results expressed in centimeters. The germination speed index was obtained by summing the number of germinated seeds each day, divided by the number of days since the test assembly, according to the Maguire's formula (1962).

The first germination evaluation occurred on the 4th day after the test assembly and the second on the 10th day, when the percentage of seed germination was quantified, which corresponds to the ratio of the number of seeds that produced seedlings classified as normal in accordance with the described recommendations in MAPA (2009).

For the evaluation of the seedlings length, the tests were performed in a germinator on germ test paper rolls packed in vertically positioned plastic bags for seven days in the absence of light and at 25 °C. The experimental design was completely randomized with four replications.

The data were submitted to normality test and analysis of variance, and in the case of statistically significant differences, the means were compared by Tukey's test at 5% of probability using the ExpDes package from the statistical R program (Ferreira et al., 2013).

#### **RESULTS AND DISCUSSION**

The results obtained in the seed germination test are shown in Table 1. The treatment which used sandpaper provided the highest germination percentage, but there were not statistically significant differences (p> 0.05) comparing with the treatment with  $H_2SO_4$ .

The scarification with sandpaper increases the percentage of germination, because it allows the formation of cracks in the seed tegument, which favors the start of imbibition and germination process (Guedes et al., 2013), while the use of  $H_2SO_4$  promotes abrasion of the seed tegument, due its corrosive action. The use of scarification with sandpaper, presents advantages over sulfuric acid in the relationship acquisition cost and the risk of manipulation that acid presents (Scheffer-Basso and Vendrusculo, 1997), besides the amount that can be used and the difficulty of acquiring it in the market (Bruno et al., 2001).

Paiva et al. (2008) obtained an increase of the archer germination percentage, seed (cv. Java) with scarification, by removing the seed tegument. Literature describes out the recommendation of the use of scarification with sandpaper to make the dormancy break of seeds of forage legumes: Calopogonium mucunoides (Morais et al., 2014), Clitorea ternatea (Deminicis et al., 2006), Neonotonia wightii (Deminicis et al., 2012) Stylosanthes macrocephala and Stylosanthes capitata (Carmona et al., 1986). The use of H<sub>2</sub>SO<sub>4</sub> was indicated for Mimosa caesalpiniaefolia (Bruno et al., 2001) and Piptadenia viridiflora (Santos et al., 2014).

Chikumba et al. (2006), they studied methods to overcome the dormancy of two seed lots of *Macrotyloma daltonii* (Webb) Verdc, which were submitted to the treatments: Removal of the integument, application of dry heat, hot water, acid scarification, sandpaper scarification, Table 1. The results of germination tests of archer seeds submitted to different methods for overcoming dormancy.

Variable	Treatments				
	Control	Sandpaper	H₂SO₄	Sodium hypochlorite	
Germination (%)	37.50 <sup>C</sup>	54.5 <sup>A</sup>	53.50 <sup>A</sup>	44.00 <sup>B</sup>	
CV (%)			7.27		

Means followed by the same letter do not differ significantly at 5% of probability (Tukey's test).

Table 2. Speed of germination index (SGI) and seedlings length (cm) after the application of different treatments to break seed dormancy of archer seeds.

Variable		Tre	atments		<b>C)/ (</b> 9/)	
	Control	Sandpaper	H <sub>2</sub> SO <sub>4</sub>	Sodium hypochlorite	CV (%)	
SGI	3.10 <sup>8</sup>	4.56 <sup>A</sup>	3.04 <sup>B</sup>	4.18 <sup>A</sup>	6.14	Ì
Seedlings length	4.08 <sup>°</sup>	8.37 <sup>A</sup>	5.66 <sup>8</sup>	5.15 <sup>BC</sup>	12.23	

Means followed by the same letter in the same line do not differ significantly at 5% of probability (Tukey's test).

pre-chiling and combination with  $H_2SO_4$  and dry heat, and concluded that immersion in  $H_2SO_4$  for 20 min was more effective at increasing germination from 10 to 80%.

Table 2 represents the speed of germination index (SGI) and seedlings length of archer seeds after submission to different treatment methods for overcoming dormancy. The treatment using sandpaper and immersion in sodium hypochlorite for 15 min showed higher SGI and the values were not statistically different (p> 0.05). The control and immersion in H<sub>2</sub>SO<sub>4</sub> presented lower SGI and values were not statistically different (p>0.05) from each other.

The results of this study are in agreement with those reported by Deminicis et al. (2006) which showed higher SGI values for eight tropical forage legume seeds submitted to scarification with sandpaper and the use of H<sub>2</sub>SO<sub>4</sub>. Lima et al. (2013) evaluated different methods of scarification (immersion in water, hot water immersion, with sandpaper and scarification  $H_2SO_4$ and combinations of these methods) in Delonix regia seeds, and found a higher speed of emergence index in the treatment with scarification by sandpaper, when compared to other treatments.

The treatment that used sandpaper had as a result higher average lengths of seedlings. Immersion in  $H_2SO_4$ did not differ significantly from immersion in sodium hypochlorite solution (p> 0.05), and the control had lower seedlings length. The seedlings length has the potential to provide additional information to those obtained in the germination test which makes it possible to estimate the potential for seedling emergence in the field (Guedes et al., 2015). Therefore, the use of scarification with sandpaper presented itself as the most efficient; this indicates that in addition to a better germination, an improving of the plants vigour will prepare them for the occurrence of stresses and weathering effects in the field.

#### Conclusion

The scarification with sandpaper provided higher germination percentage, speed of germination index and seedlings length, suggesting its recommendation as a method for overcoming dormancy in archer seeds.

#### CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

## Extract of *Persea* americana (Mill.) used for the control of *Meloidogyne incognita* in tomato plant

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The study aimed to evaluate the control of *Meloidogyne incognita* in resistant and susceptible tomato genotypes treated with hydrogel containing avocado extracts (Persea americana Mill.). The methanolic extract of avocado seeds was evaporated and re-suspended in distilled water containing Tween 80 (0.6%). The extract was prepared with the following concentrations: 100, 200, 400, 800 and 1000 mg L<sup>-1</sup>. The hatching, motility and mortality of juvenile were evaluated, in vitro, and from these results, we selected the most nematocide concentration which was incorporated into different hydrogel doses (0.1, 0.25, 0.5, 0.75 and 1.0 g pit<sup>-1</sup>), for *in vivo* testing. The hydrogel containing avocado extract was added to the pits of tomato plants during transplanting, and after three days, *M. incognita* was inoculated. After 30 days, the relative chlorophyll content was assessed, along with the total volume of root, the number of galls and egg masses, viability of the egg mass, and number of eggs and juveniles per root system in 100 cm<sup>3</sup> of soil. From these assessments, the most effective dose in the control of nematodes was tested again with the extract concentrations of 1000, 2000, 4000, 6000 and 8000 mg L<sup>-1</sup> in the hydrogel, which was added to the pits at the time of transplantation. After 30 days of inoculation the same evaluations were performed. In vitro, the concentration of 1000 mg L<sup>-1</sup> of the avocado extract was the most effective in reducing the hatched juveniles, while motility and mortality were not influenced. In vivo, 1.0 g pit<sup>1</sup> dose in the first test had greater control of nematodes in susceptible plants for all variables assessed. It was incorporated into the pits of tomato plants for the realization of the second test. In this, the concentration of 8000 mg L<sup>-1</sup> was the most effective in controlling *M. incognita*. Therefore, the avocado seed extract served in hydrogel has the potential to control *M. incognita* in tomato plants.

Key words: Avocado tree, alternative control, plant extract, hydrogel, motility, mortality, galls nematode.

#### INTRODUCTION

The galls nematodes, belonging to the genus *Meloidogyne*, are pathogens of major agricultural importance, because they cause direct and indirect damage to plants. Direct damage occurs in the root system. This is time when the juveniles penetrate the

plant, the second stage of the nematodes, which entails the formation of gall that clogs the conducting vessels of the xylem and phloem compromising thus the absorption of water and nutrients. From this, the indirect symptoms arise, such as chlorosis and wilt in the shoot and formation of patch contour in the useful area (Ferraz and Monteiro, 2011).

When nematodes are present in a specific area they are hardly eradicated, because they may remain viable for long periods in the soil. For this reason, a number of measures must be employed in order to decrease the inoculum, including: The use of crop rotation, flood, solarization, genetic resistance, synthetic nematicides and biological and alternative controls (Dias-Arieira et al., 2013).

The use of plant extracts as an alternative control has been widely studied in order to reduce their environmental impact (Salgado and Campos, 2003a). There are several plants which contain secondary metabolites with nematotoxic properties. But many of these plants have not been studied, such as avocado (*Persea americana* Mill.), whose seeds contain large quantities of phenolic compounds (Daiuto et al., 2014), providing greater antioxidant activity, and consequently, may be involved in plant defense against pathogens (Soares, 2002).

These antioxidant compounds are generally sensitive to ultraviolet radiation and therefore must be conveyed in formulations such as hydrogel in order to prevent them from damage by radiation (Souza et al., 2013). Moreover, it has as an advantage, which is the slow release of the toxic substances present in the extract around roots infected with the nematode (Byrne et al., 2002).

The aim of this study is to evaluate the effect of different concentrations of crude extracts of avocado seeds on hatching, motility and mortality of second stage juveniles of *M. incognita* and the control of this nematode in tomato susceptible and resistant to the pathogen, using extract of formulated hydrogel.

#### MATERIALS AND METHODS

#### **Experimental design**

The experimental design used for all tests was totally randomized. For the *in vitro* assays (hatching, motility and mortality of second stage juveniles (J2)) the factorial used was  $2\times7$ , considering two types of avocado extracts (red color extract and brown color extract) and seven concentrations (0, 100, 200, 400, 600, 800 and 1000 mg L<sup>-1</sup>), with five repetitions, each represented by a plastic flask (70 ml) with lid.

In a greenhouse, two experiments were performed, both in factorial  $2\times6$ , with two tomato genotypes (Santa Cruz Kada and Ivety, susceptible and resistant to *M. incognita*, respectively), and six doses of hydrogel or six extract concentrations. For the first experiment, six hydrogel doses were used (0, 0.1, 0.25, 0.5, 0.75 and 1.0 g pit<sup>-1</sup>) containing avocado extract that was more nematotoxic in the experiment *in vitro*. For the second experiment, the dose of the hydrogel + extract which gave greater control in the

first test was used adding different concentrations of the avocado extract (0, 1000, 2000, 4000, 6000 and 8000 mg L<sup>-1</sup>). Each treatment consisted of five repetitions, represented by a 2 L vessel containing a tomato plant.

#### Acquisition of the inoculum of *M. incognita*

Populations of *M. incognita* were obtained from okra plants presenting symptoms of galls and identified by perineal configuration technique proposed by Hartman and Sasser (1985). This population was multiplied in tomato Santa Cruz Kada, in a greenhouse, to serve as inoculum. The eggs and J2 for inoculation were obtained by the method of Coolen and D'Herde (1972).

#### Preparation of the avocado seed extracts

Crude extracts were prepared from fresh avocado seeds. For the preparation of each extract, an avocado seed with about 75 g each was used, totalizing two seeds in the experiment. The seeds were cut into pieces of two sizes to obtain two types of extracts: In one smaller pieces were cut (0.5 cm<sup>3</sup>), shown with a red color after cutting, being called red color extract (RE), and in another, the pieces were larger (1.5 cm<sup>3</sup>) and had brown color, calling the brown coloring extract (BE).

These seed pieces were placed in flasks containing 100 mL of methanol P.A., so as to cover the seed. The vials were sealed and protected from light, remaining under stirring for 24 h at 150 rpm. After this period, the solution passed through two filters, being first on paper filter (8  $\mu$ m of pore diameter) and then on filter membrane (0.45  $\mu$ m of pore diameter).

The solution obtained in the second filtration was evaporated in a rotary evaporator at 45°C and 60 rpm for 4 h to obtain the crude extract. For each extract was added 600  $\mu$ m of Tween 80 (0.6%), to promote homogenization of the solutions. From this, the different concentrations were prepared: 100, 200, 400, 600, 800 and 1000 mg L<sup>-1</sup> of crude extracts of avocado seeds. Control used was distilled water containing Tween 80 (0.6%).

#### In vitro tests - nematotoxic activity

To test the hatching of *M. incognita* juveniles, 1 ml of suspension containing 500 eggs and 5 mL of the treatments were incubated for 15 days at  $25^{\circ}$ C in the dark in a plastic container (70 ml) with lid. After this period, there was an evaluation under the light microscope, counting the first 100 eggs, and thereby determining the percentage of hatching (Costa et al., 2001).

For the motility and mortality tests, the juveniles were obtained through a hatching chamber by the Baermann funnel methodology (Baermann, 1917).

In each plastic container, 5 mL of the treatments and 1 ml of the suspension containing 500 juveniles of *M. incognita* were placed. The assessment of motility, conducted to check the nematostatic activity of the extracts was performed after 24 h, measuring the percentage of apparently immobile juveniles. Subsequently, the juveniles were transferred to a sieve of 400 Mesh, replacing the treatments of distilled water and collecting the suspension. This remained in the containers for more 24 h. Juveniles that remained motionless, straight or slightly bent were considered dead, thus

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Author(s) agree that this article remain permanently open access under the terms of the <u>Creative Commons Attribution</u> <u>License 4.0 International License</u> proving the nematicide activity of the extracts. The evaluations of the tests were performed in Peters slides and observed under an optical microscope (Rocha et al., 2005).

#### Hydrogel preparation containing avocado extract

With the results of the *in vitro* tests, it was determined that the concentration of one of the extracts that was more active against against *M. incognita* be used for the first assay in the greenhouse. In this test, the hydrogel containing avocado seed extract was placed in holes made for the transplanting of the tomato seedlings.

For the preparation of this mixture hydrogel + extract, 6 L of the chosen extract and 6 g of the hydrogel were used. After its homogenization, the mixture remained in the oven at 50°C for five days, until complete drying of the material. This dry and impregnated extract hydrogel was crushed with the aid of a mortar and a pistil to obtain a powder. Soon after, the different hydrogel doses containing the extract: 0.1, 0.25, 0.5, 0.75 and 1.0 g pit<sup>-1</sup> were prepared. As control, only the hydrogel without extract incorporation was used.

#### Sowing of tomato plants and inoculation of M. incognita

Susceptible tomato seeds (Santa Cruz Kada) and resistant (Ivety) were placed in trays of 128 cells containing commercial substrate. After 25 days, the seedlings were transplanted into 2 L pots containing a mixture of soil, sand and organic material (2:2:1, v:v:v), pre-autoclaved at 120°C and 1 atm for 1 h.

At the time of the transplantation, the different doses of hydrogel containing avocado seed extract were placed on each pit made. After three days of transplanting, the seedlings were inoculated with a suspension containing 2067 eggs and juveniles per plant, remaining in a greenhouse for 30 days.

## Evaluation of different doses of hydrogel per pit containing avocado extract

At 30 days after the inoculation, the relative chlorophyll content (SPAD index) in the leaves of tomato plants susceptible and resistant to *M. incognita* was assessed, using a portable measurer SPAD 502 *Plus Daminolta*. Five readings were carried out for each repetition, and subsequently made the means, which were expressed as chlorophyll content in cm<sup>3</sup>.

After this evaluation, plants of tomato plants were removed from pots and the roots were washed in water and dried on absorbent paper to obtain the total volume of the root (TVR). The number of galls (NG) and egg masses (NEM) in the root system were counted, the latter being performed according to Taylor and Sasser methodology (1978).

To determine the total volume of the root (TVR), it was placed in a graduated cylinder containing a known volume of distilled water (450 mL), thereby measuring the displacement of the water column. Through the difference obtained, the root volume was expressed in cm<sup>3</sup>.

For the viability analysis of eggs produced by the pathogen in the treated plants, the masses were removed and placed in wells containing 250 µl of distilled water, on ELISA plates. These were sealed with plastic film, keeping them in the dark at 25°C for 15 days. Viability was determined by assessing the number of hatched juveniles. For each treatment, five repetitions were performed, each in duplicate.

The determination of the population of *M. incognita* was performed by quantifying the number of eggs and juveniles on the root system (Coolen and D'Herde, 1972) of the susceptible tomato plants resistant to nematodes, and in 100 cm<sup>3</sup> of soil. The latter was

carried out according to the procedure described by Jenkins (1964).

## Evaluation of different avocado extracts concentrations in the hydrogel

From the results of the first test, where it was determined the best dose of the hydrogel + extract placed in the pits, the second trial testing different avocado extract concentrations was carried out (1000, 2000, 4000, 6000 and 8000 mg  $L^{-1}$ ) with this hydrogel dose.

At the time of the transplanting of the tomato seedlings, on each pit held we placed the different avocado extract concentrations in the hydrogel, and three days later, the seedlings were inoculated with a suspension containing 2184 eggs and juveniles per plant. They stayed in a greenhouse for 30 days later for measurement of chlorophyll content, total volume of the root (TVR), counting of the number of galls (NG) and egg mass (NEM) and determination of the population of *M. incognita* on the root system.

#### Statistical analysis

The data obtained from *in vitro* assays and the two experiments in the greenhouse were subjected to analysis of variance at 5% probability, with the help of statistical program SISVAR 5.3 (Ferreira, 2011). Checking a significant interaction between the factors, the developments were carried out. The average comparison was made for the types of extracts or genotypes using Tukey test at 5% probability; while regression analysis was done for the doses or concentrations.

#### RESULTS

With respect to the *in vitro* tests, for the variable juvenile hatching, it was observed that the increase of the avocado extract concentration afforded a linear decrease in hatching; 1000 mg L<sup>-1</sup> concentration was more effective in this reduction compared to the others, regardless of the type of extract used (Figure 1). When analyzing the extracts (Table 1), it is possible to note statistical differences only in the range of concentrations between 400 and 800 mg L<sup>-1</sup>, showing minor hatching of juveniles in red color extract compared to brown coloring extract.

With regard to motility and juvenile mortality, none of the treatments were significant (p>0.05). It is possible to infer that there was no nematostatic and nematicide effect of the avocado seed extract on *M. incognita*.

These results obtained from the in vitro evaluations indicated the concentration of 1000  $\text{mg}^{-1}$  of the red extract of the avocado seed as the most efficient against *M. incognita*, therefore, being chosen to be incorporated into the hydrogel, for tests in a greenhouse.

For the variable chlorophyll content (SPAD index), there was significant difference (p<0.05) only for the tomato genotypes, with averages for the susceptible and resistant of 35.46 and 39.05 cm<sup>3</sup>, respectively (data not shown). With regard to the variable total volume of the root, none of the factors were significant (p>0.05), indicating that regardless of the genotype of tomato and the extract concentration used, the root volume is not



**Figure 1.** Hatch of second stage juveniles (J2) of *Meloidogyne incognita* in the different concentrations of avocado extracts, after 15 days of incubation. RE: Red color extracts ( $\circ$ ); BE: Brown color extract ( $\diamond$ ).

Table 1.	In	vitro	hatching	g of	second	stage	juveniles	(J2)	of	М.	incognita	in	different	concentrations	s of	two	kinds	of	avocado	seed
extracts.																				

Extract -	Concentration (mg L <sup>-1</sup> )										
	0	100	200	400	600	800	1000	Average			
RE	41.40 <sup>a</sup>	53.40 <sup>a</sup>	46.60 <sup>a</sup>	26.40 <sup>a</sup>	16.60 <sup>a</sup>	14.20 <sup>a</sup>	9.80 <sup>a</sup>	29.77 <sup>a</sup>			
BE	48.20 <sup>a</sup>	55.40 <sup>a</sup>	51.00 <sup>a</sup>	41.80 <sup>b</sup>	49.00 <sup>b</sup>	38.60 <sup>b</sup>	17.06 <sup>a</sup>	43.09 <sup>b</sup>			

The averages followed by the same letter in the columns do not differ statistically by Tukey test ( $p \le 0.05$ ). Average values obtained of five repetitions of each treatment, expressed as a percentage. RE: Red color extracts; BE: Brown color extract.

interfered with (data not shown).

For the variables number of galls, egg masses and hatched juveniles, the interaction between the factors was significant (p<0.05), indicating that the increase in the hydrogel dose containing avocado extract promotes a reduction of these variables in tomato roots susceptible to nematode and the dose of 1.0 g pit<sup>-1</sup> that showed the lowest means. This, however, was not observed in resistant tomato plants (Figure 2A, B and C).

Regarding the genotypes (Table 2), for the variables number of galls, masses of eggs and hatched juveniles, there were statistical differences in all concentrations, with lower values for the resistant genotype.

The number of eggs and juveniles per root system was not influenced by different doses, only the factor genotype was significant (p>0.05), with averages of 560.5 and 8.4 + juvenile eggs per root system, for the susceptible and resistant genotypes, respectively (data not shown).

With regard to the number of eggs and juveniles in the soil, the interaction between the factors was significant (p<0.05), being only the susceptible genotype influenced by different hydrogel concentrations containing the extract. Therefore, with this increase, there is an increase

in the number of eggs and juveniles up to a certain concentration, followed by a reduction of these nematodes in soil up to a dose of 1.0 g pit<sup>-1</sup>. In this, the susceptible plant behaves like the resistant, not showing significant difference between them (Table 2D and Figure 2).

From the data resulting from this test, the dose of 1.0 g pit<sup>-1</sup> of the hydrogel containing the avocado extract showed the highest reduction of *M. incognita*, in comparison to the other tested doses and, therefore, chosen to perform the second test.

Similar to the results found in the first test, for the variable total volume of root, none of the factors were significant (p>0.05), and the variable chlorophyll content (SPAD index), only the tomato genotypes were influenced, with averages of 39.60 cm<sup>3</sup> for the susceptible and 42.62 cm<sup>3</sup> for the resistant (data not shown).

For the variables number of galls, egg masses and eggs and juveniles in the root system, the interaction between the factors was significant (p<0.05), because with the increase in avocado extract concentrations in hydrogel there was a linear decrease of these variables. 8000 mg  $L^{-1}$  concentration was the most efficient in this reduction. Regarding resistant tomato roots, there was no



**Figure 2.** Effect of different doses of the hydrogel containing the red color of avocado extract (RE) in susceptible tomato plants ( $\diamond$ ) (Santa Cruz Kada) and resistant ( $\circ$ ) (Ivety) to *M. incognita*. Variables analyzed: number of galls (NG) (A); number of egg masses (NEM) (B); viability of egg masses assessed by the number of second stage juveniles (J2) hatched (C); number of eggs and second stage juveniles (J2) in 100 cm<sup>3</sup> of soil (D).

**Table 2.** Average of the variables number of galls (NG), number of egg masses (NEM), viability of egg masses assessed by the number of second stage juveniles (J2) hatched, and number of eggs and second stage juveniles (J2) in 100 cm<sup>3</sup> of soil, on susceptible tomato genotypes (S) (Santa Cruz Kada) and resistant (R) (Ivety) to *M. incognita*, when subjected to various doses of hydrogel containing avocado seed extracts.

$D_{a} = a \left( \sigma \right)^{\frac{1}{4}}$	NC	<b>3</b> <sup>1</sup>	NE	И <sup>1</sup>	J2 ha	tched <sup>1</sup>	Eggs and J2 – soil <sup>1</sup>		
Dose (g pit )	S	R	S	R	S	R	S	R	
Witness	276.4 <sup>b</sup>	9.8 <sup>a</sup>	260.2 <sup>b</sup>	8.2 <sup>a</sup>	30.2 <sup>b</sup>	2.0 <sup>a</sup>	33.6 <sup>b</sup>	25.2 <sup>a</sup>	
0.1	275.4 <sup>b</sup>	10.6 <sup>a</sup>	236.0 <sup>b</sup>	7.8 <sup>a</sup>	25.4 <sup>b</sup>	1.4 <sup>a</sup>	40.2 <sup>b</sup>	23.0 <sup>a</sup>	
0.25	272.4 <sup>b</sup>	8.8 <sup>a</sup>	225.2 <sup>b</sup>	6.6 <sup>a</sup>	17.8 <sup>b</sup>	1.4 <sup>a</sup>	54.6 <sup>b</sup>	23.4 <sup>a</sup>	
0.5	269.4 <sup>b</sup>	8.0 <sup>a</sup>	223.8 <sup>b</sup>	5.0 <sup>a</sup>	8.0 <sup>b</sup>	1.8 <sup>a</sup>	44.6 <sup>b</sup>	25.2 <sup>a</sup>	
0.75	246.6 <sup>b</sup>	7.0 <sup>a</sup>	226.0 <sup>b</sup>	5.2 <sup>a</sup>	8.8 <sup>b</sup>	0.6 <sup>a</sup>	33.8 <sup>b</sup>	21.4 <sup>a</sup>	
1.0	238.0 <sup>b</sup>	7.8 <sup>a</sup>	220.0 <sup>b</sup>	4.8 <sup>a</sup>	7.0 <sup>b</sup>	0.8 <sup>a</sup>	16.2 <sup>ª</sup>	18.8 <sup>ª</sup>	
CV(%)	5.	.05	6.	56	22	2.02	17	.93	

The averages followed by the same letters in the lines, for each variable, do not differ statistically by Tukey test ( $p \le 0.05$ ); <sup>1</sup>Average value obtained from five repetitions of each treatment for each variable. Hydrogel incorporated with avocado seed extract in concentration of 1000 mg L<sup>-1</sup> of extract.

statistical difference between the treatments used in the study (Figures 3A, B and C).

With relation to the variable number of eggs and

juveniles in the soil, similar behavior was observed in the first test, by providing an increased number of nematodes as the concentration increased.  $4000 \text{ mg L}^{-1}$  concentration



**Figure 3.** Effect of different concentrations of the red color of avocado extract (RE) in the hydrogel, used at a dose of 1.0 g pit<sup>-1</sup>, in susceptible tomato plants ( $\diamond$ ) (Santa Cruz Kada) and resistant ( $\circ$ ) (Ivety) to *M. incognita*. Variables analyzed: number of galls (NG) (A); number of egg masses (NEM) (B); number of eggs and second stage juveniles (J2) on the root system (SR) (C) and 100 cm<sup>3</sup> of soil (D).

had the highest number of eggs and juveniles. From this, the final population of *M. incognita* decreases until it reaches the maximum concentration ( $8000 \text{ mg L}^{-1}$ ) used in the study, in which there was 52.57% of reduction of this nematode compared to the control (Figure 3D).

Evaluating the genotypes of tomato (Table 3), for the variable number of galls, mass of eggs, eggs and juveniles in the root system and in the soil, it is possible to observe significant differences in all tested concentrations, being the resistant genotype, in all cases, which had the lowest averages.

#### DISCUSSION

The lowest hatching of the juveniles seen in red color extract (RE) compared to the brown color extract (BE) shows a possible toxic effect on these nematodes and this toxicity can be due to the presence of substances in the extract that inhibit the hatching or affect phases of cell proliferation, embryonic development and change of the cuticle of the nematode (Salgado and Campos, 2003a). The avocado seed is rich in phenolic compounds such as catechins, hydroxybenzoic acids, hydroxycinnamic acids, flavanols and procyanidins, which give it a greater antioxidant power (Rodríguez-Carpena et al., 2011).

The difference in inhibition of juveniles hatching observed among extracts is probably due to the size of the cuts made in each of the seeds. For the RE, which was obtained from smaller seed pieces, a larger surface area was exposed for the occurrence of lipid oxidation process and the formation of potentially toxic compounds, such as free radicals, and due to this, the number of phenolic compounds released would be increased, in order to sequester these radicals, thus inhibiting the oxidation. As for the BE obtained from larger seed

Table 3. Averages of the variables number of galls (NG), number of egg masses (NEM), and number of eggs and second stage
juveniles (J2) on the root system (RS) and 100 cm <sup>3</sup> of soil in susceptible tomato genotypes (S) (Santa Cruz Kada) and resistant
(R) (Ivety) to <i>M. incognita</i> , when subjected to different avocado seed extract concentrations contained in the hydrogel.

Concentration	N	<b>G</b> <sup>1</sup>	NE	M <sup>1</sup>	Eggs and	J2 – RS	Eggs and J2 – soil <sup>1</sup>		
(mg L <sup>-1</sup> )	S	R	S	R	S	R	S	R	
Witness	59.6 <sup>b</sup>	6.6 <sup>a</sup>	56.4 <sup>b</sup>	5.8 <sup>a</sup>	1099,2 <sup>b</sup>	25.2 <sup>a</sup>	124.4 <sup>b</sup>	41.6 <sup>a</sup>	
1000	50.8 <sup>b</sup>	6.4 <sup>a</sup>	48.6 <sup>b</sup>	5.4 <sup>a</sup>	844.0 <sup>b</sup>	29.2 <sup>a</sup>	162.0 <sup>b</sup>	39.6 <sup>a</sup>	
2000	47.4 <sup>b</sup>	2.8 <sup>a</sup>	45.6 <sup>b</sup>	2.2 <sup>a</sup>	782.4 <sup>b</sup>	28.8 <sup>a</sup>	182.0 <sup>b</sup>	41.8 <sup>a</sup>	
4000	46.2 <sup>b</sup>	4.4 <sup>a</sup>	43.8 <sup>b</sup>	3.6 <sup>a</sup>	549.2 <sup>b</sup>	29.6 <sup>a</sup>	211.6 <sup>b</sup>	38.6 <sup>a</sup>	
6000	37.8 <sup>b</sup>	5.8 <sup>a</sup>	36.4 <sup>b</sup>	5.6 <sup>a</sup>	366.0 <sup>b</sup>	25.6 <sup>a</sup>	159.2 <sup>b</sup>	43.0 <sup>a</sup>	
8000	38.0 <sup>b</sup>	2.6 <sup>a</sup>	36.8 <sup>b</sup>	2.4 <sup>a</sup>	358.0 <sup>b</sup>	24.2 <sup>a</sup>	59,0 <sup>b</sup>	32.4 <sup>a</sup>	
CV (%)	17.	57	17.47		5.4	18	8.25		

The averages followed by the same letters in the lines, for each variable, do not differ statistically by Tukey test (p≤0.05);

<sup>1</sup>Average value obtained from five repetitions of each treatment for each variable. The hydrogel containing the different concentrations of avocado seed extract was used at a dose of 1.0 g pit<sup>-1</sup>.

pieces, a minor number of phenolic compounds may have been released because of the smaller area subject to oxidation.

In the case of motility and mortality of juveniles, the concentration range used in this study may not have shown a deleterious effect on the juvenile already formed (Salgado and Campos, 2003a), or, according to Chitwood (2002), due to the fact that the cuticle of the nematode may be impervious to many organic molecules such as phenolics.

The results of the chlorophyll content, obtained in two trials in the greenhouse, were expected due to the fact that the susceptible plant directs a greater amount of its assimilates to the formation of galls on the root system, thereby compromising the direction for other plant structures.

For the root volume, the fact that this variable was not influenced by the different varieties and concentrations of extract used indicates that the experimental period cannot have been enough for the vegetative recovery of plants after application of the extracts to reduce nematodes (Salgado and Campos, 2003b).

The reduction in the number of galls, egg masses, hatched juveniles and eggs and juveniles in the root system can be due to the higher concentration of tannins in the avocado seeds, as the extract concentrations are increased. This substance, present in large quantities in avocado seeds (Soares et al., 2012), when applied prior to transplantation or at planting, has as function the disorientation of nematodes, hindering thus the location of the root systems and, consequently, reducing the damage caused to plants (Maistrello et al., 2010).

Associated with this, the presence of organic matter in the soil solution can suppress nematodes due to the release of toxic metabolites such as phenolic compounds from decomposition. Furthermore, the increase of the population of parasitic microorganisms and/or predators of nematodes may also assist in reducing the population of *M. incognita* (Ritzinger and Fancelli, 2006).

When evaluating the number of eggs and juveniles on the ground in both tests in the greenhouse, the slight increase in initial doses may be due to the presence of minerals or some compound found in avocado extract, which may have been released after its application into the soil, favoring population increase in the nematode (Salgado and Campos, 2003b). Along with this increase, there has been a sharp drop in the number of eggs and juveniles in the soil, probably due to increased concentration and release of toxic substances contained in the extract.

These substances can often be considered as repellents to nematodes, promoting, according to Morillo and Silva (2015), modifications of the chemical composition of the exudates, thus affecting the reception of stimuli by the chemoreceptors of the juveniles. This behavior causes that the juvenile continually moves in the soil in order to find a host, depleting its energy reserves, and committing thus their penetration ability (Rocha et al., 2008).

For all variables assessed in the greenhouse, the susceptible genotype showed averages higher than the resistant genotype, highlighting that the latter has more effective defense mechanisms than the susceptible, preventing or delaying the nematode entrance into their root systems.

#### Conclusion

In the *in vitro* assays, the concentration of 1000 mg L<sup>-1</sup> obtained from avocado red color extract seems to be more effective in reducing hatched juveniles of *M. incognita*. Motility and juvenile mortality were not affected by the extracts and their concentrations. The dose of 1.0 g of hydrogel per pit, impregnated with avocado seed extract at a concentration of 8000 mg L<sup>-1</sup> was the most

efficient combination in the control of *M. incognita* in susceptible tomato.

#### **CONFLICTS OF INTERESTS**

The authors have not declared any conflict of interests.

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Full Length Research Paper

## Cauliflower quality and yield under tropical conditions are influenced by boron fertilization

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The aim of this study was to evaluate the influence of boron fertilization on the quality and yield of cauliflower growing under tropical conditions. The study was carried out in randomized block design in split plot design. The main plots four boron doses (0, 2, 4, 6 kg ha<sup>-1</sup>) whereas subplots with 3 cauliflower genotypes (Verona CMS, Sarah and Sharon) with three replicates. Among the genotypes, the higher fresh curd weight means were observed to Sharon followed by Verona and Sarah with 1.007, 0.801 and 0.583kg plant respectively. In relation to boron doses, genotypes of Sharon, Verona and Sarah showed responsive to boron doses with maximum fresh curd mass of 1.378, 1.216 and 0.804 kg plant<sup>1</sup> with doses of 6, 4.18 and 4.06 kg B ha<sup>-1</sup>. Total yield showed variation up to 307.96% positive with boron added. In contrast, there was observed a high loss ratio in the harvest mainly due to "hollow stem" incidence. Correlations were observed between the incidence of hollow stem, cultivars and boron doses. Analyzing hollow stem disorder between genotypes, Sharon presented higher incidence than Sarah and Verona CMS with values of 88.1%; 73.10% and 66.7%, respectively. Sharon and Verona CMS presented low incidence on the doses of 2 and 4 kg B ha<sup>-1</sup> (57.1 and 42.9%) respectively. For Sarah genotype boron dose was not significant whereas 85.7% of the curds were checked with hollow stem disorder. Overall, in this study we can conclude that: (i) Sharon genotype presented the higher productivity, whereas demand more boron fertilization than the other genotypes evaluated: (ii) Under tropical conditions Verona CMS is more tolerant to high temperature than Sarah and Sharon exhibiting more marketable products on postharvest by reducing hollow stem and physiological disorders.

Key words: Brassica oleracea, quality, mineral nutrition, tropical horticulture.

#### INTRODUCTION

Cauliflower (*Brassica oleraceae* var. botrytis L. Family: *Brassicaceae*) is a functional vegetable by presenting high nutritional value because of its high levels of antioxidants and anticarcino-genic compounds, such as

ascorbic acid (AA), carotenoids, and glucosinolates, besides of vitamins and minerals (Gu et al., 2014). This properties makes it very attractive to the consumer, generating high demand and significant economic value.
Your production is being introduced to new areas, mainly in tropical regions characterized by high temperatures, so that, breeding programs may choose lines able to tolerate temperatures higher than 30°C, which makes possible the cultivation in tropical regions (May et al., 2007). However, there is restriction of cultivation in some Brazilian regions and growing seasons, because majority of cauliflowers cultivars have long cycles, greater susceptibility to boron deficiency and poor inflorescence formation especially in areas with high thermal amplitude (Arahida, 2014).

The lack of adaptability and/or information to different environmental cauliflower cultivars cultivation reflected directly in the yield and inflorescence quality, especially in regions with high temperatures, where the genotypes are being adapted for this conditions with nights hot impair the performance of most cauliflower cultivars, leading to a reduction of leaves size and, consequently, low yield and curd standard (Kimoto, 1993). In addition to reduced productivity, high temperatures also cause physiological disorders that interfere with commercial standardization, including serious defects (leaves in the head, riceness and wine stain) that reduce marketable yield and quality (Caixeta et al., 2000).

Not only climatic disorders but also nutritional disorders are attributed to mineral fertilization. Many authors have cited boron as a crucial micronutrient, crosslink with curd quality in cauliflower (Batal et al., 1997; Dhakal et al., 2009; Mello et al., 2009; Hussain et al., 2012; Chander et al., 2014). Boron besides changing yield and quality, this nutrient has been suggested with post-harvest quality associating defects and / or anomalies caused by disability and / or excess of this micronutrient as hollow stem, brown rot and tightened curd. These physiological disorders lead to great lost on postharvest by reducing shelf-life and commercial standardization.

Others authors such as Everaarts and Putter (2003) cited that the incidence of hollow stem is related to growth shoot ratio, and not boron absent. This may occur by different conditions by assimilation and growth speed taxes of cauliflower. Campagnol et al. (2009) has noticed that may be related to the combine between environmental conditions, technical management and genotype susceptibility. Several studies were carried out to evaluating boron fertilization effects as soon as on the productivity aspects and also on the qualitative of cauliflower (Carneiro et al., 1995; Everaarts and Putter, 2003; Pizetta et al., 2005; Mello et al., 2009).

The most problem to disseminate this species under tropical conditions is the lack of technical and scientific support information about it.

Considering the lack of technological information within

the mineral fertilization with boron micronutrient and adaptability of cultivars under tropical regions, this study aimed to evaluate the quality and productivity aspects of cauliflower genotypes in tropical conditions.

#### MATERIALS AND METHODS

The assay was carried out from 22th February to 15 June of 2015, on Sinop-MT Brazil (11° 50' 53" S; 55° 38' 57" W; 384 m). The climate classification is Aw (Tropical climate with dry winter season).

Soil characteristics was red-yellow oxisol (LVA), clayey and chemical analysis (0-20cm) showed pH in  $CaCl_2 = 4.8$ ; MO = 21.3 g dm<sup>-3</sup>; Q = 2.7 mg dm<sup>-3</sup>; K = 34 mg.dm<sup>-3</sup>; Ca = 1.6 mmol dm<sup>-3</sup>; Mg = 0.7 mmol dm<sup>-3</sup>; H + Al = 3.5 mmol dm<sup>-3</sup>; SB = 2.4 mmol dm<sup>-3</sup>; % V = 41.6 and B = 0.26 mgdm<sup>-3</sup>.

Prior to seedling, dolomitic liming at the rate of 2.47 ton ha-1 (PRNT 100%) was distributed in total with harrow together with reactive phosphate natural. just to reach V% (80) following recommendations of Trani and Raij (1997).

The experimental set up was carried out in a split-plot design with three replicates. Four levels of boron: (i) 0 kg B ha<sup>-1</sup>; (ii) 2 kg B ha<sup>-1</sup>; (iii) 4 kg B ha<sup>-1</sup>; (iv) 180 kg N ha<sup>-1</sup> were assigned in the main plots and three cauliflower cv. in the subplots (Verona CMS, Sarah e Sharon).

Temperature (°C) and relative humidity (RH%) during assay were acquired by Universidade Federal de Mato Grosso.

The seedlings were grown in a greenhouse of polystyrene type 128/6 trays filled with commercial substrate. The transplant was performed at 33 days (5 to 6 leaves), arranged  $0.90 \times 0.80$  m.

The irrigation system was used with drip type emitters spaced 0.20 m and flow rate of 7.5 L h<sup>-1</sup>, applying a irrigation water level of  $5.54 \text{ mm}^{-1}$  daily.

During planting, fertilizer 4-30-16 was applied at 142 g pit<sup>-1</sup> of 600 kg ha<sup>-1</sup> of  $P_20_5$ , 80 kg ha<sup>-1</sup> of N and 320 kg ha<sup>-1</sup> of K<sub>2</sub>O. In topdressing, 160 kg ha<sup>-1</sup> N and 60 kg ha<sup>-1</sup> K<sub>2</sub>O in the form of urea and potassium chloride, parceled at 15, 30, 45 and 60 days after transplanting (DAT). Leaf fertilization with 1 g L<sup>-1</sup> sodium molybdate was practised at 45 and 60 DAT.

Boric acid (17% B) was used as a source of boron fertilization in different treatments. The fertilizer was diluted in hot water (40°C) and applied by fertigation system in the crown region at 10 DAT. Each sub-plots were composed by twelve plants and for evaluation on purpose four central plants were used and others were considered as border plants. The harvest was performed when the inflorescences were well developed and with linked flower buds as described by May et al. (2007). Cultivar Sarah (earlier) was harvested at 68 days after planting (DAP), Verona CMS at82 DAP and Sharon at 87 days DAP.

Evaluations parameters was: Curd mass - CM (kg plant<sup>-1</sup>); total yield - TP (t ha<sup>-1</sup>); Curd diameter - Cd (cm); Curd deformation - DIF (%); Average cycle - CM (days) and specific density of inflorescence - DEI (g cm<sup>-3</sup>) to measure from parameter was used to calculate the ratio of the curd mass (g) and the inflorescence displacement volume (cm<sup>3</sup>).

For qualitative analysis of commercial curd classification as soon as class and category were proceeds by HortiBrasil Program (2008). In this program, the classification is evaluated by transverse curd diameter, compactness, severe defects or physiological

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Figure 1. Fresh curd weight (CFW) (A); total yield (TY) (B) and curd diameter (CD) to Sharon, Verona CMS and Sarah cultivars under boron doses.

disorders (Caixeta et al., 2000). It was observed as an indication of deficiency or excess of boron defects as incidence of hollow stem, browning and rot. As an indication of non-adaptability to growing conditions physiological defects such as riceness, purplish stain and leafy curd appear.

The quantitative results were submitted to analysis of variance (F test, p <0.05) and the split of interactions and / or study of the isolated effect of factors was performed by regression analysis taking into account the significance of the coefficients of the models. From the regression models doses of B were estimated, to obtain the maximum points (PM) of the variables and their equivalent doses, with the help of the statistical program Sisvar® (Ferreira, 2011) and means were compared by the test Scott-Knott (p<0.05).  $_{\chi}2$  (chi-square) test was performed to all qualitative variable. When the adjust was linear, it was considered the lower or

higher dose applied as equivalent to the PM dose. Pearson correlation was performed among the variables analyzed with (p <0.05).

#### **RESULTS AND DISCUSSION**

In this study, a significant interactions between curd fresh weight (CFW) and total yield (TY) were registered (Figure 1 and Table 1), showing that boron fertilization is able to change yield parameters from each cauliflower genotype. To curd diameter (CD) there was only significant effect by dose and genotype. On other hand, specific density (DEI)

Treatment	CFW (kg plant <sup>-1</sup> )	CD (cm)	TY (t ha⁻¹)	ECD (g cm <sup>-3</sup> )	DCI (%)	CM (Days)
Variance analysis	(Fcal)					
Block	0.327 <sup>ns</sup>	0.101 <sup>ns</sup>	0.378 <sup>ns</sup>	0.660 <sup>ns</sup>	0.565 <sup>ns</sup>	0.863 <sup>ns</sup>
Dose	0. 000*	0.004*	0.000*	0.249 <sup>ns</sup>	0.280 <sup>ns</sup>	0.587 <sup>ns</sup>
Genotye	0.000*	0.001*	0.000*	0.037*	0.398 <sup>ns</sup>	0.000*
Dose × Genotype	0.000*	0.166 <sup>ns</sup>	0.000*	0.077 <sup>ns</sup>	0.469 <sup>ns</sup>	0.123 <sup>ns</sup>
Cauliflower genoty	ypes					
Verona	0.801 <sup>b</sup>	16.27 <sup>b</sup>	11.20 <sup>b</sup>	0.95 <sup>a</sup>	0.93 <sup>a</sup>	112.83 <sup>b</sup>
Sarah	0.583 <sup>c</sup>	15.03 <sup>c</sup>	8.16 <sup>°</sup>	0.87 <sup>b</sup>	0.90 <sup>a</sup>	96.50 <sup>a</sup>
Sharon	1.007 <sup>a</sup>	20.75 <sup>a</sup>	14.06 <sup>a</sup>	0.82 <sup>b</sup>	0.93 <sup>a</sup>	115.00 <sup>b</sup>
Boron dose (kg ha	ı-¹)					
0	0.521	15.37	7.29	0.89	0.94	108.55
2	0.585	15.97	8.19	0.82	0.92	109.33
4	1.294	21.94	18.11	0.93	0.96	109.66
6	0.788	16.11	11.02	0.87	0.96	106.22
CV1(%)	8.35	14.42	8.35	14.50	5.35	7.12
CV2(%)	9.35	14.39	9.35	12.18	5.59	7.58

**Table 1.** Variance analysis resume (Fcal), average and interactions to fresh curd weight (CFW), curd diameter (CD), total yield(TY), specific curd density (ECD), deformation curd index (DCI) and development cycle (CM) in function of cultivars and boron doses (Sinop/MT, UFMT, 2016).

<sup>ns</sup> Not significant; \* Significant at p≤0,05; Means followed by the same letter in the column do not differ by the Scott Knott test with p (≤0.05).

and development cycle there was only significant effect to genotype and to curd deformation (DI) there was not influence by factors and yours interactions.

The fresh curd weight was registered highest in Sharon genotype (1.01 kg planta<sup>-1</sup>) followed by Verona CMS (0.80 kg plant<sup>-1</sup>) and Sarah (0.58 kg plant<sup>-1</sup>) genotypes, respectively. Total yield followed the same behavior of CFW analysis with Sharon to 14.06 ton ha<sup>-1</sup> followed by Verona and Sarah genotypes with 11.20 and 8.16 ton ha<sup>-1</sup>, respectively. Both variables CFW and TY showed perfect correlation with r<sup>2</sup> = 0.99 (Figure 1).

The genotypes showed different behaviours to boron doses. Verona CMS genotype showed quadratic regression analyses with CFW and TY, with maximum peak of 1.216 kg planta<sup>-1</sup> and 17.02 ton ha<sup>-1</sup> (Figure 1). This total yield was obtained with maximum boron dose of 3.14 kg B ha<sup>-1</sup>, leading to an increment of CFW by 3.02 more times. This CFW was higher than observed by Zanuzo et al. (2013). These same authors observed to Verona 184 genotype, average of 0.91 kg planta<sup>-1</sup> using 2 kg B ha<sup>-1</sup>. This difference about MFI between the authors can be related to spacing transplant (0.80×0.60 m) and/or by gentoype. Verona CMS genotype is a breeding management of Verona 184.

On the Sarah genotype, optimal dose estimated was 4.06 kg B ha<sup>-1</sup> with 0.804 kg plant<sup>-1</sup> of CFW and 11.25 ton ha<sup>-1</sup> of TY (Figure 1). This genotype showed an increment of 3.86 times in comparison to control (0 kgB) ha<sup>-1</sup> with CFW of 0.204 kg plant<sup>-1</sup>. Therefore, a linear regression was observed for Sharon genotype reaching maximum CFW of 1.378 kg plant<sup>-1</sup> and TY of 19.44 t ha<sup>-1</sup>. These

datas were obtained by boron doses of 6 kg ha<sup>-1</sup>.

Mello et al. (2009), while working with Sharon genotype involving N and B, showed values of 0.949 kg plant<sup>-1</sup> to CFW independently to boron dose applied. In fact, this probably must be linked to boron source (Boromol (8% B and 0.8% Mo) and the dose applied (0 and 3 kg ha<sup>-1</sup> from B). Analyzing all the genotypes evaluated in this study, Sharon showed more dependence and quantities of B productive potential when compared to Verona and Sarah genotypes.

Monteiro et al. (2010), evaluating cauliflower cultivars, found values of 1.06 and 1.11 kg plant<sup>-1</sup> and 21.25 and 22.43 t ha<sup>-1</sup> of TY to Sharon and Sarah respectively using as boron source Borax at 3 kg ha<sup>-1</sup>. In fact, the higher total yield reach by these authors is justified by population density (20.000 plants ha<sup>-1</sup>) against 13.988 plants ha<sup>-1</sup> from this study besides soil and climatic conditions. It is acknowledged that high temperatures are able to change growth and development parameters in cauliflower genotypes (Zanuzo et al., 2013; Trevisan et al., 2003).

According to Trani and Raij (1997) values between 8 to 16 ton ha<sup>-1</sup> can be consider as good for better cauliflower yield. The best results for total yield were observed in Verona CMS and Sharon with total yield of 17.02 and 19.44 ton ha<sup>-1</sup>, with doses of 3.14 and 6 kg B ha<sup>-1</sup>, respectively.

Otherwise, doses of 0 and 6 kg ha<sup>-1</sup> of B to Verona CMS and Sarah, gave low yield as compared to Trani and Raij (1997), with values of 6.79 and 3.85 t ha<sup>-1</sup>, respectively. These results pointed out that each

Dama da a	TV	0)/						Class <sup>2</sup>				
Boron dose	ΙΥ (the <sup>-1</sup> )	UY (the <sup>-1</sup> )	Type <sup>1</sup>	1	2	3	4	5	6	7	8	9
(Kg na ')	(tha)	(tha)						%				
Verona CMS (	Genotype)											
0	7.2	1.03	CI	-	16.7	83.3	-	-	-	-	-	-
2	10.79	0	FE	-	-	-	30	50	20	-	-	-
4	20.89	11.93	CI	-	-	-	-	-	16.7	33.3	50	-
6	5.94	1.7	CI	-	57.1	28.6	-	-	-	14.3	-	-
					Sara	h						
0	4.36	0.61	CI	33.3	16.7	33.3	16.7	-	-	-	-	-
2	4.76	0.68	CII	33.3	16.7	50	0	-	-	-	-	-
4	15.59	4.46	CII	-	-	-	-	50	16.7	16.7	16.7	-
6	7.91	2.26	CIII	33.3	16.7	-	16.7	33.3	-	-	-	-
					Share	on						
0	10.29	2.95	CI	-	-	-	-	33.3	-	33.3	33.3	-
2	9.01	3.87	CI	-	-	-	-	-	9.7	85.2	5.1	-
4	17.85	2.55	CI		-	-	20	-	40	-	-	40
6	19.22	0	FE	-	-	-	-	9.6	33.3	57.1	-	-

Table 2. Commercial classification and distribution of curds by diameter standardization.

<sup>1</sup>Type or Category according to CAIXETA (2000): FE = out of specification; CI = category 1; CII = Category 2 and Category 3 = CIII. <sup>2</sup>Classes according to CAIXETA, (2000), curd diameter in cm 1 = <10.0 cm; 2 = 10.0 to <13,0 cm; 3 = 13.0 to <15.0 cm; 4 = 15.0 to <17.0 cm; 5 to 17.0 <19.0 cm; 6 = 19.0 at <21.0 cm; 7 = 21.0 to <23.0 cm; and 8≥23.0 cm; PC classification by type: FE, Out of specification; TY = total yield; CY = commercial yield.

genotype is dependent of boron calibration to maximize total yield and the range of lack and phytotoxicity for boron is very close.

Curd diameter (CD) is an important factor to be evaluated in cauliflower so this parameter is used by marketplace to quality factor. It was observed that Sharon genotype presented high CD (20.75 cm) between other genotypes (Table 1), however the value obtained was lower than Monteiro et al. (2010) and Massaroto et al. (2008) study that obtained values of 26.37 and 25.25 cm, respectively. These differences can be related to temperature as well as spacing, so that, temperature is able to changing plant development, bud development, shape and curd quality, yield and cycle time (Trevisan et al., 2003).

The data for CD analysis showed a quadratic adjust model to Verona CMS with maximum estimate value of 20.25 cm and optimal dose of 3.14 kg ha<sup>-1</sup> of B (Figure 1C). Verona CMS and Sarah presented high correlation between CD and CFW (r = 0.94 and 0.87), respectively. On the other hand, Sharon genotype presented poor correlation (r=0.35) between the variables (Figura 1C). Zanuzo et al. (2013) verified high correlation (r=0.73) for Verona between MFI and DI.at 4 kg B ha<sup>-1</sup>

Other important quality attribute to be evaluated was specific curd density (ECD) in cauliflower. This parameter can be associated to compactness and can show the grade of deformation of the curd. The result nearby one means good compactness (harvest point) and above of one show that the plant has already started the flowering process in advance. This situation is characterized by non-commercial curds. For ECD analysis, Verona CMS genotype presented 0.95 g cm<sup>-3</sup> when compared with Sarah and Sharon (0.87 and 0.82g cm<sup>-3</sup>) respectively (Table 1). This variable is pointed out as a genotype morphological characterized and not shows correlation to boron doses (Table 5).

In relation to development cycle, it was observed that Sarah was more precocity with 96.5 days (Table 1), when compared with Verona CMS and Sharon (112.83 and 115 days) respectively. It can note that Sarah has precocity of 16.33 and 18.50 day respectively (Table 1). The development cycle was more than described by Seed Company to Verona CMS (95 to 100 days) and Sharon (100-105 days). This increase of the cycle probability occurred by temperature effects. It was observed during all the cycle maximum and minimum of 32.86 and 17.67°C, respectively (Figure 2A). High temperature to cauliflower can stimulate more vegetative cycle than reproductive leading to change physiological, biochemical and photosynthetic parameters contributing to low yield efficiency as fast as qualitative parameters in cauliflower.

These results corroborate with Zanuzo et al. (2013) that reported a growing cycle of 106 days of Verona genotype in Sinop-MT Brazil with medium average temperature of 28°C. Therefore, Monteiro et al. (2010) reported to Sharon and Sarah cycle of 119 and 108 days, respectively when grown in temperatures of 22°C.

In case of Verona CMS the best commercial yield (CY) was observed with 4 kg B ha<sup>-1</sup> with value of 11.93 ton ha<sup>-1</sup> classified on the category I. Others doses were lower than this (Table 2). This result express that maximum



**Figure 2.** (A) Rainfall, maximum temperature (T max.), minimum (Tmin.) and average (T med.), day by day, and (B) medium average of temperature and relative humidity instantaneous to each 5 min checked during 05/30/2015 to 07/15/2015).

dose for qualitative parameters (CFW and TY) was 3.14 kg ha<sup>-1</sup> of B.

To Sarah genotype, the major CY (4.46 t  $ha^{-1}$ ) was observed on 4 kg B  $ha^{-1}$ , similar trend was obtained in case of CFW and TY. Compared Verona CMS, CY to Sarah is lower than 50%.

Sharon genotype showed high CY with 2 kg ha<sup>-1</sup> of boron. The value found was 3.87 ton ha<sup>-1</sup> (Table 2). Among the others genotype, MFI showed tendency for

gradual enhancing boron doses. This may be due to morphological parameters as stomata numbers, leaf area, and number of leaves that contribute to increase respiration and reduce photo-assimilates to meristematic growth regions as curd. All this happens with enhancing temperature during curd development as presented in Figure 2. Mello et al. (2009) in other region of Brazil with thermal average of 21°C verified for Sharon genotype CY of 11.33 ton ha<sup>-1</sup>.

Sharon		aron	Sarah		Verona CMS		
Defects	No	Yes	No	Yes	No	Yes	X <sup>2</sup>
%							
IHO	11.9	88.1	26.2	73.8	33.3	66.7	0.004*
PP	32.5	67.5	69	31	64.3	35.7	0.749 <sup>NS</sup>
PO	76.2	23.8	71.4	28.6	85.7	14.3	0.076 <sup>NS</sup>
DC	73.8	26.2	47.6	52.4	83.3	16.7	0.000*

**Table 3.** Chi-square  $\chi^2$  test to each genotype according to hollow stem incidence (IHO), curd browning (PP); mechanical or bacterial rot (PO) and climate order defects (DC).

No = absent of anomaly; Yes = presence of anomaly.

**Table 4.** Chi-square  $\chi^2$  test to each cultivar according to boron doses.

Constants	Incidence -		2			
Genotype		0	2	4	6	X
		Boron dose	versus Hollow ste	em %		
01	No	28.6	42.9	14.3	0	0.000*
Sharon	Yes	71	57.1	85.7	100.0	0.000
Sarah	No	14	14.3	28.6	28.6	O E 40 <sup>ns</sup>
	Yes	85.7	85.7	71.4	71.4	0.540
Verona CMS	No	14.3	0	57.1	28.6	0.001*
	Yes	85.7	100.0	42.9	71.4	0.001

No = absent of anomaly; Yes = presence of anomaly. \*Significant at p<0.05 and <sup>ns</sup> No significant at p<0.05.

Analyzing hollow stem incidence (IHO) by genotypes, we can observed that Sharon presented higher incidence than Sarah and Verona CMS with values of 88.1, 73.10 and 66.7%, respectively (Table 3). In relation to climatic disorders (DC) Verona CMS showed more tolerance to high temperature with minor alterations in this parameters followed by Sharon and Sarah genotypes with values of 83.3, 73.8 and 47.6%. Disturbs of curd browning (PP) and mechanical or bacterial rot did not influenced by genotypes.

The incidence of hollow stem showed be influenced by boron doses and genotypes. It is possible to verify that Sharon and Verona CMS presented low incidence on the doses of 2 and 4 kg ha<sup>-1</sup> of B (57.1 and 42.9% respectively) (Table 4). On the other hand, Sarah genotype did not show any influence due to different dose. This genotype showed highly sensitive to hollow stem incidence and temperature during the cycle. An explanation to this answer is due to high sensibility to high temperature hypothesized by sugar alcohol carrier during development that must influence distribution or partition of boron to growth apical meristems.

Sharon genotype presented high incidence 100% of IHO on dose of 6 kg ha<sup>-1</sup> B. According to Everaarts and Putter (2003), the hollow stem occurrence and cavity severity are related to shoot and plant growth taxes, and not necessary to boron absent. This can occur due to the absorption of boron micronutrient do not follow the

cauliflower growth taxes. In fact, we observed this and was recorded absolute growth taxes of  $15.16 \text{ g g}^{-1}$  dia-<sup>1</sup> to 75 DAT applying 6 kg há-<sup>1</sup> of boron (Dates not presented).

Mello et al. (2009) studying Sharon genotype with boron dose of 3 kg ha<sup>-1</sup> sowed directly in the pit or divided during the cycle verified that hollow stem was reduced more than 200% when compared with control without fertilization in both ways.

Kojoi et al. (2009) studying the effect of nitrogen fertilization (120, 180 and 240 kg ha<sup>-1</sup> of N) and boron (2, 4 and 6 kg ha<sup>-1</sup> of B) on the Shiromaru III genotype, showed that boron do not change presence or absent of hollow stem. They verified that hollow stem has linked direct with nitrogen fertilizations at high concentrations.

When climatic disturbances were analyzed wine spot, riceness and curd leafly) for Sarah genotype the research showed incidence of 52.4% (Table 3), showing that this genotype is not adapted to this region during this cultivation period. Verona and Sharon genotypes showed low values 16.2 and 26.2%) of climatic disorders as riceness, wine spot and curd leafly respectively showing better adaptation to tropical regions than Sarah genotype.

On the Sarah genotype the high defects percent and/or anomaly, become easier to know that the stress by high temperature during the observation of experimental study, with average temperature on the critical hour between 11:30 and 13:30 hours at 42.07°C (Figure 2B).

Vari	ety	DCI	ECD	Doses	IHO
	DCI	1.000	0.389 <sup>*</sup>	-0.106	-0.115
Verona CMS	ECD	0.389 <sup>*</sup>	1.000	087	-0.424 <sup>*</sup>
	В	-0.106	-0.087	1.000	-0.258
	IHO	-0.115	-0.424*	-0.258	1.000
	DCI	1.000	-0.069	0.054	0.141
•	ECD	-0.069	1.000	0.146	0.075
Saran	В	0.054	0.146	1.000	-0.156
	IHO	0.141	0.075	-0.156	1.000
	DCI	1.000	0.158	-0.175	-0.103
Charan	ECD	0.158	1.000	-0.320	-0.388 <sup>*</sup>
Sharon	В	-0.175	-0.320	1.000	0.311
	IHO	-0.103	-0.388*	0.311	1.000

Table 5. Pearson correlation test to curd deformation (DCI); specific curd density-ECD, boron doses (B) and hollow stem incidence in IHO.

\*Significant at p<0.05;

According to Ferreira (1983), temperature so high and intense insolation during curd formation can speed up the growing, without reaching ideal size wanted, beside causes by other defects, as fast division and the appearance of other defects as small curds formation plus anthocyanin spots.

Analyzing the Pearson correlations between boron rates and IHO variables, DCI and ECD, we observed that the boron doses have no relationship with these variables for any of the cultivars (Table 5). However, genotype Verona and Sharon showed a moderate and weak negative correlation between the IHO respectively and gave variables, namely, the incidence of hollow stem, decreased the specific density of the inflorescence, probably caused by the incidence of severity of the hollow stem. Based on the results it is evident that boron fertilization alone is not able to elucidate the defects and/or anomalies observed during the experiment, but when added to climatic factors for the conditions of high temperatures has shown corroborative for these factors beyond expression genotypic potential of each cultivar.

#### Conclusions

Overall, in this study we can conclude that: (i) Sharon genotype presented the higher productivity, whereas demand more boron fertilization than the other genotypes evaluated. (ii) Under tropical conditions Verona CMS is more tolerant to high temperature than Sarah and Sharon exhibiting more marketable products on postharvest by reducing hollow stem and physiological disorders

#### **CONFLICTS OF INTERESTS**

The authors have not declared any conflict of interests.

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# Kappaphycus alvarezii extract used for the seed treatment of soybean culture

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Marine macroalgae, Kappaphycus alvarezii has economic importance which is considered as the main source of Kappa carrageenan, a hydrocolloid used in various industries as an emulsifying agent and stabilizer. In the production process of this raw material, the fresh seaweed is subjected to grinding and filtering process, yielding a wet solid fraction rich in carrageenan and other liquid fractions. The latter has been studied for its use in pulverization of bean leaves, wheat, soybean, rice, among others. Several reports have shown positive effects on the growth, yield and quality of vegetables and grains treated with the extract of this alga. However, there are few reports on the use of the liquid extract of K. alvarezii for seed treatment, and also of its use in conjunction with the solid fraction of seaweed. In this context, this study aims to evaluate the physiological quality of soybean seeds treated with liquid extract of this alga and, with the mixture of liquid extract and solid biomass, submitted to a hydrolysis process. Two soybean seed treatment tests were performed in laboratory: one treatment contained pure liquid extract (100%) while the other was mixed with two samples of solid biomass hydrolyzed with concentrations of 25 and 75%. For comparison of their results, there was a positive control treatment and one negative control treatment. The results showed that, within the present working conditions, treatments with seaweed extract can increase the length of the aerial part of the seedling up to 28% and the length of the primary root up to 19% as compared to the negative control; thus, it improves the physiological performance of seeds.

Key words: *Glycine max*, macroalgae, agriculture, bioregulator.

#### INTRODUCTION

*Kappaphycus alvarezii* (Doty) Doty ex P.C. Silva is one of the main algae grown in the world. Along with other red

algae, *Eucheuma* are responsible for about 34% of world production of seaweed (FAO, 2012). The commercial

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Author(s) agree that this article remain permanently open access under the terms of the <u>Creative Commons Attribution</u> <u>License 4.0 International License</u> importance of these organisms is because it is the source of carragegenan, a phycocolloid which has many industrial applications due to its emulsifying and stabilizing properties (Yong et al., 2013). The cultivation technique mastery and the increase in world demand of this phycocolloid made *K. alvarezii* widely cultivated in several countries (Ask and Azanza, 2002).

The introduction of this species in Brazil took place in 1995 in experimental scale cultivation in Northern São Paulo State coast and aimed to make the country selfsufficient in the production of carrageenan. From this moment, several studies have been conducted to evaluate the commercial viability of marine farms of *K. alvarezii*, but despite the acknowledged progress in this matter, Brazil is not yet part of the world's leading producers and imports most of its domestic demand of carrageenan and agar, primarily from Philippines and Chile (Paula et al., 1998; Oliveira et al., 2009). However, today, *K. alvarezii* is the only marine macroalgae legally cultivated in Brazilian coast.

In addition to the global demand for carrageenan, potential new biotechnologies are being used to find these species of seaweed and its derivatives to obtain new products, and increase phycocolloid. This will encourage the cultivation of the macroalgae. An example is the work carried out to evaluate its antimicrobial potential (Abirami and Kowsalya, 2011; Prabha et al., 2013; Pushparaj et al., 2014), the profile of its nutritional and biochemical composition (Fayaz et al., 2005; Chew et al., 2008; Abirami and Kowsalya, 2011) and the use of its extract in agriculture (Rathore et al., 2009; Zodape et al., 2009, 2010).

In particular, the use of seaweed extract in agriculture has shown interesting results in the increment of growth, yield and quality of some crops such as soybeans, wheat and beans (Rathore et al., 2009; Zodape et al., 2009, 2010). In general, the use of algae in agriculture has occurred for centuries; however, they have currently been used specifically as biostimulants or bioregulators, for application and/or seed treatment. foliar These compounds are able to act on the physiology of plants, where a number of biomolecules perform metabolic functions that contribute to a better development of the plant. Among the biomolecules usually present in these extracts, the plant hormones, such as abscisic acid, auxin, cytokinins and gibberellins can be highlighted among others (Craigie, 2011).

In literature, there are results that demonstrate the effectiveness of products with growth promoter or promoter action, as is the case of stimulatant. The results obtained by the authors (Vieira and Castro, 2001; Ávila et al., 2008) with the product, or other regulators are applied exogenously. Such research has attested that there is a real possibility of bioregulators to not only change the agronomic performance of soybeans, but also to produce seeds with different physiological potential and incidence of pathogens, as well as seed treatment, to promote

better initial growth, which contributes to a better development of culture and derivations, best yields (Marcos Filho, 2005).

In addition to the use of liquid seaweed extract, studies suggest other applications for solid biomass of K. alvarezii like acid hydrolysis process that can serve as raw material for bioethanol production. Although, the primary application for the acid hydrolysis of biomass is the production of raw material to obtain bioethanol, there biological are several activities related to derived from molecules such oligosaccharides as carrageenan, which may appear as other applications, such as antiviral (Katsuraya et al., 1999), antitumor (Yuan et al., 2006), anti-inflammatory (Sanders et al., 1999), anticoagulants (Wall et al., 2001) and antiangiogenic (Kãsbauer et al., 2001).

In this context, considering the proven potential of the liquid extract of *K. alvarezii* in agriculture and the possible biological activity of the polymers present in the solid fraction of the algae and its derivatives, the common use of these biomasses can expand the positive physiological responses already obtained in vegetable treatment with the seaweed liquid extract. And considering that these liquid extracts contain products with potential hormonal action, the present study aimed to evaluate the use of liquid extract of *K. alvarezii* and also its use in conjunction with the liquid extract and algae hydrolyzate solid biomass, in soybean crop, for verification of their effects in the physiological quality of the seeds.

#### MATERIALS AND METHODS

Two seed treatment trials were conducted at the Seed Technology Laboratory, Department of Agricultural Sciences, Federal University of Parana, Palotina Sector.

The extracts used in the work are derived from seaweed cultivated in Brazil coastline with the tubular net technique. They were obtained according to the method described by Eswaran et al. (2005). After harvesting, the algae were subjected to the following processes: washing in running water in order to remove any residue or marine impurity; mechanical trituration with industrial shredder to break the cell wall and release the sap; filtration of the aqueous biomass through a cotton cloth, then obtaining two biomass, liquid extract and a moist solid phase. The wet solid phase was exposed to sunlight for a period of approximately 72 h, until the moisture content reached a percentage (%) below 25 and it was stored in a suitable place for its conservation. The liquid phase was bottled and stored in the proper location for conservation.

After grinding and sieving, the solid biomass of algae was subjected to acid hydrolysis. The process was carried out using an autoclave and glass jars with capacity of 500 mL, where the working volume was approximately 200 ml employing a seaweed substrate concentration of 12 g 100 ml<sup>-1</sup> solution of sulfuric acid. The concentration of the sulfuric acid solution was 0.2 molar, with reaction temperature of 127°C and two times reactions of 15 and 30 min. This resulted in samples called solid biomass hydrolyzate 1 and 2, respectively.

For each seed treatment test, the experimental design was completely randomized with twelve treatments, of four replications. In each case these was analyzed: one negative control treatment (T0); a control positive treatment (T1) consisting of the commercial product Stimulate®, a plant growth regulator promoters-based: cytokinin, auxin and gibberellin (kinetin, as gibberellic acid GA3 form; 4-indol-3-ilbutírico, respectively); two treatments with pure liquid extract (T2 and T3); two treatments with the solid biomass hydrolyzed 1 (one) in mixture with the liquid extract in concentration of 25% (T4, T5); two treatments with the solid biomass hydrolyzate 1 in mixture with the liquid extract in concentration of 75% (T6, T7); two treatments with the solid biomass hydrolyzate 2 in mixture with the liquid extract in concentration of 25% (T8 and T9); and two treatments with the solid biomass hydrolyzate 2 in mixture with the liquid extract in concentration of 75% (T10 and T11).

The two trials only differ in the groups of doses used, which in the first test treatment with seaweed had two doses of 2.5 and 5.0 mL for 100 kg<sup>-1</sup> of seed. The negative control consisted of no treatment and positive witness the Bioregulator Stimulate® with dose of 5.0 mL for 100 kg<sup>-1</sup> of seeds. And, for the second experiment, the doses of treatment with seaweed were 4.0 and 8.0 mL for 100 kg<sup>-1</sup> of seed, also constituting negative control of no treatment, and the positive had the Bioregulator Stimulate®, with dose of 8.0 mL for 100 kg<sup>-1</sup> of seed. The soybean cultivar used for the seed treatment was Monsoy 5947 IPRO.

To evaluate the physiological quality of the seeds, the percentage of germination was determined, performed with four subsamples of 50 seeds each, with the volume of water equivalent to two and a half times the mass of the dry paper. The rolls were placed in germinator Mangelsdorf, at constant temperature of 25 ± 2°C. The percentage of normal seedlings, according to the Rules for Seed Analysis (MAPA, 2009), was analyzed on the eighth day at the beginning of the experiment. The performance of seedlings was also evaluated through the length of the seedlings, using four replications of 20 seeds for each treatment, germinated in the same conditions of the germination test. The seeds were distributed in the longitudinal direction of the sheets, with the thread facing the lower end of the substrate. The rolls were placed vertically in the germinator, at a temperature of 25 ± 2°C. The length of normal seedlings (primary root and aerial part) was assessed on the seventh day, with the aid of a millimeter ruler, getting the results in cm/seedling (Nakagawa, 1999).

Finally, the biomass of the average dry weight (g of seedling) of the seedlings after the length evaluation was determined. Normal seedlings were placed in paper bags and dried in an oven with forced air circulation, at  $80 \pm 2^{\circ}$ C for 24 h and then the weigh-in analytical balance was held (Nakagawa, 1999).

The effects of the treatments were checked by analysis of variance and the averages compared by Tukey test at 5% significance through the Sisvar® computer program.

#### **RESULTS AND DISCUSSION**

According to statistical analysis, the results obtained in the first assay, doses varied between 2.5 and 5.0 mL for 100 kg<sup>-1</sup> of seed, and are shown in Table 1. Some treatments showed significant differences for aerial part length parameters and primary root.

According to the results, only the treatments derived from the mixture of liquid extract with solid biomass hydrolyzate presented statistics superior to the negative control (T0) for the length of the aerial part of the seedlings. However, these treatments did not differ significantly among themselves and with the positive control (T1). On average, treatment with lower dose and lower concentrations of liquid extract (T10), presented an increase of more than 28% as compared to the negative control (T0).

Regarding the length character of primary root, only two treatments showed significant difference as compared to the negative control (T0), which is the treatments with lower dose and lower concentrations of liquid extract (T6 and T10), with higher increase to 11%. However, they did not present significant difference between themselves and in relation to the positive control (T1).

The results of the second assay, where the doses vary ranging between 4.0 and 8.0 mL for 100 kg-1 of seed, are shown in Table 2. Similar to the first experiment, some treatments showed significant difference only for the shoot variable length and primary root. In this case, considering the results of the aerial part length, only the treatments derived from the mixture of liquid extract with the hydrolyzed biomass were statistically higher than the negative control (T0), an increase of up to 23%. However, they did not present significant difference between themselves and in relation to the positive control (T1).

Finally, in the case of aerial part length, only the treatment with lower dose of pure liquid extract (T2) and the positive control (T1) showed a significant difference as compared to the negative control (T0), with an increase of up to 19%. However, they did not present differences when they were compared.

Considering the possible content of phytohormones (or similar) of the liquid extract, these results are consistent with the report of Moterle et al. (2011) and Vieira and Castro (2001) who found that there were positive changes in the performance of seedlings, increase in the length of the aerial part of the soybean seedlings treated with Stimulate®. One was Bioregulator registered and recommended for soybean culture (EMBRAPA, 2014). And, also with the results obtained by Santos (2009), where the same plant growth regulator was able to promote the initial growth of soybean plants, increasing the average height. Further, Wang et al. (2008) observed another effect, that there was a delay in the emergence and early development of soybean seedlings when treated with high doses of gibberellin and cytokinin. However, the results tend to show promoting answers. For instance, França-Neto et al. (2011), using biostimulants seaweed extract derived from the soybean seed treatment with micronutrients, reported positive effects on seedling length.

The literature has several reports of significant expressive effects of commercial bioregulators (Klahold et al., 2006; Ávila et al., 2008), as well as the potential use of new substances, associated or not with nutrients and phytosanitary products (Ferreira et al., 2007; Castro et al., 2008). This emphasizes the need for continuing the researches, seeking alternatives not only for the best physiological performance of the seeds, but also for the culture as a whole, in terms of agronomic yields.

In this context, the results suggest the biostimulant potential of the K. alvarezii extract, which showed

Tracting and	Evaluated characteristics <sup>1</sup>						
Treatment	Germination (%)	Aerial Part (cm)	Primary root (cm)	Dry biomass (g seedling <sup>-1</sup> )			
Т0.	76.0 <sup>a</sup>	7.9004 <sup>c</sup>	16.8770 <sup>c</sup>	0.147124 <sup>a</sup>			
T1.	78.5 <sup>a</sup>	9.1491 <sup>bc</sup>	17.5394 <sup>abc</sup>	0.147392 <sup>a</sup>			
T2.	80.0 <sup>a</sup>	9.1293 <sup>bc</sup>	17.4777 <sup>bc</sup>	0,147424 <sup>a</sup>			
Т3.	80.0 <sup>a</sup>	8.8440 <sup>bc</sup>	17.7019 <sup>abc</sup>	0.147339 <sup>a</sup>			
T4.	74.5 <sup>a</sup>	9.7049 <sup>ab</sup>	18.0983 <sup>abc</sup>	0.150501 <sup>a</sup>			
T5.	79.0 <sup>a</sup>	9.8489 <sup>ab</sup>	17.3228 <sup>c</sup>	0.146604 <sup>a</sup>			
Т6.	76.0 <sup>a</sup>	10.0455 <sup>ab</sup>	18.8578 <sup>a</sup>	0.151099 <sup>a</sup>			
T7.	74.5 <sup>a</sup>	9.4452 <sup>ab</sup>	17.9204 <sup>abc</sup>	0.146123 <sup>a</sup>			
Т8.	73.0 <sup>a</sup>	10.0317 <sup>ab</sup>	17.8510 <sup>abc</sup>	0.150394 <sup>a</sup>			
Т9.	78.0 <sup>a</sup>	9.7890 <sup>ab</sup>	17.2986 <sup>c</sup>	0.147372 <sup>a</sup>			
T10.	76.5 <sup>a</sup>	10.1623 <sup>a</sup>	18.7726 <sup>ab</sup>	0.151336 <sup>a</sup>			
T11.	73.5 <sup>ª</sup>	9.7863 <sup>ab</sup>	17.5779 <sup>abc</sup>	0.155146 <sup>a</sup>			
C.V (%)	11.86	5.43	3.08	3.57			

Table 1. Physiological performance of soybean seeds of cultivar M 5947 IPRO, subjected to different treatments with extract of *K. alvarezii.* 

<sup>1</sup>Averages followed by the same letter in the column do not differ significantly by Tukey test at 5% probability level; T0 = negative control; T1 = positive control; T2 = pure liquid extract (2.5 ml/kg); T3 = pure liquid extract (5 mL kg<sup>-1</sup>); T4 = liquid extract + Biomass Hydroponics 1.25% (2.5 ml kg<sup>-1</sup>); T5 = liquid extract + Biomass Hydroponics 1.25% (5 ml kg<sup>-1</sup>); T6 = liquid extract + biomass hydroponics 1.75%; T8 = liquid extract + biomass hydroponics 2.25% (2.5 ml kg<sup>-1</sup>); T7 = liquid extract + biomass hydroponics 1.75%; T8 = liquid extract + biomass hydroponics 2.25% (2.5 ml kg<sup>-1</sup>); T9 = liquid extract + biomass hydroponics 2.25% (5 ml kg<sup>-1</sup>); T10 = liquid extract + biomass hydroponics 2.75% (2.5 ml kg<sup>-1</sup>); T11 = liquid extract + biomass hydroponics 2.75% (5 ml kg<sup>-1</sup>).

	Evaluated characteristics <sup>1</sup>							
Treatment	Germination (%)	Aerial part (cm)	Primary root (cm)	Dry biomass (g seedling⁻¹)				
Т0.	73.0 <sup>a</sup>	12.1075 <sup>°</sup>	18.3782 <sup>c</sup>	0.140685 <sup>a</sup>				
T1.	77.5 <sup>a</sup>	13.5972 <sup>abc</sup>	21.1247 <sup>ab</sup>	0.146438 <sup>a</sup>				
T2.	79.0 <sup>a</sup>	13.3537 <sup>abc</sup>	21.8962 <sup>a</sup>	0,146963 <sup>a</sup>				
ТЗ.	78.5 <sup>a</sup>	13.1827 <sup>bc</sup>	18.4802 <sup>bc</sup>	0.140763 <sup>a</sup>				
T4.	73.0 <sup>a</sup>	14.0140 <sup>ab</sup>	20.8805 <sup>abc</sup>	0.143243 <sup>a</sup>				
T5.	79.0 <sup>a</sup>	13.9872 <sup>ab</sup>	20.4355 <sup>abc</sup>	0.144020 <sup>a</sup>				
T6.	74.5 <sup>a</sup>	14.0587 <sup>ab</sup>	20.4345 <sup>abc</sup>	0.147120 <sup>a</sup>				
T7.	76.5 <sup>a</sup>	13.5397 <sup>abc</sup>	19.7275 <sup>abc</sup>	0.144605 <sup>a</sup>				
Т8.	75.0 <sup>a</sup>	14.7857 <sup>a</sup>	19.8627 <sup>abc</sup>	0.145063 <sup>a</sup>				
Т9.	78.0 <sup>a</sup>	14.1697 <sup>ab</sup>	19.7332 <sup>abc</sup>	0.145018 <sup>a</sup>				
T10.	75.5 <sup>a</sup>	14.0320 <sup>ab</sup>	21.0490 <sup>abc</sup>	0.146070 <sup>a</sup>				
T11.	76.0 <sup>a</sup>	14.9162 <sup>a</sup>	20.1502 <sup>abc</sup>	0.144948 <sup>a</sup>				
C.V (%)	9.96	4.63	5.43	3.01				

 Table 2. Physiological performance of soybean cultivar M 5947 IPRO, subjected to different treatments with extract of K. alvarezii.

<sup>1</sup>Averages followed by the same letter in the column do not differ significantly by Tukey test at 5% probability level; T0 = negative control; T1 = positive control; T2 = pure liquid extract (4 ml kg<sup>-1</sup>); T3 = pure liquid extract (8 mL kg<sup>-1</sup>); T4 = hydroponics biomass 1 + liquid extract 25% (4 ml kg<sup>-1</sup>); T5 = hydroponics biomass 1 + liquid extract 25% (8 ml kg<sup>-1</sup>); T6 = Hydroponics biomass 1 + liquid extract 75% (4 ml kg<sup>-1</sup>); T7 = hydroponics biomass 1 + liquid extract 75% (8 ml kg<sup>-1</sup>); T8 = hydroponics biomass 2 + liquid extract 25% (4 ml kg<sup>-1</sup>); T9 = hydroponics biomass 2 + liquid extract 25% (8 ml kg<sup>-1</sup>); T10 = hydroponics biomass 2 + liquid extract 75% (8 ml kg<sup>-1</sup>); T11 = hydroponics biomass 2 + liquid extract 75% (8 ml kg<sup>-1</sup>).

tendency to increase length of soybean seedlings, where the use of some products of the extract have evident technological potential.

#### Conclusion

The use of seaweed, K. alvarezii extract can improve the

physiological quality of soybean seeds, providing the best performance seedlings. Some products were superior and similar to commercial bioregulator. However, more studies are necessary to understand the real promoter effect of extracts and their preparations.

#### CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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