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African Journal of Agricultural Research

Full Length Research Paper

Sweetpotato seed exchange systems and knowledge on sweetpotato viral diseases among local farmers in Acholi Sub Region-Northern Uganda

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Viral infections greatly limit sweetpotato yields. Good farming practices are critical for effective disease management. 383 Ugandan farmers were surveyed to document symptom incidence, crop-management practices, and buyer preferences. Results showed that 89.27% farmers grow sweet potatoes yearly and 62.76% of these farmers were female. A total of 56.83% farmers obtained vine seeds from their previous gardens, 25.85% from neighbours, and 12.20% purchased. Only ~8% of sellers and ~4% of buyers were selected for disease-free materials. None of the farmers who used vine-cutting knives sterilised them. Almost half of farmers (47%) observed whitefly or aphids but most were unaware they are viral vectors. Most farmers (77%) observed viral symptoms, but few (<2%) recognised them as infections. Insufficient knowledge of sweetpotato viruses and their vectors is common and increases the risk of spread. Practices like vine selling, sharing of vines coupled with insufficient knowledge on sweet potato viruses and its vectors among farmers increase the risk of virus spread among different farms.

Key words: Sweet potato, seed exchange, local farmers, farming practices, vine selling.

INTRODUCTION

Sweetpotato (*Ipomoea batatas* (L.) Lam.), is considered to be the third most important root crop after cassava and potato (Mukasa et al., 2003; Kashif et al., 2012). In Uganda, the crop is grown both at subsistence level for home consumption and for sale in local markets (Aritua et al., 2007; Kivuva et al., 2014). Sweetpotato is an excellent crop for small household farmers because it yields relatively well in poor soil, provides an important source of carbohydrates, and its tubers can remain in the soil for extended periods thus providing a continuous

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food source. The production of orange-fleshed sweetpotato is being particularly encouraged among local farmers because it contains beta-carotene, which is a precursor of vitamin A, and is thus one of the cheapest means of alleviating vitamin A deficiency (Kivuva et al., 2014).

In East Africa, sweetpotato production is concentrated around Lake Victoria and Uganda is among the largest producers of sweetpotato in Africa (Karyeija et al., 1998; Byamukama et al., 2004). Sweetpotato is the second most important root crop in Uganda and is grown in almost every district (Mukasa et al., 2003). In many areas of East Africa, where sweetpotato production is high, production has not yet attained its full potential (Kivuva et al., 2014); the high production of sweetpotato in Uganda is attributed to the large cultivated area rather than high yield (Byamukama et al., 2004). The total area of sweetpotato in Uganda is estimated at 4,440,000 ha (Uganda Bureau of Statistics, 2010). Given production potential of 25 t/ha for virus free vines (Clark et al., 2012), Uganda's expected production capacity is predicted to be about 1.11×10^8 t. The yield attained has, however, been lower than expected ranging from 1.8×10^6 t in 2009 to 2.55×10^6 t in 2011 (Caliskan et al., 2007; Uganda Bureau of Statistics, 2010; Okonya and Kroschel, 2014). Yields of sweetpotato below production potential can be a result of either abiotic or biotic constraints (Kivuva et al., 2014). Sweetpotato yield loss due to viral infections ranks second after weevil infestation (Opiyo et al., 2010). Co-infection of sweetpotato plant with sweetpotato feathery mottle virus and sweetpotato chlorotic stunt virus produce severe disease syndrome known as sweet potato virus disease (SPVD) (Gutiérrez et al., 2003). SPVD is the major sweet potato disease in East Africa which cause yield loss of up to 98% (Mukasa et al., 2003).

Vegetative propagation of sweetpotato remains the most important mechanisms for the spread, survival and transmission of sweet potato viruses from generation to generation (Adane, 2010). Farmers often have the tendency to leave their sweetpotato vines from previous seasons to sprouts and provide planting materials for next season. As result, this promotes the accumulation of the viruses each season (Karyeija et al., 1998). The practices increase the chances of distribution of viruses over wider areas.

In Uganda, most farmers obtained planting materials from previous own sweetpotato farm (Bashaasha et al., 1995). However, some farmers obtained vines from neighbours' sweetpotato garden usually for free. Under extreme condition farmers reported buying of sweetpotato vines (Bashaasha et al., 1995). Recently non-governmental organisation like world vision and harvest plus were involved in sweetpotato vine multiplication and distribution among local farmers (Gibson, 2013). As result informal vine multiplication dealers were established by the organisations to increase accessibility of orange flesh cultivars among local sweetpotato farmers (Gibson, 2013).

Northern Uganda suffered from nearly two decades (1986 to 2006) of war, which led to breakdown in the agriculture sector as most people were settled in camps for internally displaced people. Since 2006, people have resettled in their former villages and struggled to recover from effects of the war by engaging in agricultural activities to improve their livelihoods. However, most farmers in Africa use traditional practices that unknowingly enhance the spread of crop diseases (Karyeija et al., 1998; Kivuva et al., 2014). Viral diseases are the most difficult to manage and therefore good farming practices are critical for effective disease management, but there is little information on farming practices for sweetpotato in this region. As previous studies on sweetpotato virus in Uganda did not extensively cover northern Uganda due to war at that time. As result little information is available on sweetpotato farming practices in Acholi sub region which was at the epicentre of the two decades of insurgency. The aim of this study was to explore the different farming practices of local farmers in Northern Uganda that promote transmission and spread of sweetpotato viruses among farmers' fields. A cross-sectional survey was performed in three districts and 383 questionnaires were administered.

MATERIALS AND METHODS

The survey was conducted in Gulu, Kitgum and Lamwo out of the seven districts comprising the Acholi sub-region in Northern Uganda. Kitgum and Gulu were chosen because they were the two pioneer districts for production of sweetpotato in Acholi. It was assumed that the farming practices used for growing sweetpotato in all newly formed districts would not deviate much from the two districts (that is, Kitgum and Gulu) from which they were derived. The Lamwo district borders South Sudan and there is frequent interaction between the two populations. Lamwo was therefore the third logical addition to explore any hybrid farming practices that may have arisen from people moving between Lamwo and South Sudan. Northern Uganda is located at about 1100 m above sea level. The area experience unimodal pattern of rainfall that last from April to October every year. The temperature varies from 26 to 29°C between April and November. The temperature can rise to 37°C during dry season usually from December to March

A total of 383 farmers in the three districts were interviewed. The number of farmers interviewed was determined using a statistical formula described by Krejcie and Morgan (1970). The population of smallholder farmers who grow sweetpotato in Northern Uganda was estimated at 100,512 (Uganda Bureau of Statistics, 2010). The C-survey 2.0 (UCLA/Fogarty AIDS International Training and Research Program, Los Angeles, CA, USA) was used to generate and assign random numbers to the complete list of sub-counties in each district. Two sub-counties were then selected using a random-numbers table. Questionnaires were administered in person to farmers in these sub-counties. A total of 152 questionnaires were administered in Gulu as it is a bigger producer of sweetpotato than the other two districts, with a production capacity of 61,732 t annually (Uganda Bureau of Statistics, 2010). Questionnaires were administered to 124 farmers in Kitgum and 107 in Lamwo. The farmers were presented with picture of plants showing sign of viral infections (purple chlorotic spot on leaf, yellow chlorosis of the leaves, vein clearing, leaf mottling, and leaf mosaic) and then asked questions to assess their understanding. Similarly, farmers were presented with pictures of *Bemisia tabaci* (Gennadius) or *Myzus persicae.*

Questionnaires were administered to farmers with sweetpotato gardens and to farmers who sold vines. Those farmers who sold vines were required to answer section 3 of the questionnaire, which contained questions on how vines were sold. The study was approved by the Department of Crop Protection unit under the Ministry of Agriculture Animal Industry and Fishery with file number CCP/95. Oral informed consent was obtained for every individual farmer interviewed. The questionnaires were entered into the database created in EPI info 7 (CDC, Atlanta, USA). Raw data were exported to Microsoft excel for data validation. The validated data were uploaded back to EPI info 7. Frequency of response to each question was computed and expressed as percentage. The data were presented inform of table and figure.

RESULTS

Sweetpotato vine exchange system

Majority of farmers, 63.71% interviewed were female and male were only 36.29% (Supplementary Table 1). Most farmers, 89.3% grew sweetpotato every year; the other 10.7% skipped some years (Table 1). Of the farmers who grow sweetpotato every year, 62.76% (Table 1) were female. Females were 1.43 times (Supplementary Table 2) more likely to grow sweetpotato yearly than males. Sources of sweetpotato vines for most farmers 75.25% where near their homestead and only 5.10% farmers move to another districts sourcing sweetpotato vines for planting (Supplementary Table 3). Most of the farmers surveyed 56.83% (Table 1) obtained vine cuttings from their previous gardens/fields (volunteer vines). Meanwhile, 12.2 and 5.12% of farmers obtained vines from market and their relatives, respectively (Table 1). Females who grow sweetpotato are 1.94 times (Supplementary Table 4) more likely to buy sweetpotato vines for planting compared to male sweetpotato farmers.

In total, 21% of farmers travelled out of their district to obtain planting materials. A higher proportion of farmers who source vines outside their districts were from Kitgum district and followed by farmers from Lamwo district. A total of 78.72% of farmers in Kitgum and 40% of farmers in Lamwo district who travelled to obtain vines from another district reported Gulu district as their source. Female farmers were 0.69 time less likely to source sweetpotato planting materials beyond the boundary of their district of residence compared with male farmers. Of the farmers interviewed, 82.5% deliberately selected sweetpotato varieties to plant, whereas only 17.5% did not select vines (Table 2). The majority of farmers (33.9%) favoured high-yielding vines, 31.3% liked early maturity and only 6.0% preferred healthy vines (Table 2).

Sweetpotato harvest practices

A total of 59.8% farmers were unable to complete harvest of their sweetpotato from old gardens before the new planting season commenced in which they expected to plant a new crop. By contrast, 40.2% farmers completed harvest before the new season commenced. It was found that 97.0% farmers preferred piecemeal harvest, compared to 3.0% of farmers who harvested by clearing the garden all at once. About one-fifth (21%) of farmers preferred intercropping sweetpotato compared to 79.0% who grew sweetpotato as a monocrop.

A total of 83.0% of farmers preserved vines to plant in the new season, in contrast to only 17.0% who never preserved vines for the new season. There were 66.1% of farmers who kept vines and preferred to leave vine remnants in their gardens, to sprout spontaneously when rain commenced in a new rainy season (Table 3). In addition, 16.2% of farmers preferred growing vines in moist areas during the dry season to prevent them from desiccating and only 3.7% planted vines in protected areas to prevent animals from destroying them. A total of 99.4% of farmers used a knife to cut sweetpotato vines and, surprisingly, none of these farmers sterilised their knife during vine cutting. Only 9.2% of farmers reported that they cleaned their knife with non-disinfectant agent like clothes, stones or soap (Table 3).

Vine selling

Only 5.48% of farmers have ever engaged in selling of sweetpotato vines. Most farmers who sold vines (71.4%), came from Gulu; 19.0% were from Kitgum and 9.5% were from Lamwo (Table 4). Of the 21 vine sellers, 19 reported that buyers generally request vines with the attributes they liked the most. Only two sellers reported they had buyers who bought any kind of vine. About half (12 of 21) of sellers recorded that buyers preferred vines with high-yield attributes; seven sellers said their buyers preferred vines that matured rapidly (Table 4). Only one vine seller reported that buyers selected vines based on

Table 1. Frequency that local farmers grow sweetpotato and the sources of their vines.

Variable	Frequency	Percentage
Frequency that farmers grow sweetpotato		
Yearly	341	89.27
Not yearly	41	10.73
Total responses	382	100.00
Grow sweetpotato yearly		
Male	127	37.24
Female	214	62.76
Total	341	100
Sources of sweetpotato vines		
Own farm	233	56.83
Neighbour's farm	106	25.85
Market	50	12.20
Relative's farm	21	5.12
Total responses	410	100

Table 2. Criteria used by farmers when selecting sweetpotato vines.

Variable	Frequency	Percentage
Select vines for planting when cutting		
Yes	316	82.51
No	67	17.49
Total responses	383	100
Qualities of vines that farmers prefer		
High yield	130	33.33
Mature quickly	120	30.77
Other (specify)	34	8.72
Healthy vines	23	5.90
Taste preference	23	5.90
Broad leaves	20	5.12
Disease tolerance	15	3.85
Drought tolerance	13	3.33
Delayed rotting of tubers in field	8	2.05
Ease of access to vines	4	1.03
Total responses	390	100

the taste of tubers and only two sellers reported that buyers preferred healthy vines. Of the 21 vine sellers, 15 preferred selling vines with high-yield traits, four opted for vines that were easy to get and only two opted to sell disease-free vines (Table 4).

Knowledge about sweetpotato viruses and their vectors

About half (46.7%) of farmers had observed *Bemisia tabaci* (Gennadius) or *Myzus persicae* (aphid) in their

Table 3. Methods for preserving vines and tools used for vine cutting

Variable	Frequency	Percentage
Preserve vines for planting		
Yes	318	83.03
No	65	16.97
Total responses	383	100
Methods for preserving vines		
Leaving them to sprout in garden (volunteer vines)	253	66.06
Growing them in moist place	62	16.19
Growing them in a conserved area	14	3.66
Keeping them in refrigerator	0	0.00
No response	54	14.09
Total responses	383	100
Tools used for cutting vines		
Knife	381	99.48
Others (specify)	0	0.00
Sickle	0	0.00
Scissors	0	0.00
No response	2	0.52
Total responses	383	100
Clean tools during vine cutting		
Yes	35	9.21
No	345	90.79
Total responses	380	100
Materials used for cleaning vine-cutting tools		
Cotton	1	3.23
Files	1	3.23
Piece of cloth	8	25.80
Stones	3	9.68
Water	2	6.45
Water and soap	15	48.38
Water and stones	1	3.23
Total responses	31	100

sweetpotato gardens compared with 53.3% who had never observed the vectors in their gardens. Most (78.7%) of the interviewed farmers were unaware of the viral threats associated with whitefly and aphids. Only 14.1% of farmers reported use of pesticides in their sweetpotato gardens. Female farmers were 0.73 times (Supplementary Table 5) less likely to know the vectors of sweetpotato viruses compared to male farmers and thus females were 0.70 time (Supplementary Table 6) less likely to assess for the presence of the vectors compared with male farmers.

Most (77%) sweetpotato farmers said they had seen symptoms of infection as presented to them in the questionnaire pictures. A small number of farmers (14.3%) had no idea of the possible cause of symptoms they had seen in sweetpotato plants, 15.15% attributed the symptoms to disease infection and 13.5% to insect pest infestation (Table 5).

Only 31% of farmers regularly checked vines for symptoms of sweetpotato viruses. Most (74.5%) of

Table 4. Vine sellers and qualities of vines preferred by buyers based on a survey of 21 sellers.

Variable	Frequency	Percentage
Vine selling per district		
Gulu	15	71.43
Kitgum	4	19.05
Lamwo	2	9.52
Total responses	21	100
Inquiry on vine quality being sold by sellers		
Yes	19	90.48
No	2	9.52
Total responses	21	100
Qualities of vines from sellers		
High yield	12	52.17
Early maturity	7	30.43
Others	2	8.70
Disease free	1	4.35
Taste preference	1	4.35
Total responses	23	100
Qualities of the vines that sellers take to market		
High yield	15	60.00
Easy to obtain	4	16.00
Variety	2	8.00
Disease free	2	8.00
Drought resistant	1	4.00
Taste preference	1	4.00
Total responses	25	100

farmers took no action even when they observed virus-like symptoms; 9.6% sprayed their gardens with some kind of pesticide once such symptoms appeared (Figure 1).

DISCUSSION

This study confirmed that the majority of sweetpotato farmers were women, as was reported in other studies (Karyeija et al., 1998; Kivuva et al., 2014). It was reported that men tend to engage more in production of crops with high economic value (such as *Sesamum indicum*) that fetch higher prices in the market than sweetpotato in order to meet the financial demands of the family (Kivuva et al., 2014). Men are more likely than women to hold salary jobs in the urban and semi-urban centres, which tend to keep them away from their villages and their farms (Kivuva et al., 2014). Regarding sweetpotato production, men may only participate in heaping mounds whereas most of the other activities (e.g., vine gathering, planting and harvesting) are done by women and children. Reports show that women have limited access to agriculture extension services, which has serious implications for dissemination of knowledge and the fostering of good farming and production practices (Karyeija et al., 1998; Okonya and Kroschel, 2014).

Most farmers grow sweetpotato yearly, indicating that it is a priority crop for most farmers, especially for women who grow it for household consumption. Sweetpotato can thrive in poor soils, requires few inputs and can be harvested many times within a season (Clark et al., 2012), making it preferred by rural farmers as a safeguard against extreme food shortages. Some farmers also use sweetpotato as a source of income, especially for women who sell the tubers in the local

Table 5. Farmers' beliefs about what causes abnormal appearance of sweetpotato vines. Abnormal appearance refers to all symptoms of viral infection and includes curled leaves, mosaic leaves, vein clearing, mottled leaves, yellow chlorosis, necrotic spots on leaves, purple chlorosis and stunted plants.

Causes of abnormal appearance of sweetpotato	Frequency	Percentage
Ash deposited in field	1	0.34
Caterpillars	18	6.06
Excess vines in the garden	1	0.34
Heavy rain	1	0.34
Infection	45	15.15
Insects	40	13.47
Millipedes	15	5.05
Mixture of varieties	4	1.35
Nematodes	33	11.11
No idea	55	18.52
No response	4	1.35
Old age	7	2.36
Pests	16	5.39
Soil infertility	10	3.37
Sun burn	36	12.12
Viruses	5	1.68
Weeds	6	2.02
Total responses	297	100.00

markets. Some farmers have shifted to growing sweetpotato because of the continued devastating effect of cassava mosaic disease and cassava brown streak disease on cassava production in the region (Scott et al., 1997; Kumakech et al., 2013).

Most farmers get their sweetpotato vines near their homes from their own, neighbours' or relatives' gardens (Table 1). The practice of sharing sweetpotato vines among farmers is very common and this tradition was previously reported in among different sweetpotato growing communities (Karyeija et al., 1998). The practice has been implicated as the major contributing factor in the spread of sweetpotato viruses among distant farms because the exchange of vines among farmers not only occurs within villages or sub-counties but also among farmers in different districts and countries. It was found that cross-boundary movement of vines among different districts was common. Movement of vines is greatly facilitated by the extreme shortage of vines or the presence of unique traits that are attractive to farmers. We found a high degree of movement of sweetpotato vines from Gulu to the neighbouring districts of Kitgum and Lamwo. This study suggests that it will be important to perform extensive and frequent virus surveillance in Gulu specifically because it is an important centre for supply and distribution of sweetpotato vines in the northern part of Uganda. The results also suggest that Gulu could be a major centre for distribution of sweetpotato viruses in Northern Uganda.

Although no farmer reported obtaining sweetpotato materials from districts in Central and Western Uganda where the sweetpotato virus burden is reportedly very high (Aritua et al., 2007), we cannot rule out exchange of vines among farmers in the three districts of the present study with those in other regions of Uganda. Additionally, there was evidence of imported vines in the present study; one person reported getting vines from Democratic Republic of Congo. Surveillance of the movement of sweetpotato materials across country borders is very important because such movements can lead to the introduction of additional viruses and viral strains, including those with more serious effects (Mukasa et al., 2003; Aritua et al., 2007).

The preferences for most farmers to grow specific sweetpotato varieties were driven by local preferences about vine varieties, mainly high yield and early maturity rather than the health status and taste preference (Table 2). To minimise the spread of sweetpotato viruses, farmers first need to select vines that are visually healthy. Of course, not all vines that look healthy are virus-free (Aritua et al., 2007), so additional practices must be in place. The most effective choice is to use virus-free

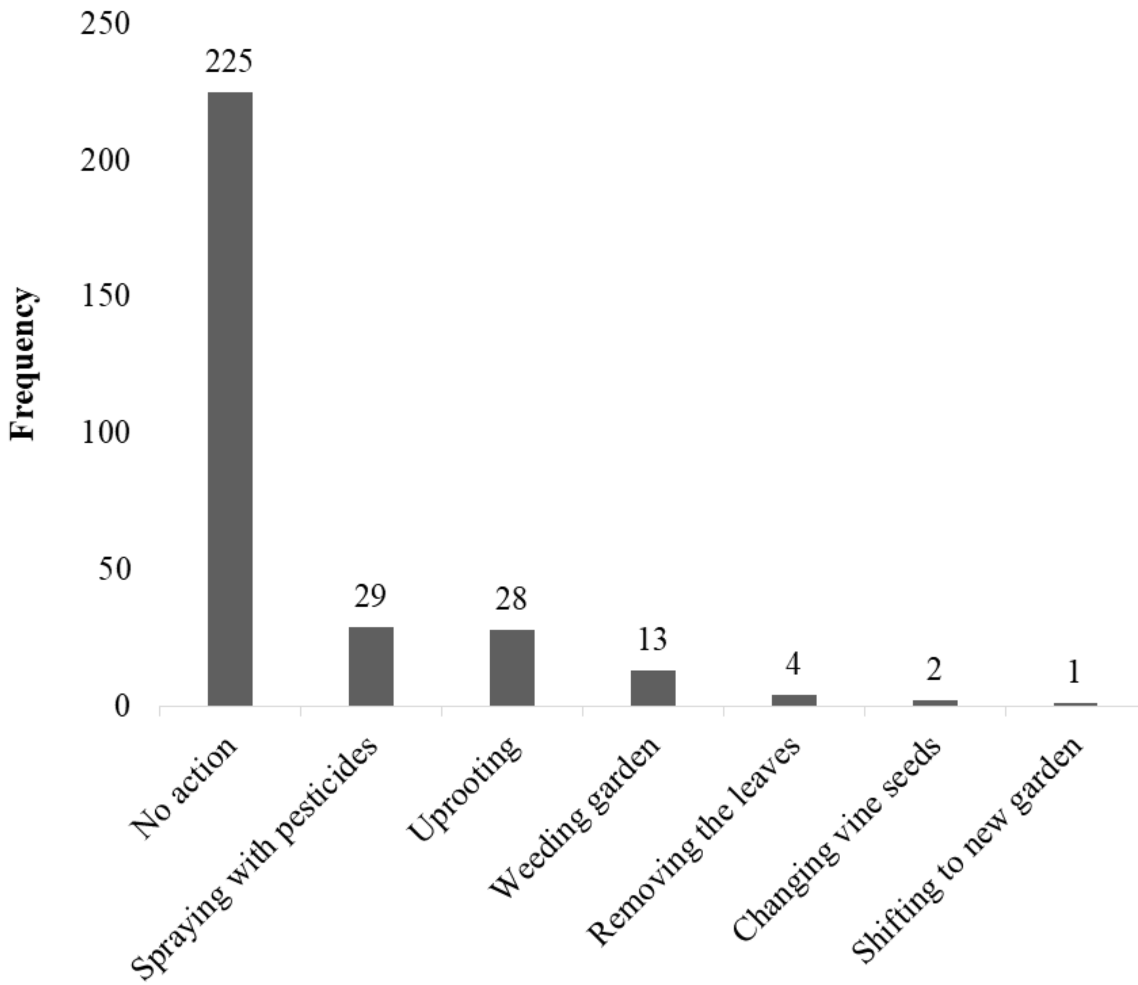

Figure 1. Management strategies taken by 383 surveyed farmers when sweetpotato plants exhibited symptoms of viral infection.

germplasm cuttings from seed multiplication centres or tissue culture labs; however, access to such services is very hard for local farmers in rural settings and the number of vines supplied is insufficient for all farmers. Farmers need to be trained in viral symptom identification so that they can select healthy vines.

In addition to growing sweetpotato in wetlands during the dry season, most farmers preserve their vines by allowing remnants of vines of the previous season to sprout (so-called "volunteer vines"). Regardless of the techniques used for preservation of these vines, the consequences of this practice are very important. This practice allows cyclic use and reuse of the same planting materials for an extended number of years, which may favour accumulation of high virus titre within infected plants. Such vines may be distributed to wider areas by sale and sharing among friends, neighbours and relatives. Accumulation of virus titre within infected plants degenerates the potential of the plants to achieve high yields. The preferred cultivars or varieties (which are widely shared because of their high yields) will increasingly experience declined yields as a result of virus-induced degeneracy. Such cultivars with virus induced low yield will be widely rejected and their production abandoned among local farmers. This will lead to narrower pool of genetic resources and potentially the extinction of some cultivars. Despite the challenges with the "volunteer vines" method of vine preservation, this practice does result in limiting the influx of vines from other virus-prone areas, which is the case in Gulu where few farmers look for vines from other districts, compared to Kitgum where there is minimal preservation of vines.

Piecemeal harvesting is a common practice among most sweetpotato farmers in Africa because the tubers do not readily spoil (Karyeija et al., 1998). In this area, farmers usually use a stick to excavate mounds and remove the tubers. They usually take one or two tubers from each mound and leave immature tubers to continue growing for the next harvest. Farmers can therefore harvest sweetpotato on a daily or weekly basis and it can be up to 5 to 6 months before harvest is complete, which in most cases is during the dry season. The attraction of piecemeal harvest is that it regularly supplies tubers for an extended period and provides a sustainable food source for the household. However, the practice also maintains plants for long in the fields which often overlap into new planting seasons. Usually vines from such old fields form the first source of planting materials among local farmers. If they are infected, these plants become the source of infection in new sweetpotato fields.

Most farmers use knives for cutting new vines to be transferred to their new gardens or to take to markets (Table 3). The importance of sterilising cutting tools is emphasised in the sweetpotato production manual (Dennien et al., 2013); however, it is clear from our study that farmers lack this vital information. Farmers usually use a single knife to cut as many vines as possible in one round of sweetpotato vine gathering. This practice provides a substantial risk of the knife becoming contaminated and then spreading viruses to all subsequent cuttings. Sterilisation measures are requisite for effective control. Although we could not find any reports of sweetpotato virus being spread through contaminated cutting tools, mechanical transmission of some viruses to virus-free vines by inoculation with sap from infected plants has been demonstrated (Domola, 2003; Wosula et al., 2012). Use of contaminated tools has been implicated in the spread of other plant viruses, especially in citrus during pruning (Garnsey and Whidden, 1971). If this is the case with sweetpotato, then the scope for viral spread and transmission is very broad and the risk is high.

Vine selling is accelerated by extreme shortage of sweetpotato vines because of the prolonged dry spell in the dry season, which scorches most vines except those in wetlands. Most farmers have limited access to wetlands where they can preserve vines during the dry season. Conditions are worse in Lamwo and Kitgum, which experience greater heat during the dry season than Gulu. There are relatively more people who grow sweetpotato during the dry season in wetland in Gulu than in Lamwo and Kitgum. Farmers grow sweetpotato during the dry season for three main reasons: to preserve vines for themselves, to preserve vines for sale at the start of the rainy season and for sale of tubers during the period of shortage. Gulu district therefore becomes a major centre where there are vine sellers, and most farmers can get sweetpotato vines for the new rainy season. Sweetpotato vines that are sold in the open market by farmers do not undergo a process of virus-free certification and the chances of buying infected vines are very high. In addition, most farmers who buy such vines tend to focus on traits such as early maturity, yield and tuber taste; they do not consider the disease status of vines.

B. tabaci and *M. persicae* are the two main insect vectors that spread sweetpotato viruses and aid in co-infection of sweetpotato plant by two or more viruses. The present study reveals that farmers have insufficient knowledge concerning these vectors. This limited knowledge can be attributed to the rarity of vectors within their farms or inadequate sensitisation of farmers to the presence and potential threat of these vectors. Reports indicate that women have limited access to essential agricultural information such as that concerning vectors and pests (Okonya et al., 2014), and women are the predominant sweetpotato farmers in this area. It has also been reported that extreme weather during the dry season does not favour rapid multiplication of the vectors and consequently their populations drop in this area, which experiences a prolonged dry spell during the dry season, which may account for a rarity of vectors. Further studies should be performed to adequately assess the populations of these two vectors.

Only a few farmers reported the use of pesticides to control insect pests. Similarly, Bashaasha et al. (1995) found that only a few sweetpotato farmers in Gulu and other districts in Uganda use pesticides in controlling insect pests of sweetpotato. Pesticide or insecticide application would reduce the population of whitefly and aphids and subsequently reduce spread viruses among sweetpotato plants (Opiyo et al., 2010). However, the costs associated with pesticides results in most local farmers adopting less expensive methods; for example, some farmers slash off the dense vegetative cover of sweetpotato to remove the food source for major pests such as caterpillars. Thus, vectors could potentially be regulated by such natural means as predators, parasitoids and prolonged dry spells, rather than farmer intervention, but more studies need to be done before management suggestions can be made.

Most farmers are unaware of sweetpotato virus diseases, the symptoms of virus infection and the burden associated with viruses. Limited knowledge of sweetpotato viruses is not unique to Northern Uganda but is commonly reported among farmers in other parts of

Africa (Domola, 2003). Limited knowledge was evident in the present study by many farmers mistaking damage on sweetpotato by insect pests to be virus symptoms. Such inadequate knowledge has implications for the management and control strategies farmers take on diseased plants. Most farmers tend to keep diseased vines because the vines have known good attributes. Additionally, some of the actions ordinary farmers take (Figure 1) are insufficient to curtail spread of sweetpotato viruses because they do not focus on destroying but rather maintaining the diseased plants. Thus, the vines continue to be used as planting materials, maintaining the virus for longer and potentially acting as a source of further infection. There is thus an urgent need to train farmers on symptom identification and possible measures concerning vines that look infected.

Conclusions

Exchange of sweetpotato planting materials among local farmers was high. In most cases such exchange occurs among neighbourhoods, relatives or farmers from different districts by sale of vines. The exchange of vines at different levels among local farmers risks spreading infected vines to wider geographical areas within the country. Additionally, limited knowledge of the symptoms of sweetpotato viruses among local farmers results in the use of infected vines for an extended period and the planting of new infected vines into their fields. This results in the perpetuation of virus-infected sweetpotato in the production cycle. Thus, there is a great need for local farmers in this area to be sensitised to the presence of viruses, be familiar with the symptoms of virus manifestation and understand the extent of damage caused to sweetpotato production. It is also important that local farmers be taught and encouraged to use phyto-sanitation measures such as uprooting diseased plants and planting visually healthy vines.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Supplementary Table 1. Socio demography of the respondents.

Variable	Frequency	Percentage
Age (years)		
11-20	15	3.93
21-30	132	34.55
31-40	112	29.32
41-50	61	15.97
51-60	28	7.33
61-70	25	6.54
71-80	5	1.31
81-90	4	1.05
Total	382	100
Marital status		
Single	45	11.87
Married	286	75.46
Divorced	30	7.92
Others (widow, widower and co-habiting)	18	4.75
Total	379	100
Sex of respondents		
Male	139	36.29
Female	244	63.71
Total	383	100

Supplementary Table 2. Odds of growing sweetpotato yearly between male and female farmers

How often you grow sweetpotato	Gender		Odds ratio		
	Male	Female	Odds estimate	Upper odds	Lower odds
Yearly	27	214			
Not yearly	2	29	1.4342	0.7068	2.9104

Supplementary Table 3. Distance of vine source from farmers gardens.

Variable	Frequency	Percentage
Distance of vine source from farmer's garden		
Near home	295	75.25
Another village	64	16.33
Another district	20	5.10
Another sub-county	13	3.32
Total	392	100

Supplementary Table 4. Odds of buying vines between female and male.

Buy vines from market	Gender		Odds ratio		
	Female	Male	Odds estimate	95% Upper interval	95% Lower interval
Yes	41	13			
No	203	126	1.95	1.009	3.796

Supplementary Table 5. Odds of knowledge on vectors between male and female.

Knowledge on vectors	Gender		Odds ratio		
	Female	Male	Odds estimate	95% Upper interval	95% Lower interval
Yes	107	72			
No	137	67	0.73	0.478	1.1038

Supplementary Table 6. Odds of farmers who asses presence of vectors on gardens.

Asses presence of vectors on garden	Gender		Odds ratio		
	Female	Male	Odds estimate	95% Upper interval	95% Lower interval
Yes	46	35			
No	195	104	0.70	0.425	1.1556

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Water excess in different phenological stages of canola cultivars

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The objective of this study was to determine the stage of development with greater sensitivity to water excess and the period of time required to compromise the emergence and grain yield components of canola. The experiments were performed in a greenhouse at the Federal University of Santa Maria and at the Farroupilha Federal Institute, Campus of São Vicente do Sul, RS during the 2015 agricultural year. The completely randomized experimental design was utilized to investigate phenological stages and periods of continuous water excess in the soil. Also, factors like percentage of emergence, emergence speed index, grain yield, number of siliques per plant, one hundred grains weight, dry matter of aerial part, silique length, number of grains per silique, and weight of 20 siliques were determined. The stages of rosette leaf formation and beginning of anthesis are the most sensitive to water excess in the soil. Water excess for 24 h is enough to reduce the emergence speed index. However, the percentage of emergence is not compromised by water excess up to 192 continuous hours. 24 h of water excess reduces the number of siliques per plant, dry matter of aerial part and grain yield of canola.

Key words: *Brassica napus*, grain yield, lowland cultivation, waterlogging.

INTRODUCTION

In Brazil, Rio Grande do Sul is the largest canola producing state, with potential for expansion due to the difference of six million hectares between the area cultivated with summer crops and winter crops (CONAB, 2015). However, most of these fields cultivated in the summer present edaphic problems such as low natural drainage of the soil and elevated water table, originating areas with frequent water excess, which can limit the development of certain crops.

Water excess is an abiotic stress that compromises plant growth by reducing the oxygen diffusion into the soil and hindering root cellular respiration (Bailey-Serres and Voesenek, 2008). It is caused mainly by excessive rainfall, irrigation, superficial water table, and poor natural drainage of the soils (Sairam et al., 2008), which jointly with the low atmospheric demand during the winter cause excessive accumulation of water in the soil.

The inadequate oxygen supply to the roots is considered the main problem for plants not adapted to water excess, mainly for inhibiting root respiration and

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energy production as ATP (Taiz et al., 2017). It compromises the growth of roots and aerial part (Liao and Lin, 2001) and the absorption of nutrients and water, which causes plant wilt (Ahmed et al., 2013; Loose et al., 2017), hormonal imbalance, early leaf senescence, and subsequent death of plants unadapted to these conditions (Rodríguez-Gamir et al., 2011).

Despite canola (*Brassica napus*) being considered a crop sensible to soil water excess (Zhou and Lin, 1995; Gutierrez Boem et al., 1996; Ku et al., 2009; Xu et al., 2015), recent studies in China have painted this crop as an alternative for cultivation in areas with water excess such as on the banks of the Yangtze River, which irrigated rice is the main crop. Cultivars tolerant to water excess have been developed such as ZS9 (Cheng et al., 2010; Zou et al., 2013, 2014), which presents a low reduction in yield when cultivated under these conditions (Zou et al., 2014).

Determining the phenological stage and period of tolerance of canola to water excess is required for the adoption of techniques that avoid water excess in stages of greater sensitivity. In winter canola, Zhou and Lin (1995) verified that rosette leaf formation stage is the most susceptible to prolonged water excess in the soil, followed by appearance of the floral bud and silique formation. Moreover, Ku et al. (2009) observed that although water excess in the vegetative phase reduces stomatal conductance and photosynthetic rate, the greatest negative effects are observed when the stress is applied in the reproductive phase. In addition to the reduction of stomatal conductance and photosynthetic rate, it causes reduction in shoot growth and later plant death after two weeks of exposure to water excess.

The duration of water excess is also of great importance, since only 3 days under these conditions, canola presents a reduction in grain yield (Gutierrez Boem et al., 1996). Hereupon, determining the most sensitive stage and period of time of water excess tolerated by canola cultivars is important, since better planning of the sowing date can be performed and even increasing the areas suitable for cultivation through agricultural zoning, allowing, in the future, the agricultural financing in areas formerly considered unsuitable for canola cultivation.

Given the aforementioned, the objective of this study was to determine the stage of development with greater sensitivity to water excess and the period of time required to compromise the emergence and grain yield components of canola.

MATERIALS AND METHODS

The experiments were carried out in greenhouse in two locations at the Crop Science Department of the Federal University of Santa Maria (UFSM), Santa Maria, RS (29°43'23" S; 53°43'15" W; 95) and at the Farroupilha Federal Institute (IFFar), São Vicente do Sul Campus, RS (29°42'21''S; 54°41'39'' W; 129 m), during the 2015 agricultural year. According to the Köppen climate classification, the climate of the region is Cfa, subtropical humid with warm summers and without defined dry season (Heldwein et al., 2009).

In Santa Maria, the experiment was performed in a greenhouse with transparent polyethylene coverage, sides closed by screen with protection against aphids, and oriented in the north-south direction. In São Vicente do Sul, the experiment was carried out in a greenhouse with transparent polyethylene coverage and open sides.

The experimental units were polyethylene vessels externally painted with white paint, filled with soil and placed in a bucket larger than the vessel. Wooden blocks were placed at the bottom of each bucket in order to support the vessels with soil that stays suspended three centimeters from the bottom to enable drainage of water when necessary.

Sixty days before sowing the soil was sieved, homogenized and calcareated. Both the application of limestone and the fertilization (base and cover) were carried out according to the chemical analysis of the soil, following the recommendations of the manual of fertilization and liming for the states of Rio Grande do Sul and Santa Catarina.

The water level during the application of water excess treatment was maintained at a height of 15 cm by a perforation on the lateral border of each bucket (Loose et al., 2017). In this way, the water level was maintained around two centimeters below the seeds. The vessels were randomized and arranged in benches 50 cm high and in two simple rows on each bench.

In the treatments that received excess water, the water was applied daily directly on the soil of the vessels and inside the buckets. The water was removed from the bucket after the period of water excess, allowing drainage of the excess through the holes in the bottom of the vessel. The soil moisture of the other treatments that did not have water excess treatment was kept close to the field capacity.

In Santa Maria, the experimental design used was completely randomized with five replications in a 4 × 5 factorial scheme (phenological stages and periods of water excess), totaling 100 experimental units. In São Vicente do Sul, a completely randomized design with four replications was used in a 5 × 4 + 1 factorial scheme (phenological stages and periods of water excess) with one additional treatment, without water excess, totaling 69 experimental units.

The qualitative factor phenological stage comprised four levels in Santa Maria, being sowing (S), rosette leaf formation (RF), beginning of anthesis (BA), and end of anthesis (EA). In São Vicente do Sul, in addition to those levels mentioned previously, water excess was also applied in the emergence stage (EM), totaling five levels for this factor. The phenological stages were characterized according to Iriarte and Valetti (2008).

The quantitative factor period of water excess was comprised five periods of continuous water excess in the soil: 0, 24, 48, 96 and 192 h applied at each level of the phenological stage, when 50% of the plants were in the desired phenological stage. Water excess was maintained in each vessel for the corresponding period of each treatment. After removal of the excess water, the plants received the same management as the other plants without water excess.

The sowing procedure was carried out with the soil under field capacity conditions on 28 May, 2015 in Santa Maria, with the hybrid Hyola 411. In São Vicente do Sul, sowing was performed on 08 May, 2015 with the hybrid Hyola 433. Both cultivars presented germination percentage of 87% and were treated with fungicide. Water excess treatments were started after sowing in the experimental units in which the water excess treatment was applied at sowing.

Thinning was performed when the plants were with two definitive leaves, leaving one plant per hole and two plants per vessel that were conducted until harvest. The harvest of two plants of each experimental unit was carried out on 10 October, 2015 (129 days after sowing) in Santa Maria and on 09 September, 2015 (121 days after sowing) in São Vicente do Sul.

The following variables were analyzed: percentage of emergence, emergence speed index (Maguire, 1962), dry matter of aerial part, grain yield per vessel, silique length, number of grains per silique, weight of 20 siliques and one hundred grains weight. The grain moisture was corrected to 10%.

Data of each variable were submitted to the Shapiro-Wilk test of normality of errors and Bartlett test of homogeneity of variances of the treatments in the Action software (Equipe Estatcamp, 2014). Data that did not meet these assumptions were transformed by log (x) and utilized for analyses of variance.

The data were submitted to analysis of variance at 5% of probability and when a significant effect was verified; data referring to the period of water excess were submitted to regression analysis while data referring to the phenological stages were compared by the Scott-Knott test at 5% probability using the Sisvar software (Ferreira, 2011).

RESULTS AND DISCUSSION

The water excess in the soil influenced emergence speed index but did not influence percentage of emergence for both canola cultivars. The emergence speed index decreased exponentially for cultivar Hyola 411 with the increase in the period of water excess, being maximum without water excess (5.67) and minimum with the application of 192 h of water excess (0.85). The greater period of water excess in the soil (192 h) reduced the emergence speed index by 85% when compared to the treatment without the application of water excess (Figure 1) and a period of only 24 h of water excess reduced emergence speed index by 21.3%.

Similar results were obtained by Loose et al. (2017) with sunflower, where the increase in periods of water excess from zero to 240 h exponentially reduced emergence speed index. Moreover, the 48 h period of water excess was enough to compromise and reduce the emergence speed index.

A linear decrease in the emergence speed index with the increase of the period of water excess in the soil occurred for the cultivar Hyola 433, being maximum without water excess (3.33) and minimum with the application of 192 h of water excess (1.46). The greater period of water excess in the soil (192 h) reduced the emergence speed index of canola seedlings by 46.4% when compared to the treatment without water excess (Figure 1) and a period of only 24 h of water excess reduced emergence speed index by 5.8%.

The emergence speed index in soils with water excess reflects seed vigor, that is, the capacity that the seeds have for a rapid emergence and establishment in unfavorable conditions. Thus, water excess in the soil reduces the vigor of canola seeds and this may cause a reduction of plant stand in the field. Furthermore, seedlings may die due to the lack of oxygen in the soil if the water excess persists for many days due to frequent rainfall, low atmospheric demand, poor soil drainage, or groundwater level near the surface.

The reduction of emergence speed index in canola is probably due to the reduction in soil oxygen content, which temporarily inhibits or reduces the physiological activity of the seeds by reducing the respiratory process, reducing germination speed (Marcos Filho, 2005) and consequently the emergence of seedlings. However, oxygen returns to the soil after the stress removal, giving continuity to the germination process.

The emergence speed index under water excess can be used to select canola cultivars tolerant to water excess in an early stage of development (Cheng et al., 2010). Selection of cultivars from germination tests is a relatively inexpensive and effective option and the tolerance to water excess is a trait transmitted during plant ontogeny (Zou et al., 2014).

Although there was a great reduction in emergence speed index, no differences occurred in percentage of emergence. A delay in the emergence time of the plants with longer periods of water excess was noticed during the conduction of the experiment. However the seeds remained viable, since the emergence of the seedlings occurred a few days after removing the water excess.

The capacity of Hyola 411 and Hyola 433 cultivars to maintain an elevated percentage of emergence when submitted to water excess may be related to the melanin content of the seed integument, since these seeds have a darker coloration. Therefore, the greater the melanin content (darker integument), the slower the water absorption and lower the loss of solutes by the seeds (Zhang et al., 2008), explaining the delay in the emergence without the loss of seed viability.

The phenological stage of application of water excess influenced the number of siliques per plant and grain yield in the Hyola 411 cultivar. Water excess influenced dry matter of the aerial part and grain yield. Treatments interacted with and influenced dry matter of the aerial part, number of siliques per plant, and grain yield.

The dry matter of aerial part adjusted to the linear model when the water excess was applied at sowing, increasing the dry matter of aerial part with increased exposure time of the crop to water excess (Figure 2A). When water excess was applied at the beginning of anthesis, the values also adjusted to the linear model but the dry matter of aerial part decreased with increasing exposure time of the crop to the water excess. Water excess of 192 h applied at sowing and at the beginning of anthesis caused respectively an increase of 21.2% and a reduction of 22.9% of the dry matter of aerial part of canola when compared to the control treatment without water excess.

When the water excess was applied at the rosette leaf formation stage and at the end of anthesis, the values did not fit to the mathematical models tested (linear, quadratic, exponential, and power model). Although, there was a tendency (not significant at 5% probability) of dry matter of aerial part reduction with the increase in the exposure time of the crop to water excess wherein the continuous occurrence of water excess for 192 h reduced

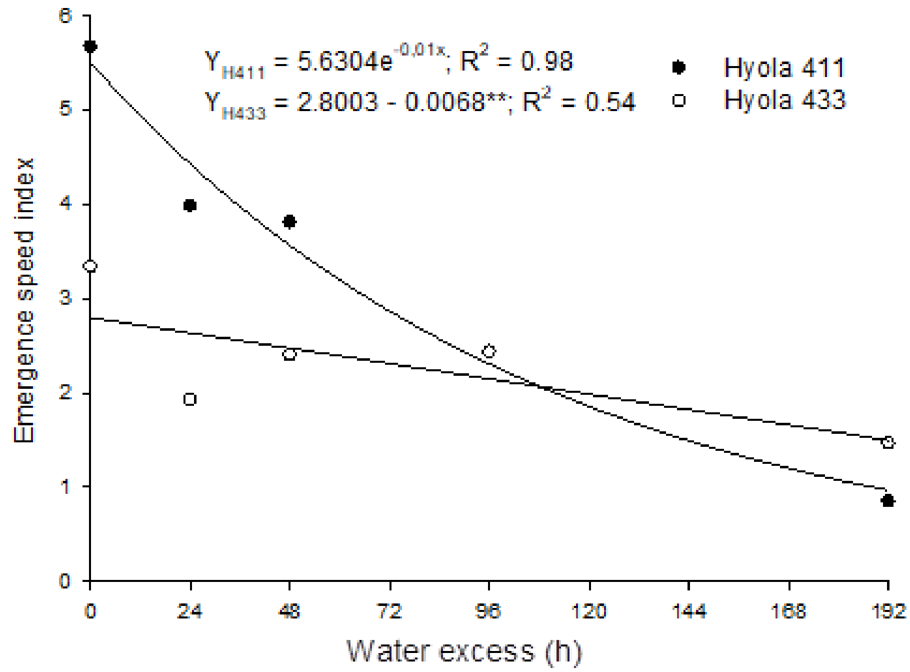

Figure 1. Emergence speed index of the cultivars Hyola 411 and Hyola 433 as a function of the period of water excess in the soil (h).

the canola dry matter of aerial part in 7.6 and 1% when applied respectively at the rosette leaf formation and at the end of anthesis.

These results somewhat disagree from those obtained by Issarakraisila et al. (2007), who studying two species of Brassicas reported a reduction of 81% in the dry matter of aerial part when the plants were submitted to water excess at the rosette leaf formation stage (with four to six leaves). Moreover, Gutierrez Boem et al. (1996) concluded that the rosette leaf formation stage is the most sensitive in relation to the grain filling and that the longer the period of exposure of the crop to water excess, the greater the reduction of dry matter of aerial part. In this study, sowing and early stages of anthesis were more sensitive to water excess for Hyola 411 cultivar regarding the dry matter of aerial part.

The number of siliques per plant increased with increasing exposure time of the crop to water excess applied at sowing (Figure 2B). Water excess of 192 h increased the number of siliques per plant by 46.4%. Although water excess in other stages did not fit to any mathematical model tested, there was a tendency to reduce the number of siliques per plant with increased exposure time of canola to water excess mainly at the rosette leaf formation stage. Water excess of 192 h at the rosette leaf formation stage reduced the number of siliques per plant by 26.5% when compared to the control treatment. Similar results were obtained by Zhou and Lin (1995) and Leul and Zhou (1998) with winter canola, with a reduction in number of siliques per plant respectively of 14.2 and 28.2% when water excess was applied at the rosette leaf formation stage (five leaves).

Smaller reduction in number of siliques per plant occurred when water excess was applied at the end of anthesis, which may be due to siliques being already formed at this stage of development with no occurrence of abortion. This revealed that at this stage the negative effects of the water excess are smaller for the plant than in the rosette leaf formation, when the plant yield is being defined.

Water excess applied for 192 h at the beginning of anthesis reduced the number of siliques per plant compared to the control treatment (without water excess) by 5.2%. This reduction is smaller when compared with that obtained by Ku et al. (2009) and by Xu et al. (2015), in which the water excess in the reproductive phase and at the beginning of anthesis reduced respectively, the number of siliques per plant on average 65.9 and 24.3%.

The grain yield with application of water excess at sowing and at the end of anthesis did not adjust to the mathematical models tested but increasing the period of water excess tended to decrease the grain yield (Figure 2C). When the water excess was applied at the stages of rosette leaf formation and at the beginning of anthesis, the grain yield was reduced linearly with the increase of the exposure time of canola to water excess.

The stages of rosette leaf formation and the beginning of anthesis in canola can be considered the most sensitive to water excess in the soil after the plant emergence, since the application of 192 h of water

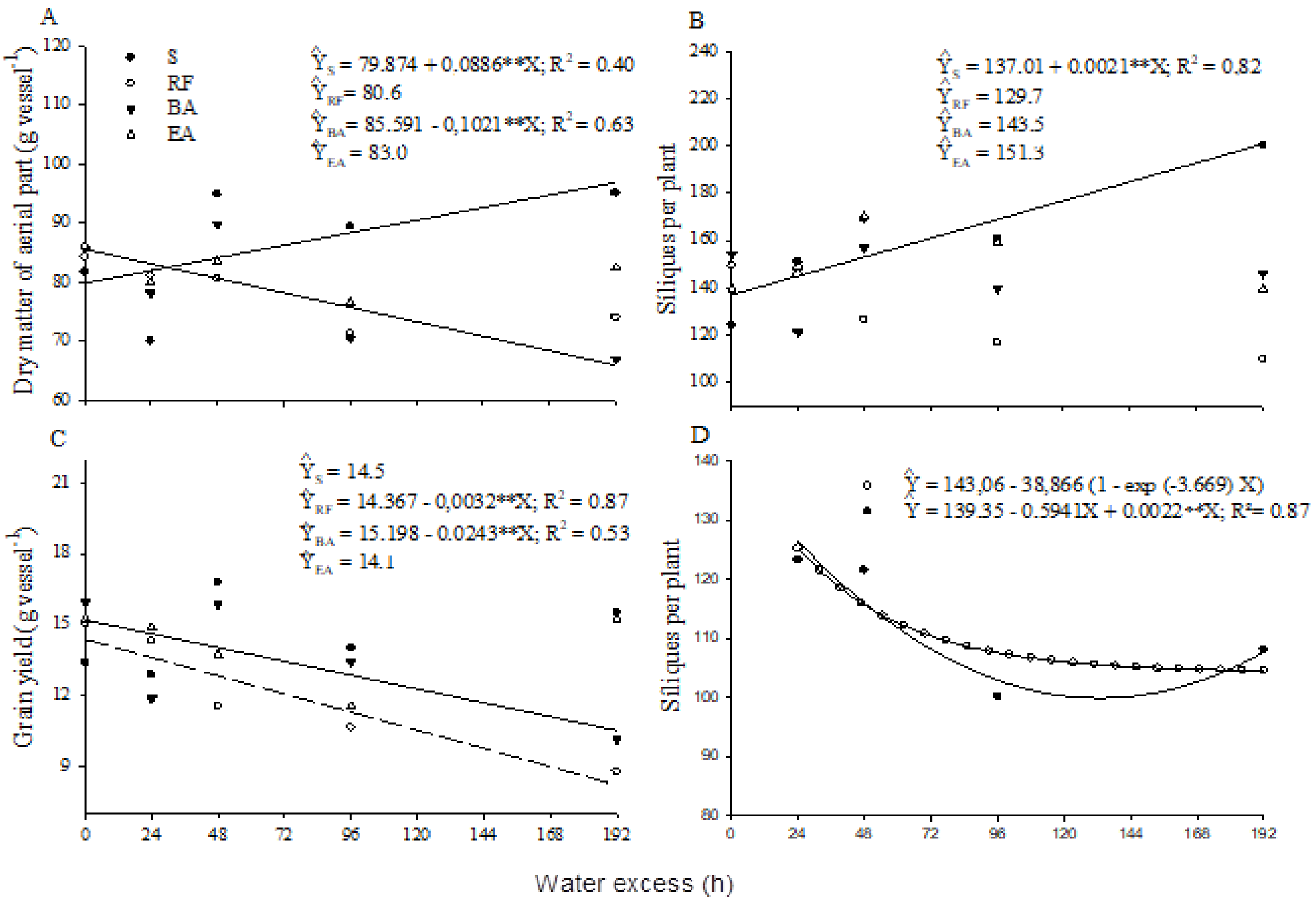

Figure 2. Relation between the dry matter of aerial part (A), number of siliques per plant (B) and grain yield (C) of the canola cultivar Hyola 411; and number of siliques per plant of the canola cultivar Hyola 433 subjected to different periods of water excess at sowing (S) and at the stages of rosette leaf formation (RF), beginning of anthesis (BA), and end of anthesis (EA). Only the data that fit the mathematical models were plotted with a line.

excess in the soil caused respectively a reduction of 42.7 and 30.7% in grain yield when compared to the control treatment (without water excess). Some authors (Zhou and Lin, 1995; Leul and Zhou, 1998; Zou et al., 2014) also found a reduction in grain yield when the water excess was applied at the rosette leaf formation stage, with reduction respectively of 26.2, 21.3, and 50%. At the beginning of anthesis, Xu et al. (2015) found an average reduction of 20% in the grain yield of canola but some cultivars presented a reduction of 30 to 41.9% when compared to the control treatment.

The grain yield reduction with increased exposure time of canola to water excess at the rosette leaf formation stage may be due to the plants being juvenile and fragile (Zou et al., 2014). At this stage, the plants present lower nutrient reserves and smaller root system, reducing the absorption of water and nutrients. In addition to having a small root system, the absence of oxygen in the soil reduces the root growth rate (Vidal, 2011), causing a reduction in ATP production and in the transport of photoassimilates in the aerial part (Wample and Davis, 1983), lower production of dry matter of aerial part (Figure 2A), and lower grain yield. In addition, tissues exhibit greater respiration rates because they require more energy and carbon skeletons for dividing and lengthening cells in the early stages of development such as in the rosette leaf formation (Taiz et al., 2017). Thus, the effects of absence of oxygen are more pronounced and more strongly affect growth and subsequently, productivity.

At the beginning of anthesis, the reduction in grain yield with the increased periods of water excess can be due to the abortion of flowers and siliques by the reduction in the transport of photoassimilates to these structures as the water excess compromises the transport in the phloem (Wample and Davis, 1983), besides reducing the production of photoassimilates (Liao and Lin, 2001). Thus, water excess reduces the production of chemical energy by the plant and its transport and distribution to the sinks, compromising the production and fixation of reproductive structures such as flowers and siliques.

No significant difference was observed for one hundred grains weight and grain yield for the cultivar Hyola 433 when the control treatment (without water excess) was compared with the other treatments (with water excess). However, water excess in the soil reduced number of siliques per plant. Within the water excess treatments, the phenological stage and the interaction of the factors did not influence any of the studied variables, even though the water excess factor influenced the number of siliques per plant (Figure 2D).

The greatest number of siliques per plant for the cultivar Hyola 433 was obtained without the application of water excess in the soil, with a mean of 131 siliques per plant. A second-degree concave curve is one option to represent the decrease in the number of siliques per plant as a function of increased periods of water excess, which would imply an increase in the number of siliques with periods of water excess greater than 192 h. However, this variation is not logical, since longer periods of water excess would cause root respiration problems and from a certain critical value would possibly cause the plants to die.

Thus, another explanation for the number of siliques per plant can be given with an asymptotic curve, which from 96 h of water excess there would be a tendency to stabilize the reduction of the number of siliques per plant near a minimum limit value (104 siliques), which would result from the plant energy expenditure to modify morphological or physiological structures to acclimatize to the condition of water excess. Exposure of canola for 24, 48, 96 and 192 h to water excess caused a reduction in the number of siliques per plant respectively of 9.3, 16.8, 26.3, and 23.6% when compared to the control treatment (without water excess). The results corroborated with those obtained by Zhou and Lin (1995), Leul and Zhou (1998), Xu et al. (2015) and Zou et al. (2014).

The number of siliques per plant is an important component of grain yield in canola (Diepenbrock, 2000; Xu et al., 2015) and plants that produce the greatest number of siliques possibly reach higher yields. However, no difference was obtained in grain yield for the cultivar Hyola 433 in the present study, although there was a difference for number of siliques per plant. Another reason may have been the partial recovery of the plant after the removal of excess water in the soil that allowed a good nutrition of the grains in formation with less competition between sinks in the plant, partially compensating the reduction of number of siliques per plant. Further studies should be developed to confirm the results obtained.

The grain yield components obtained for the Hyola 433 and Hyola 411 cultivars were divergent in the direction of the variables that suffered an effect of the factors studied but the trend remained: lower grain yield with longer period with water excess.

Conclusions

1. For canola, the stages of rosette leaf formation and beginning of anthesis are the most sensitive to water excess in the soil.
2. Water excess for 24 h is enough to reduce the emergence speed index of canola. However, the percentage of emergence is not compromised by water excess up to 192 continuous hours.
3. The tolerance period to water excess varies according to the phenological stage although 24 h of water excess reduces the grain yield components, essentially the number of siliques per plant, dry matter of aerial part, and grain yield of canola.
4. The canola cultivars Hyola 411 and Hyola 433 respond differently when subjected to water excess in the soil, however, both present the tendency of reduction in grain yield with increased period of water excess.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Effects of biochar on carbon pool, N mineralization, microbial biomass and microbial respiration from mollisol

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Biochar incorporation as a soil amendment has been shown to enhance soil quality. However, there has been conflicting reports on its short term effects on C and N mineralization and microbial biomass. An incubation experiment was conducted to determine the effects of three different levels (0.5, 1 and 2%) of biochar on carbon mineralization, soil organic carbon, nitrogen mineralization, microbial biomass and total nitrogen from mollisols of two different organic matter (high organic matter soil and low organic matter soil) levels. The experiment consisted of four treatments (Soil, Soil + 0.5% biochar, Soil + 1% biochar and Soil + 2% biochar) and each was replicated three times. Overall, soil respiration rate was reduced by biochar additions over a 100-day period. Two percent biochar application rate showed greatest CO_2-C reduction. Soil respiration in high organic matter soil was higher than low organic matter soil. NO_3^--N level was reduced by biochar addition in both high and low organic matter soils. Control (Soil) of the high organic matter soil showed the highest NO_3^--N (33.79 mg kg^{-1}) and NH_4^+-N (7.23 mg kg^{-1}) values at 70 days. The total nitrogen was increased by biochar additions; 1 and 2% application rates showed the highest total nitrogen values. Biochar additions also increased soil microbial biomass carbon and soil microbial biomass nitrogen of both soils.

Key words: Biochar, C mineralization, N mineralization, microbial biomass.

INTRODUCTION

Biochar is the product of the thermal degradation of organic materials in the absence of air (pyrolysis) and is distinguished from charcoal by its use as soil amendment (Lehmann et al., 2011). It is considered as a carbon-rich organic matter with long residence time up to hundreds of years (Kuzyakov et al., 2009; Lehmann et al., 2015). Biochars made from diverse biomass species (feedstock) are characterized by different morphological and chemical properties but also characteristically differ based on specific pyrolysis conditions (that is, final pyrolysis temperature or peak temperature, rate of charring or ramp rate, and duration of charring time) (Mukherjee and Zimmerman, 2013). Hence biochar properties and the effect on crop production depend

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on feedstock, pyrolysis conditions and soil type (Jeffery et al., 2011). The effect of biochar is said to be strongly connected to the soil properties and the climate but the correlations with crop yield are not completely clear (Cornelissen et al., 2018). Several authors (Agegnehu et al., 2016; Jeffery et al., 2017) have stated that the yield increases are related to an overall improvement of soil qualities. Biochar application is reported to stimulate (Wardle et al., 2008; Luo et al., 2011) or conversely, to suppress (Keith et al., 2011; Zimmerman et al., 2011) the mineralization of native soil organic carbon (SOC); these effects are termed positive and negative priming, respectively (Luo et al., 2011; Keith et al., 2011; Zimmerman et al., 2011). The differing results observed in previous studies may have been due to variations in the proportion of labile C in biochars (Luo et al., 2011), the presence or absence of plant-derived labile organic matter input in soil (Keith et al., 2011), and the degree of biochar ageing in soil (Zimmerman et al., 2011; Cross and Sohi, 2011; Liang et al., 2010).

Soil respiration is a product of several rhizospheric processes, which is root exudation, root respiration, and root turnover, as well as decomposition of litter and bulk soil organic matter from various pools with different characteristic turnover times (Luo et al., 2001). Release of CO_2 from soils due to the production of CO_2 by roots and soil organisms and, to a lesser extent, chemical oxidation of carbon compounds is commonly referred to as soil respiration. Soil surface CO_2 efflux, or soil respiration, is a major component of the biosphere's carbon cycle which is influenced by the environment change because it may constitute about three-quarters of total ecosystem respiration (Law et al., 2001). Application of biochar has been shown to have a variety of effects on the soil biota which may be associated with its impacts on C and N cycling. Biochar has the capacity to potentially sequester C, and also has agronomic benefits such as improving soil quality, nutrient availability and crop yield (Sohi et al., 2010; Spokas et al., 2012; Schulz and Glaser, 2012). Biochar amendments can alter soil N dynamics (Clough and Condron, 2010), increase soil pH, base saturation, available nutrient content, nutrient retention and CEC (Cation Exchange Capacity) (Tiessen et al., 1994; Glaser et al., 2002; Moreira et al., 2005; Mukherjee and Zimmerman, 2013), and decrease Al toxicity (Glaser et al., 2002).

Many research works (Spokas and Reicosky, 2009; Prayogo et al., 2014) have been done to determine how mineralization of C and N is affected by biochar application but few emphases have been made on testing the effects of different biochar application rates on microbial respiration, nitrogen mineralization, microbial biomass and soil organic carbon pool from mollisol of two different suborders with different organic matter levels. The rate of biochar application on the soil is expected to have serious influence on the impact of biochar on soil processes, including microbial soil respiration and nitrogen mineralization. Therefore, a laboratory incubation method in which soil temperature and moisture regimes could be manipulated was employed. The objective of this study is to determine the effects of low and high biochar application rates on microbial soil respiration, soil organic carbon content, N mineralization as well as microbial biomass from mollisol of two different suborders with varying organic matter levels.

MATERIALS AND METHODS

Soil and biochar

Mollisol consisting of two levels of organic matter, high organic matter and low organic matter, was used for the experiment. The high organic matter soil which is of the Suborder Udolls (Dark colored) was obtained from the Experimental and Practical Basement of Northeast Agricultural University while the low organic matter soil which is of the Suborder Albolls (Light colored and high concentration of sand and silt) was obtained from Northeast Forestry University. The crop planted in the previous season on both soils was maize. The soils were collected randomly at a depth of 0-20 cm, after sieving to < 2 mm and the basic properties determined (Table 1). The biochar which was produced from corn at a pyrolysis temperature of 450°C in an oxygen-restricted environment in a batch system was provided by Jin and Fu Agriculture Co., China and was crushed to pass through 2 mm sieve. The properties of biochar and soils are listed in Table 1.

Incubation procedure and soil respiration

Soil sieved to 2 mm was amended with biochar at different rates, 0.5, 1 and 2% which are equivalent to 10, 20 and 40 t ha^{-1} respectively. For incubation, dry weight soil equivalent to 25 g were placed in air-tight glass jars (0.3 L) in a completely randomized design for anaerobic incubation with 3 replicates. All treatments and control were moistened to 60% of their water holding capacity and incubated for 100 days at 25°C in the dark. Water content was regularly checked gravimetrically and adjusted with de-ionized water. Carbon mineralization was measured as CO_2-C using alkaline trap (Tufekcioglu et al., 2001) during 100 days of incubation. The emitted CO_2 was trapped in 10 ml of NaOH which was titrated with HCl on days 5, 10, 15, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90 and 100 after carbonate precipitation with $BaCl_2$.

Analytical methods

Soils from the jars were analyzed for selected chemical properties; soil organic carbon, microbial biomass C and N, Total N. Ammonium nitrogen and nitrate nitrogen contents were extracted with 2M KCl (1:10 w/v) after shaking for 2 h and determined colorimetrically, using the salicylate method as the variation of Berthelot-Phenate method. Microbial biomass C and N were determined by fumigation and extraction technique as described by Vance et al. (1987). SOC was measured using wet oxidation with $K_2Cr_2O_7$; while Total N was measured using Kjeldahl method as described by Gupta (2006).

Statistical analysis

The statistical analyses were performed using SPSS 19.0 program.

Table 1. Physical and chemical properties of soils and biochar.

Parameter	Soil (High OM)	Soil (Low OM)	Biochar
pH	6.12	6.02	9.89
Total N (g kg^{-1})	0.7	0.34	6.89
Available N (mg kg^{-1})	Nd	Nd	*
Organic Carbon (g kg^{-1})	38.2	10.1	415.3
Available P (mg kg^{-1})	23.8	19.3	*
Total P (g kg^{-1})	Nd	Nd	10.26
Available K (mg kg^{-1})	185.6	180.1	25.9
Particle Size (g kg^{-1})			
Sand	500	520	Nd
Silt	190	200	Nd
Clay	310	280	Nd
Textural Class (USDA)	Clay Loam	Clay Loam	Nd
Bulk Density (g cm^{-3})	1.38	1.42	Nd

*<LOD of the technique, Nd = Not determined.

After testing of assumptions, One-Way analysis of variance (ANOVA) was performed followed by Duncan Multiple Range Test (DMRT). Results marked as significantly different are different at P<0.05 unless specified in text. All reported values are means of three replicates.

RESULTS

Microbial soil respiration

Biochar application to soil had effects on CO_2-C release (Figure 1) with respect to soil alone. Difference was found for CO_2-C among the rates of application in the two soils. The mineralization of C was slightly depressed by 1% biochar addition and 2% biochar addition; however, stimulated by 0.5% biochar addition. The respiration rate of 0.5% biochar was higher at the beginning than the control until 20 days incubation (15 mg CO_2-C $g^{-1}d^{-1}$) for high organic matter soil (Figure 1a) and 15 days incubation (15.4 mg CO_2-C $g^{-1}d^{-1}$) for the low organic matter soil (Figure 1b). There was a sharp decline in all respiration rates during the first 25 days of incubation after which the respiration rate became steady. It was observed that the higher the rate of biochar applied, the lower the amount of CO_2-C released, which is an indication that there is decreased decomposition following biochar application to soils. High organic matter soil recorded higher soil respiration rate than low organic matter soil throughout the incubation period.

Soil organic carbon (SOC)

SOC increased with the increasing biochar application in all treatments for both high organic matter soil and low organic matter soil at 50 and 100 days of incubation (Table 2). Soil + 1% biochar and Soil + 2% biochar were significantly (P<0.01) higher than control (soil). The highest SOC value (58.8 g kg^{-1}) was shown by Soil + 2% biochar of the high organic matter soil at 100 days incubation.

N mineralization

The levels of NO_3^--N increased between 20 and 40 days for both soils (Table 3). Mineralization was highest in control (Soil), followed by Soil + 0.5% biochar. Biochar application significantly (P<0.05) reduced mineralization at 20, 40, 60 and 90 days for the high organic matter soil; while significant (P<0.05) reduction by biochar was observed at 40 and 100 days for the low organic matter soil. The highest NO_3^--N levels were recorded at 70 days for both soils. Between 20 and 40 days, NH_4^+-N levels reduced in all soils (Table 4). Greatest NH_4^+-N reduction was observed at 90 days, indicating net immobilization. Rates of immobilization were significantly (P<0.05) highest in treatment receiving 2% biochar relative to other treatments. Control (Soil) was significantly (P<0.05) higher than Soil + 2% biochar at 40 and 90 days for the high organic matter soil; while significant differences among treatments were observed at 20 and 90 days for the low organic matter soil.

Soil microbial biomass carbon (SMBC)

Variability of SMBC under different rates of biochar was large as shown in Figure 2. A sharp increase in SMBC was observed at 20 days for both soils with a further

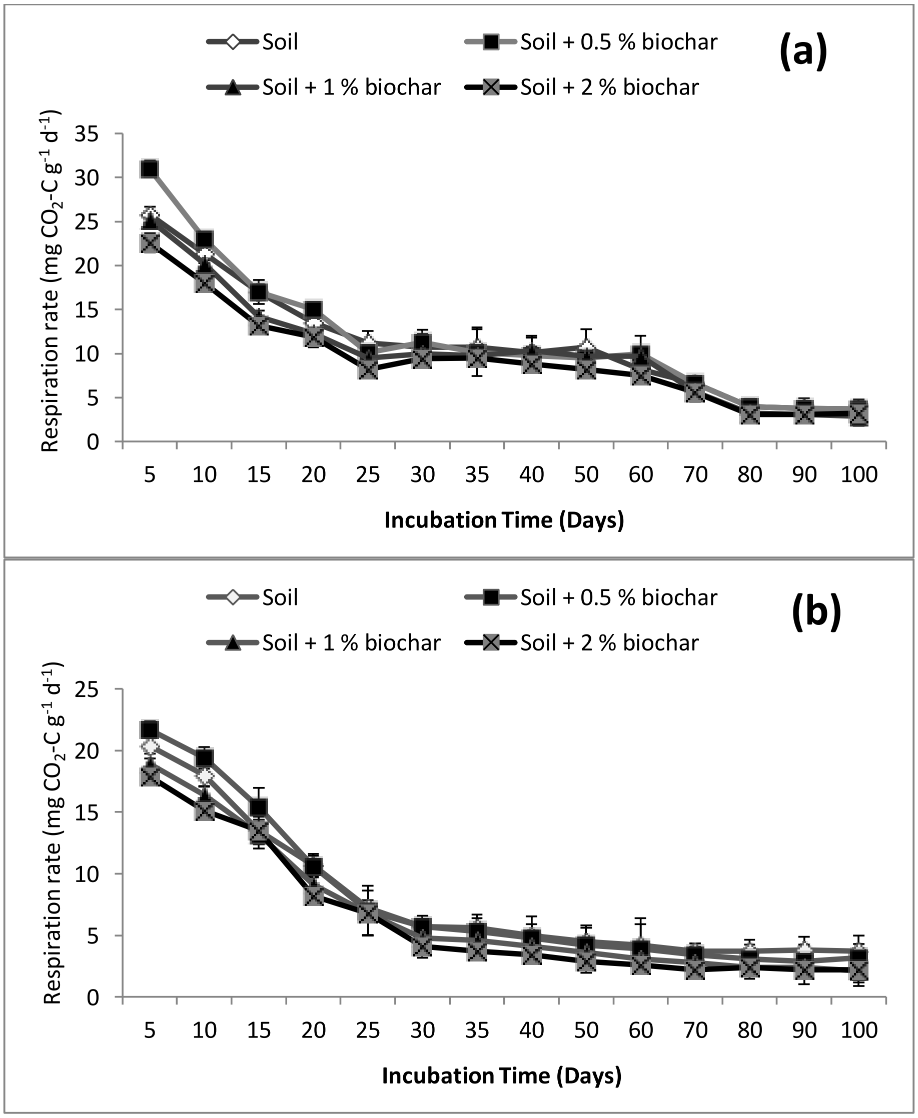

Figure 1. Respiration rates of high organic matter soil (a) and low organic matter soil (b) amended with biochar at 0.5, 1 and 2% levels (Means ± SE, n = 3).

Table 2. SOC of high organic matter soil (a) and low organic matter soil (b) at 50 days and 100 days of incubation as affected by biochar additions (Mean ± SE, n = 3).

Soil code	Treatments	SOC (g kg^{-1})	
		50d	100d
(a)	Soil	37.3±1.12 ^{d}	37.6±1.79 ^{c}
	Soil + 0.5 % biochar	53.1±0.56 ^{c}	53.1±1.12 ^{b}
	Soil + 1 % biochar	56.4±1.12 ^{b}	55.8±1.96 ^{b}
	Soil + 2 % biochar	57.8±0.56 ^{a}	58.8±0.85 ^{a}
(b)	Soil	9.7±1.71 ^{d}	10.2±1.71 ^{d}
	Soil + 0.5 % biochar	21.8±1.12 ^{c}	22.5±1.71 ^{c}
	Soil + 1 % biochar	28.1±1.71 ^{b}	27.6±1.16 ^{b}
	Soil + 2 % biochar	36.7±1.41 ^{a}	37.1±1.71 ^{a}

Means followed by different letters are significantly different (P<0.01).

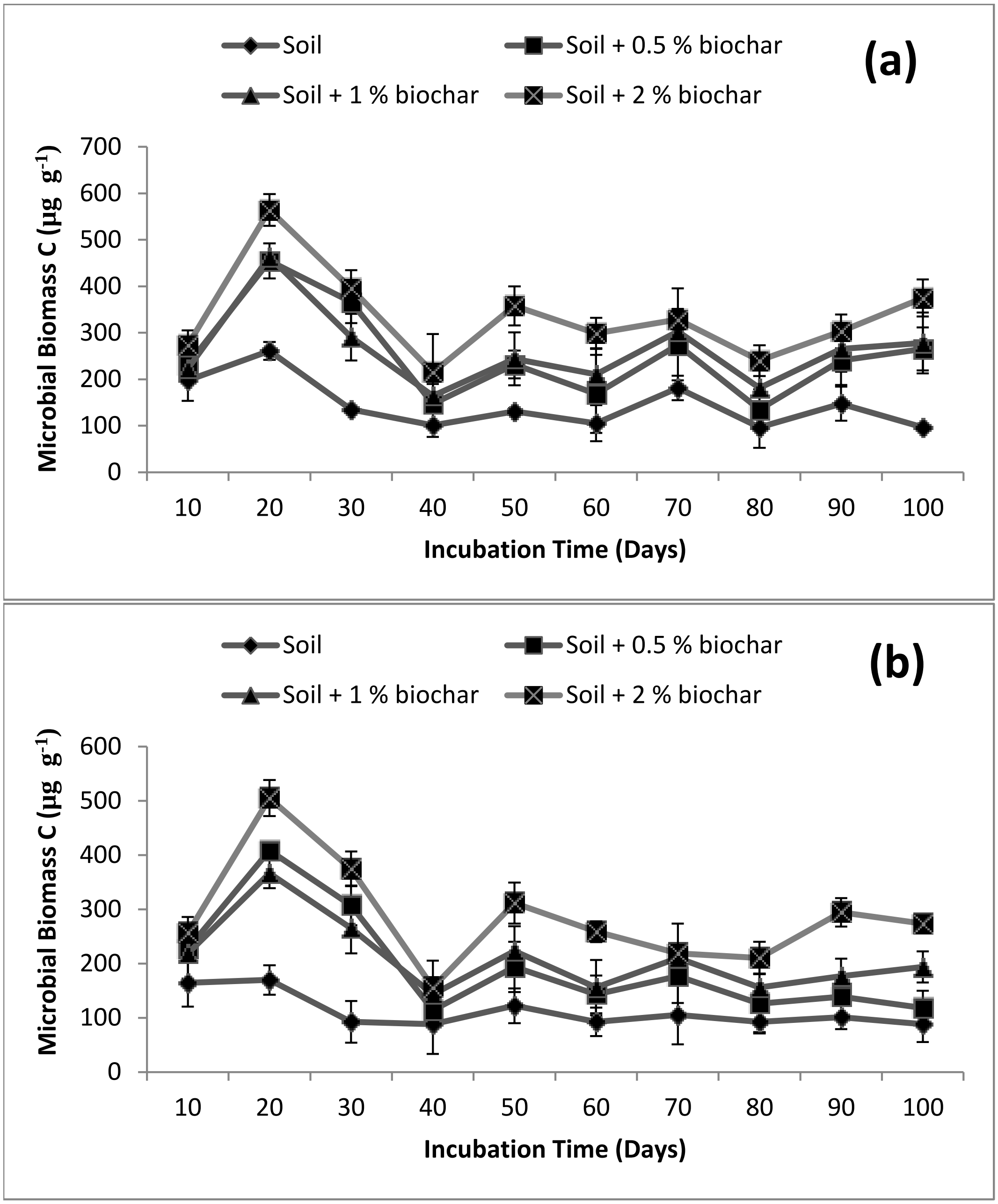

Figure 2. Soil MBC for high organic matter soil (a) and low organic matter soil (b) amended with biochar at 0.5, 1 and 2% levels (Mean ± SE, n = 3).

decline at 40 days. SMBC was highest at 20 days for both high organic matter soil and low organic matter soil (Figure 2a and b). The results of the soil microbial biomass analysis also indicate that the high organic matter soil contains the greatest microbial biomass C. Treatment receiving 2% biochar was significantly ($P<0.05$) higher than other treatments throughout the incubation period. The variations in the microbial biomass C among the different soil types is an indication of the differences in their microbial activities.

Soil microbial biomass nitrogen

At day 50 of the high organic matter soil (Table 5), treatment receiving 2% biochar showed the highest SMBN (111.9 µg g^{-1}) and it was significantly ($P<0.001$) higher than other treatments; while for the low organic matter soil, treatment receiving 2% biochar (80.8 µg g^{-1}) was also significantly ($P<0.001$) higher than other treatments. At day100 of the high organic matter soil (Table 5), the treatment receiving 2% biochar showed the

Table 3. NO_3^--N levels in biochar-amended high organic matter soil (a) and low organic matter soil (b) during incubation.

Soil code	Treatments	NO_3^--N (mg kg^{-1})					
		20 d	40 d	60 d	70 d	90 d	100 d
(a)	Soil	15.32±0.27[a]	23.37±0.90[a]	19.17±1.11[a]	33.79±1.27[a]	26.84±1.08[a]	27.13±0.55[a]
	Soil + 0.5 % BC	15.19±0.24[a]	21.47±1.59[b]	17.34±0.10[b]	33.44±2.65[a]	25.10±1.24[ab]	24.94±0.05[a]
	Soil + 1 % BC	14.15±0.47[b]	21.06±0.62[b]	16.10±1.07[b]	31.74±1.83[a]	24.22±0.57[b]	24.38±3.35[a]
	Soil + 2 % BC	13.93±0.49[b]	21.28±0.36[b]	16.77±0.34[b]	32.94±0.06[a]	23.30±1.06[b]	26.43±1.74[a]
(b)	Soil	6.52±0.47[a]	7.99±0.76[ab]	9.73±0.43[b]	26.68±2.04[a]	12.60±3.26[a]	17.56±1.76[a]
	Soil + 0.5 % BC	6.44±0.33[a]	9.44±1.42[a]	9.35±1.10[a]	25.55±2.72[a]	11.94±0.83[a]	15.67±0.56[ab]
	Soil + 1 % BC	6.51±0.22[a]	6.99±1.47[b]	8.56±0.57[a]	24.35±1.06[a]	9.81±0.17[a]	14.72±0.81[b]
	Soil + 2 % BC	5.97±0.68[a]	8.81±0.25[ab]	8.46±0.83[a]	23.68±2.05[a]	9.54±0.97[a]	14.21±1.90[b]

Means followed by different letters are significantly different (P<0.05), BC = Biochar.

Table 4. NH_4^+-N levels in biochar-amended high organic matter soil (a) and low organic matter soil (b) during incubation.

Soil code	Treatments	NH_4^+-N (mg kg^{-1})					
		20 d	40 d	60 d	70 d	90 d	100 d
(a)	Soil	3.28±2.19[a]	0.70±0.05[a]	2.42±0.59[a]	7.23±0.81[a]	0.52±0.08[a]	0.73±0.10[a]
	Soil + 0.5 % BC	2.85±0.98[a]	0.58±0.08[a]	3.37±1.05[a]	5.27±2.50[a]	0.470±0.03[a]	0.68±0.13[a]
	Soil + 1 % BC	3.26±1.14[a]	0.60±0.13[a]	2.60±0.33[a]	2.27±1.56[a]	0.30±0.05[b]	0.67±0.08[a]
	Soil + 2 % BC	2.60±035[a]	0.38±0.18[b]	2.00±1.28[a]	2.00±0.35[a]	0.28±0.03[b]	0.57±0.19[a]
(b)	Soil	4.42±2.05[a]	0.68±0.06[a]	3.15±0.33[a]	2.92±2.05[a]	0.45±0.06[a]	0.72±0.10[a]
	Soil + 0.5 % BC	1.97±0.86[b]	0.67±0.08[a]	2.70±0.61[a]	2.62±0.72[a]	0.43±0.01[a]	0.72±0.10[a]
	Soil + 1 % BC	2.62±1.17[ab]	0.78±0.03[a]	2.33±0.31[a]	2.32±0.89[a]	0.32±0.05[b]	0.72±0.14[a]
	Soil + 2 % BC	1.75±0.08[b]	0.67±0.10[a]	2.00±1.22[a]	2.90±1.33[a]	0.30±0.03[b]	0.73±0.08[a]

Means followed by different letters are significantly different (P<0.05), BC = Biochar.

highest SMBN (76.67 µg g^{-1}) and it was significantly (P<0.001) different from other treatments. Treatments receiving 0.5 and 1% biochar were not significantly different from each other. For the low organic matter soil, treatment receiving 2% biochar (74.6 µg g^{-1}) was significantly (P<0.001) higher than other treatments.

Total N

The Total N values following biochar addition are shown in Figure 3. The high organic matter soil was higher in soil Total N (Figure 3a). Higher biochar application rate increased soil Total N throughout the incubation period in relation to control. Treatment receiving 2% biochar showed the highest Total N value and it was significantly (P<0.05) higher at 70 and 100 days. Slight increase in Total N was shown by Soil + 2% biochar of the low organic matter soil in the first 20 days (Figure 3b). Soil + 2% biochar was significantly (P<0.05) higher at 10, 20, 60 and 80 days with respect to other treatments. The control soil showed the lowest soil Total N value throughout the 100 days incubation.

DISCUSSION

Many research works conducted in China have shown that biochar application to agricultural soils has little or no effect on carbon mineralization (CO_2-C efflux). There is decreased decomposition or negative priming following biochar application to soils which inadvertently leads to a reduction in soil respiration. Findings from this research showed that biochar treatment with highest application rate 2%, which is equivalent to 40 t ha^{-1} recorded the lowest mineralization, and it is in agreement with other findings (Liu et al., 2011; Case et al., 2014; Schimmelpfennig et al., 2014). Soil + 2% biochar reduced CO_2-C mineralization by 15.8 % in the high organic matter soil and 16.1 % in the low organic matter soil at 10 days incubation. Even though improvement in the growth of soil microorganisms following biochar application has been reported (Chen et al., 2015; Lu et al., 2015), it did not amount to an increase in soil respiration. The reason that can be adduced to this could be that the fine particles in biochar might have taken up the evolved CO_2-C to an extent; thereby limiting its release to the atmosphere.

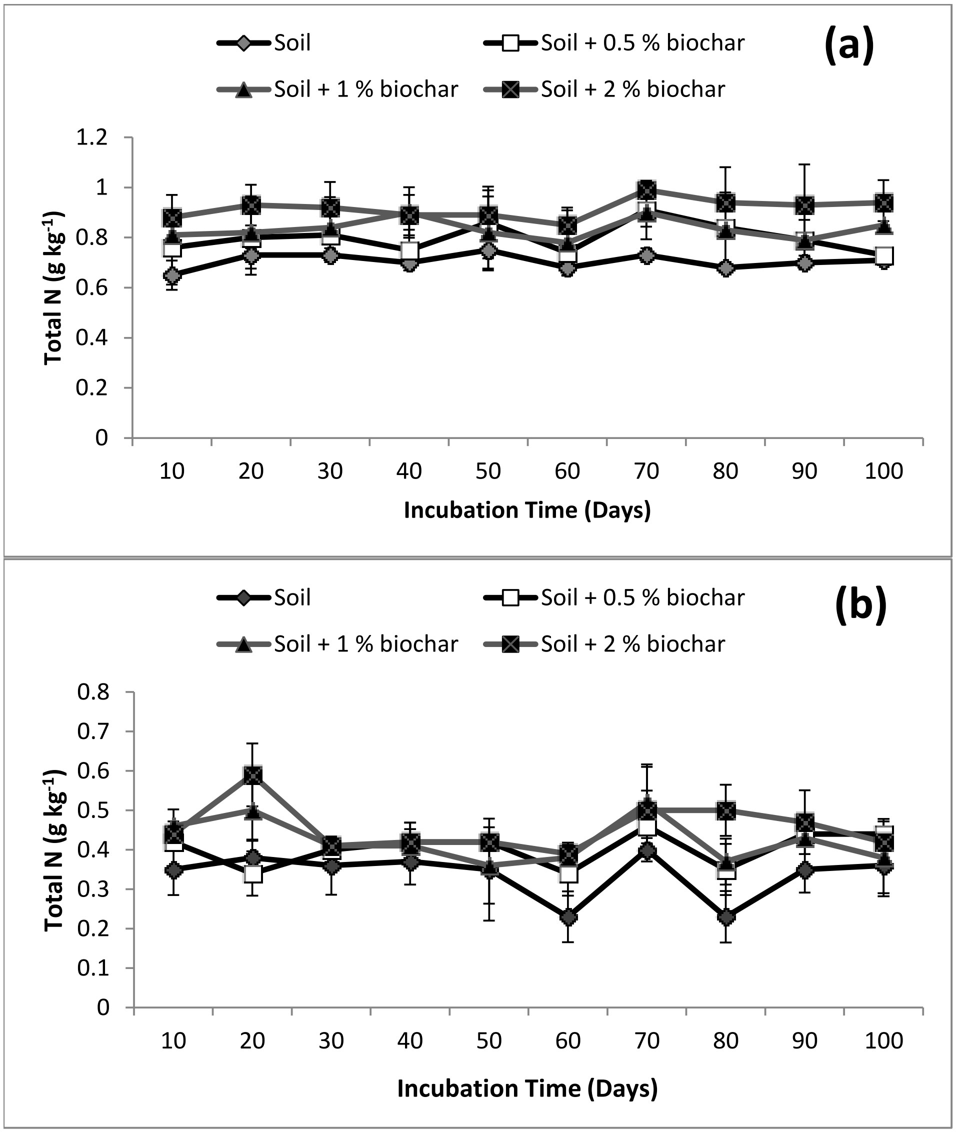

Figure 3. Soil Total N for high organic matter soil (a) and low organic matter soil (b) amended with biochar at 0.5, 1 and 2% levels (Mean ± SE, n = 3).

Biochar additions largely increased SOC and contributed to carbon storage. This finding is in agreement with the work of Biederman and Harpole (2012). The observed increases in SOC following biochar addition were expected, considering the high carbon content (81%) and recalcitrant carbon of biochar.

Mineral nitrogen content (NH_4^+-N and NO_3^--N) in the rhizosphere soil is an important indicator of threats to soil by nitrogen saturation (Fingerman et al. 2011). The N mineral forms are the forms in which agricultural crops take in nitrogen from soil. The findings from this research showed that biochar when added to soil reduced N mineralization and accumulation of NO_3^--N and NH_4^+-N which is in consonance with other works (Novak et al., 2010; Taghizadeh-Toosi et al., 2011; Zavalloni et al., 2011). High biochar concentration significantly reduced NO_3^--N and NH_4^+-N concentrations. The reason for this could be as a result of a change in soil pH that follows biochar addition which inadvertently affects the structure of soil microbial organisms.

The changes showed in SMBC among treatments points out the activities of microorganisms and breakdown of organic matter. The results from this study established that SMBC increased with biochar application relative to control, which is an indication that biochar addition to soils can accelerate the growth of microbes (mostly bacteria and fungi). Same results have been reported by several authors (Anderson and Domsch, 1993; Tian et al., 2008; Kolb et al., 2008; Aciego and Brookes, 2009; Liang et al., 2010; Lehmann et al., 2011).

The effect of biochar on soil microbial biomass and activity depends mainly on biochar type (feedstock and pyrolysis temperature). For SMBN, biochar had positive effects on soil microbial biomass nitrogen. The addition of biochar increased the microbial composition of both soils and enhanced the release of biomass N. Biochar concentration also affected SMBN release from soils following the death of these microbes. This result is in consonance with the work of Zhang et al. (2014).

Total N is the sum of total kjeldahl nitrogen (ammonia, organic and reduced nitrogen) and nitrate-nitrate. Biochar additions increased soil Total N, which is consistent with the work of Zhang et al. (2012b) who recorded a significant maize increase, accompanied by increased soil Total N content following application of different levels of a nutrient rich wheat-straw biochar (20 and 40 t ha^{-1}); but this is in contrast to the findings of Jones et al. (2012). The tendency of biochar amendment to increase soil Total N depends on the feedstock used in biochar production as well as the pyrolysis temperature. It also depends on soil properties as well as the prevailing weather and climatic conditions. Biochar properties such as large surface area and high porosity could also be responsible for the changes in Total N when added to the soil.

Conclusion

In conclusions, this study shows that the addition of biochar to Mollisols reduced soil respiration rate throughout the 100-days incubation period. Higher biochar concentration caused a further decline in soil respiration rates. Incorporation of biochar to soils increased SOC and soil Total N content; hence high biochar rate can be applied to soils as amendment to boost SOC and soil Total N. A positive effect on soil microbial biomass carbon SMBC and soil microbial biomass nitrogen SMBN was also shown by biochar. However, biochar addition reduced mineral soil nitrogen in the forms of NO_3^--N and NH_4^+-N.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Economics and variety adoption of tuberose: A farm level survey in Bangladesh

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qqqQ1`Recently, Bangladesh Government has incorporated flowers in its export policy. Flowers can be a source of earning huge foreign currency if established as a traditional export item. Using cross-sectional data obtained from a survey conducted during 2016/2017, gross margin analysis was used to calculate cost and returns for double (HYV) and single varieties tuberose farmers and multiple regressions for conducting input output relationship of different varieties of tuberose farming. Also, a probit model was performed to determine the probability of double variety tuberose by flower growers, particularly in Jessore district. Stratified random sampling technique was used to obtain data from 200 tuberose farmers. The results revealed that education, extension services training and credit access play significant roles in adoption decisions. The results also revealed that production of double variety tuberose is highly profitable than single variety. The tuberose farmers lack improved technology and are deprived of fair prices for their produces due to numerous reasons that need to be explored. Besides, the flower scientists, research managers, and policy makers in the country also lack socio-economic data and information regarding tuberose cultivation which are very much important for further development of the crop.

Key words: Economics, tuberose, variety adoption, Probit model, Bangladesh.

INTRODUCTION

Recently flower cultivation has become popular in many parts of Bangladesh, as its demand is increasing day by day. Jessore district is one of the most important flowers producing areas in Bangladesh. The increasing adaptation of flowers by the farmers has led to the costs and returns from flower cultivation to facilitate the growth of flower cultivation. Amin et al. (2017) said it has a great economic potential for cut flower trade and essential oil industry. Tuberose (*Polianthes tuberosa L.*) is one of the most important tropical ornamental bulbous flowering plants with long lasting flower spikes cultivated for production. It is popularly known as Rajanigandha or Nishigandha in Bangladesh. The main purpose of floriculture includes cultivation of flowering and ornamental plants for direct sale or for use as raw materials in cosmetic and perfume industry and in the pharmaceutical sector. In the floriculture industry, there is always demand for new and novel varieties (Datta, 2017).

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Figure 1. Single and double variety tuberose.

The growth of commercial flower production can be traced back to the early 70s that got impulsion in the mid-80s, when large-scale commercial production started in Jhikargacha Upazila of Jessore district (Sultana, 2003). Later it speeded largely in other areas of Jessore, Savar, Chuandanga, Mymensingh and Gazipur, which eventually turned into the major flower production belt in Bangladesh. Presently, Jessore is home to 70% of Bangladesh's flower production, where around 4500 growers are engaged in this small but very promising industry. The Department of Agricultural Extension depicts that in 2014, commercial floriculture has extended to 8,500 ha, while in 1990s it was less than 200 ha.

Adoption is a mental process that begins when an individual or operation learns of an innovation and ends at the final adoption stage (Rogers, 1962). The major share of total cost was for human labour (30%) followed by land use (23%) and fertilizer (17%). The total cost was 26 and 12% higher than its competitive crops, banana and papaya, respectively (Haque et al., 2012). Neill and Lee (2001) point out that majority of the existing literature on agricultural technology adoption are focused on Green Revolution (GR) technologies such as irrigation, fertilizer use and the adoption patterns of high-yield variety (HYV) seeds. Numerous studies were conducted to better understand the factors that influence adoption of conservation and organic production practices (such as Barreiro-Hurleetal, 2008; Baynard and Jolly, 2007). Bangladesh is well matched for commercial flower cultivation due to the favourable climate, cheap labour and relatively low capital investment (Khan, 2012). Seraj (2008) states that the history of commercial flower cultivation took place in Jhikorgacha upazila of Jessore during Mid-80s. Gradually, it has spread to other upazilas of Jessore and now Gadkhali bazaar of Jessore district is the biggest flower bazaar of Bangladesh. Just about thirty thousand flower farmers of Jessore region are now engaged with this bazaar. About two-thirds (65.2%) of respondents thought sustainability of floriculture was very important to the environment (Tanya et al., 2009). Similarly, more than half (63%) of the respondents had sustainable practices in their operations. Hassan (2012) estimated that the gross margin of a flower grower is $16383.424. He also estimated that, the average marketing margin for three intermediaries remained as $7.691 for wholesale-cum-retailer, $2.259 for BRAC and $8.310 for retailer in Dhaka city. He identified some problems faced by the farmers such as insecticides, lack of training and scientific knowledge, plant diseases, poor transportation system, unstructured market, lack of market information, demand fluctuation, and lack of storage facilities (Figure 1).

At present, the main problem assigned to floriculture of the country is lack of sufficient production technology for which export quality flowers and plants are not being produced in plenty. Another serious problem is that proper post harvest technology is almost absent in case of floriculture which is very necessary for after harvest supply chain management. However, it is a matter of misery that there is still no technology for reducing post harvest loss of flowers in Bangladesh. Thus, at present, adoption of HYV tuberose for the sake of improved production and quality control of floricultural commodities is considered as one of the most burning issue. As a result, probit model was adopted to investigate the key factors that influence adoption HYV tuberose seedlings. Whereas, there is insufficient evidences of substantial research work in this country concerning adoption of HYV tuberose. So, the present study is considered as a very timely and valued one to analyze the economics and adoption of tuberose in Bangladesh. Throughout the study, search for proper and more sophisticated technology was carried out so that some effective policy implications can be concluded for the wellbeing of all stakeholders engaged in this promising agricultural sub-sector.

DATA COLLECTION AND METHODOLOGY

Sampling and data

The sampling strategy for the present study is a combination of both purposive and random sampling. The two upazila, namely Jhikargacha and Sharsha under the Jessore district of Bangladesh, were chosen purposively based on the highest flower production area. Four villages were selected from each upazila; whereas the farmers at the village level were randomly selected. A complete list of tuberose growers were collected through the help of DAE personnel from the study areas. Finally, 200 tuberose farmers and 25 farmers from each village were randomly selected and data were collected during the month of February-March, 2017. A participatory methodology was followed to get information about technology adoption followed in the research locations. There are two dimensions of data available for analysis: (i) farmers who have received HYV tuberose, that is, technology receiver and (ii) farmers who did not receive any technology, that is, technology non-receiver.

Analytical technique

Data collected were analyzed using descriptive statistics and multiple regression analysis. Descriptive statistics were used to analyze socio-economic characteristics of the farmers and constraints associated with tuberose cultivation. Gross margin analysis was used in analyzing cost and returns in tuberose cultivation per hectare. The tabular method of analysis involved different descriptive statistics and land use cost was calculated based on per year lease value of land. The profitability of tuberose cultivation was estimated using gross margin, net return, and benefit cost analysis.

Linear regression analysis

Multiple regression analysis was used to determine input output relationship of tuberose farming. Four functional forms, namely, the linear, semi-log, double-log and exponential were tried out while using the ordinary least squares estimates in assessing the regression model. The one that gave the best fit in terms of the magnitude of R^2, Adjusted R^2 and the significance of the overall regression as judged by the F-ratio and the significance of the individual coefficients was chosen and reported.

The multiple regression model was implicitly stated as:

Y = f (X1, X2, X3, X4, X5, X6, X7,......................................U) (i)

where Y=output of tuberose (stick), X1=Human labour cost ($), X2=Tuberose seedling cost ($), X3=Organic fertilizer cost ($), X4=Inorganic fertilizer cost ($), X5=Irrigation cost ($), X6=Pesticides cost ($), X7=Vitamin cost ($), and U=Error term.

Gross margin is the difference between the total revenue (TR) and the total variable cost (TVC). It is a useful planning tool in situation where fixed capital is a negligible portion of farming enterprise as in the case of small-scale subsistence agriculture (Olukosi and Erhaor, 1988; Omotesha et al., 2010; Abdullahi, 2012).

Probit model for estimating the determinants of adopting tuberose farming

To analyze the adoption of tuberose cultivation, the probit model was utilize. The dependent variables in the adoption model are 0, 1 dummy variables; indicating one if a farmer adopt HYV (double varity tuberose) for flower cultivation and zero if otherwise. According to Gujarati (2004), there are three approaches for estimating the qualitative response of dummy dependent variables: (1) linear probability model (LPM); (2) logit model; and (3) probit model. The linear probability model (LPM) is a typical regression model, but the dependent variable is a dummy variable. The conditional anticipation of the dependent variable, given independent variables, is interpreted as the conditional probability. However, Wooldridge (2009) and Gujarati (2004) dispute that the linear probability model has some negative aspects, including non-normality of the error term, the probabilities can be less than zero or greater than one, and the partial effect of any independent variable (appearing in the level form) is constant. These confines of the linear probability model can be triumphed over by the logit or the probit model.

Logit and probit models are based on logistic and normal cumulative distribution functions (CDF), respectively. Gujarati (2004) disputes that logit and probit probability models have several features to rise above the shortcomings of the linear probability model, including (1) as an independent variables, X_i increases, the probability of adoption (that is, $P_i = (Y = 1 | X)$ increases, but only in the 0-1 interval; and (2) the relationship between P_i and X_i is nonlinear. Therefore, the probability approaches zero as X_i approaches negative infinity and the probability approaches one as X_i approaches positive infinity. Both logit and probit models are quite similar, but the logistic distribution has to some extent fatter tails. Therefore, the conditional probability approaches zero or one at a slower rate in the logit than in the probit model (Gujarati, 2004). This study uses the probit adoption model to analyze households' adoption decision because it is an appropriate econometric model for the binary dependent variable and the error term is assumed to be normally distributed.

The probit model, also known as the normit model, estimates the effects of X_i on the response probability, $P_i = (Y = 1 | X)$. The model presumes that households make decisions based on a utility maximization purpose. The conceptual framework of the analysis model used in this study is similar to the model that Uaiene et al. (2009) and Zavale et al. (2005) used to estimate households' technology adoption. The model presumes that households' decisions whether or not to adopt agricultural technology depend on unobservable utility index (or a latent variable) that is determined by household specific attributes X (such as, household head's gender, age, and education; access to extension services and credit; membership in an agricultural association). The probit model of HYV adoption is derived from an underlying latent variable model, which is expressed as:

$$Y_i^* = \beta_0 + \beta_{ij} X_{ij} + e_i \quad (1)$$

where Y_i^* is an underlying index reflecting the difference between the utility of adopting and not adopting HYV; β_0 is the intercept, β_{ij} is a vector of parameters to be estimated; X_{ij} is independent variables which explain HYV adoption; and e_i is the standard normally distributed error term that is independent of X_j and is symmetrically distributed about zero. From the latent variable model (1) and the assumptions given, the flower grower's adoption of HYV is derived as:

$$P(Y_i^* = 1 | X) = F(\beta_0 + \beta_{ij} X_{ij}) \quad (2)$$

where F is the function that ensures the likelihood of adopting HYV, strictly between zero and one. Therefore, a farm household adopts HYV if $Y_i^* > 0$, and otherwise if $Y_i^* \leq 0$. In the case of a normal distribution function, the model to estimate the probability of observing a farmer using a new technology can be explicitly stated as:

$$P(Y_i^* = 1/x) = F(\beta X) = \int_{-\infty}^{\beta X} \frac{1}{\sqrt{2\pi}} exp(-Z^2) dz \quad (3)$$

where P is the probability that the i^{th} farmer used HYV and 0 otherwise; X is the K by 1 vector of the independent variables; z is the standard normal variable, that is, $Z \sim N(0, \sigma^2)$; and β is the K by 1 vector of the coefficients to be estimated.

In most appliances, once parameter estimates from the probit or the logit regressions are obtained, a natural next step is to think about the marginal effects. According to Cornelibe (2005) and Gujarati (2004), regression analysis more often than not aims at estimating the marginal effect of an independent variable on the dependent variable, controlling for the influence of other independent variables. In the linear regression model, the estimated parameters can be interpreted as marginal effects. In non-linear regression models or the binary regression models (such as, probit and logit models), parameter estimates cannot be interpreted as marginal effects. The marginal effect of an independent variable (such as household had access to extension services) is attained by calculating the derivative of the result probability with respect to an independent variable. Wooldridge (2009) and Gujarati (2004) argue that in most applications of binary regression models (such as probit model), the primary goal is to explain the effects of the X_j on the probability regression $P_i(Y = 1 | X)$. The latent variable formulation (Equation 1) indicates that the probit adoption model is primarily interested in the effect of each X_i (for instance, households had access to extension services and credit) on Y_i^* (whether or not to adopt technologies).

RESULT AND DISCUSSION

Summary of descriptive statistics

The mean output (yield) of high yielding tuberose (double variety) is 301940 sticks/ha, which is 49.56% higher than the single variety. The average seeding (korom) requirement and organic fertilizer for double variety is slightly lower than the single variety. About 29% inorganic fertilizer require more for the high yielding variety of tuberose; indicating that high yielding variety tuberose requires more fertilizer for higher production. The average levels of education and farming experience for HYV tuberose adopters were 8 years of schooling and 10 years; whereas this figure for non-adopters was 2 years of schooling and 6 years, respectively. Twenty-five percent income comes from off-farm sources for adopters; whereas 31% for non-adopters from the same sources (Table 1).

Profitability of tuberose cultivation

The average per hectare yield of double variety tuberose is 301940 sticks, whereas it is around 201880 sticks for a single variety of tuberose. Similarly, adopters enjoy higher net returns ($ 7665.794) than non-adopter ($3233.905) of HYV tuberose. The net return from double variety tuberose cultivation is 57% higher than single variety. The higher costs of double variety of tuberose are compensated by higher production and profit.

Benefit cost ratio (undiscounted) for different tuberose varieties was calculated over variable cost and total cost basis. The BCR for the improved variety growers is 3.19 over total cost, which is higher than the single variety (2.13). The BCR for double variety over total cost is almost similar with the finding of Haque et al. (2012), who found BCR for tuberose cultivation over full cost was 3.39. Due to lower production and less price, single variety growers incur losses (Table 2). The break-even yield of single variety is higher than the double variety due to high price of stick of double variety (Table 2). The average numbers of tuberose plucked per hectare are around 926955 and 506721 sticks for double and single varieties in that order. The yield (sticks/ha) of tuberose is reportedly higher for double variety compared to that for single variety due to higher production for double variety (Table 2). Operating ratio and gross ratio was calculated for double and single variety tuberose farming as a ratio of total variable cost to gross income and total cost to gross income, respectively. Both ratios are higher for single variety than improved variety due to lower production and comparable lower price.

Factor influencing the key drivers of production of tuberose varieties

Functional analysis was used to determine the quantitative relationships between dependent variables and set of independent variables or explanatory variables (Gujrati, 2003). To determine the effects of the explanatory variables, multiple linear regression model was estimated for gross return of different tuberose varieties. Considering the effects of explanatory variables on gross return of double and single variety tuberose farming, six explanatory variables were considered, such as human labour cost (X_1), seedling cost (X_2), inorganic

Table 1. Summary statistics of tuberose cultivation.

Variable	Double variety		Single Variety	
	Mean	Std.	Mean	Std.
Flower output (stick/ha)	301940	83027.76	201880.9	69952.29
Flower seeding (Korom/ha)	35849.72	18990.89	40900.32	67627.41
Organic fertilizer (kg/ha)	19160.60	8619.64	22789.25	11870.52
Inorganic fertilizer (kg/ha)	2446.94	1091.38	1890.37	1054.02
Insecticides (mg/ha)	2896.13	1807.99	1890.81	1052.16
Labor(no/ha)	506.17	263.88	464.89	182.04
Output price ($/stick)	0.037	0.004	0.030	0.005
Korom price ($/piece)	0.007	0.001	0.005	0.001
Organic fertilizer price ($/kg)	0.007	0.0004	0.007	0.0004
Inorganic fertilizer price ($/kg)	0.227	0.115	0.226	0.108
Insecticides price ($/mg)	0.049	0.001	0.049	0.002
Labor price ($/m-d)	3.426	2.410	3.385	0.287
Age of tuberose farmers	45.9	9.66	42.62	10.04
Experience in tuberose production (years)	9.88	4.39	6.11	3.37
Distance from market (km)	2.95	1.97	3.75	4.13
Education level (year of schooling)	7.54	4.03	2.12	3.36
Training receipt**	0.67	0.47	0.38	0.49
Extension service**	0.80	0.40	0.94	0.24
Farm size (decimal)	40.61	17.80	37.50	17.08
Credit excess**	0.64	0.48	0.18	0.39
Off-farm income (share of total income)	0.25	0.19	0.31	0.21

**Dummy: 1 = farmers having training/extension service/credit excess, 0 = otherwise. Mean farm size and age are also higher for adopters compared with non-adopter counterparts, whereas mean of extension service is slightly higher for non-adopter group of tuberose farmers. This may be due to modify non-adopter to adoption for HYV tuberose (Table 1).

fertilizer cost (X_3), organic fertilizer cost (X_4), irrigation cost (X_5), insecticides cost (X_6) and vitamin cost (X_7). All these variables were estimated per hectare basis (Table 3).

It is clear from the model that the co-efficient of organic is positively significant at 1% level indicating an increase in the cost of this input; keeping other factors constant would increase the yield of double variety tuberose by 9.555 unit. For single variety, an increase in the cost of human labour, keeping other factors remaining constant would increase the yield of that variety by 0.807 units at 1% level of significance. The co-efficient of tuberose seedlings for double variety is positively significant at 5% level indicates an increase in the cost of tuberose seedlings; keeping other factors remaining constant would increase the yield of tuberose by 5.557 units. For single variety tuberose, the coefficient of irrigation is positively significant at 5% level; however, the coefficients of insecticides and vitamin costs' are negatively significant for double and single varieties, respectively. It is perhaps because of cost ineffectiveness of factors of production, that is, insecticides and vitamin costs born in the production of double and single varieties tuberose, respectively. Profit from production of both varieties of tuberose is offset by the costs of insecticides and vitamin incurred in the production of those varieties.

The value of coefficient of determination (R^2) were 0.404 and 0.312 for double and single varieties, respectively, which indicates that around 40 and 31% of the variation in yield were explained by the independent variables included in the model. The F-value were found as 7.932 and 3.371 correspondingly for both varieties which were significant at 1% level; implying that the variation of yield mostly depends on the explanatory variables incorporated in the model.

Factors influencing probability of adopting double variety of tuberose

The outcome of probit regression and the marginal effect of the explanatory variables are used to explain factors affecting efficiencies. Adopters and non-adopters of double variety tuberose were regressed on socioeconomic variables, which explain adoption of high yielding variety across farm households using Probit regression model (Table 4).

The access to credit is a significant variable in adopting HYV variety (double variety tuberose). The positive and significant impact of credit entails that credit availability

Table 2. Per hectare cost and return of tuberose cultivation.

	Double variety (improved) tuberose	Single variety tuberose
Input items	Cost ($/ha)	Cost ($/ha)
(A) Variable cost		
1. Human labor	1301.751 (37)	1083.296 (38)
2.Tuberose Seedling (korom)	224.600 (6)	202.038 (7)
3.Organic fertilizer	139.382 (4)	161.995 (6)
4.Inorganic fertilizer	579.778 (17)	427.725 (15)
5. Insecticides	142.872 (4)	93.173 (3)
6.Irrigation	343.373 (10)	204.819 (7)
7. Vitamin	60.240 (2)	12.048 (0.42)
Total variable cost (TVC)	2792.000 (80)	2185.096 (76)
(B) Fixed cost		
a) Interest on operating capital	111.68 (3)	87.404 (3)
b) Land use cost	586.619 (17)	598.668 (21)
Total fixed cost(TFC)	698.299 (20)	686.072 (24)
Total cost TC(TVC+TFC)	3490.299 (100)	2871.168 (100)
Returns		
Gross income (stickha-1)	11168.142	6105.073
Gross margin(tkha-1)= GI- TVC	8367.141	3919.977
Net farm income(tkha-1)=GI-TC	7665.794	3233.905
Benefit-cost ratio (GI/TC)		
Over variable cost	4.00	2.79
Over total cost	3.19	2.13
Operating ratio(TVC/GI)	0.25	0.36
Gross ratio(TC/GI)	0.31	0.47
Price received by farmers per stick	0.037	0.0302
Actual yield (sticksha-1)	302659.680	201880.9
Break even yield (sticksha-1)	94588.049	94942.99

Interest on operating capital has been calculated at 8.0% for 6 month period. Break even yield ($stickha^{-1}$) is total cost divided by average price received. Actual yield ($stickha^{-1}$) is gross income divided by average price received

Table 3. Explanatory variables on gross return of double and single variety tuberose farming.

Item	Double variety tuberose		Single variety tuberose	
	Coefficient	t-value	Coefficient	t-value
Constant	17362.566*** (5924.251)	2.931	15076.707** (6762.831)	2.229
Human labor	0.325 (0.216)	1.506	0.807*** (0.300)	2.692
Tuberose seedling (korom)	5.557** (2.572)	2.161	0.249 (0.800)	0.311
Inorganic fertilizer	0.700 (1.007)	0.695	-0.676 (1.246)	-0.543
organic fertilizer	9.555*** (3.041)	3.142	0.425 (3.949)	0.108
Irrigation	0.336 (0.492)	0.682	0.365** (0.392)	0.930
Insecticides	9.851** (4.530)	2.174	13.321 (5.109)	2.607
Vitamin	7.965* (4.275)	1.863	-7.720* (4.562)	-1.692
R - Square	0.404		0.312	
F - value	7.932		3.371	

***Significant at 1% level (p<0.01); **Significant at 5% level (p<0.05); *Significant at 10% level (p<0.10); ( ) indicates standard error

Table 4. Parameter estimates of adoption of double variety tuberose flower.

Variable	Parameter estimate	z-value	Marginal effect
Age	0.0014 (0.018)	0.08	0.000
Education	0.206 (0.043)	4.77***	0.073***
Experience	-0.000 (0.047)	-0.01	-0.000
Distance from market	-0.110 (0.082)	-1.33	-0.039
Extension visit	1.822 (0.392)	4.65***	0.618***
Non-farm income	-1.616 (0.844)	-1.91	-0.579*
Training	0.950 (0.341)	2.78***	0.337
Farm size	0.010 (0.010)	1.03	0.003
Credit access	1.308 (0.543)	2.41**	0.433***
Constant	-2.533 (1.245)	-2.03**	
Log likelihood	-40.303		
LR chi2 (9)	121.30		
Prob > Chi^2	0.0000		
Pseudo R^2	0.600		

***Significant at 1% level (p<0.01); **Significant at 5% level (p<0.05); *Significant at 10% level (p<0.10); ( ) indicates standard error.

enables the respondents to make judicious use of inputs that they cannot make available using their own capital. The impact of education on HYV tuberose adoption has a significant and positive sign. As anticipated, education is a key factor to influence the level of profitability in the production of tuberose. That is to say, farmers having high-educated family members are more profitable.

Surprisingly, the consequence of experience was not found significant for HYV adopters. It may be the fact that training and extension service play more important role than experience. The extension services provided by government, semi government and non-government agencies are very effective for adopting HYV variety.

Age cannot explain the adaptation of HYV; indicating that age is not an important factor (neither opportunity nor barrier) for achieving higher profit in tuberose farming. Like age and experience, distance from market and farm size showed insignificant impact as well. However, off farm income played significant role in HYV tuberose farming, negatively, which indicates that the appetite for maximizing profit by farming reduces when the farmers get enough income from non-firm activities.

CONCLUSIONS AND POLICY IMPLICATIONS

Improved seed technology permits farmers to save labor and management time, by this means, improving productivity of farming operation. Using the cross sectional data and the probit model, the factors affecting the adoption of HYV tuberose (double variety tuberose) by farmers particularly from the highest flower production area was observed. In doing so, it compared production and profitability between HYV and single variety grown by the farmers in the study areas. Cultivation of improved variety results in higher production though it requires more capital. Ultimately, compared to non-adopters, adopters earn a significantly higher level of return. Tuberose seedlings, organic fertilizers, insecticides and vitamin had positive effect on double variety tuberose cultivation, while vitamin and irrigation human labour had positive effect on single variety tuberose farming.

Extension services and training play a vital role in adoption of high yielding variety. Effective extension services and training considerably influence the adopters. The extension programmes for improved tuberose variety adoption are more likely to flourish among educated farmers. Those farmers who rely more on credit for their farming (such as farmers having more credit access) are more likely to be adopters. Access to credit assists farmers to adopt. Since farmers with access to credit are more capable in accumulating capital than their counterparts who do not have access, these farmers adopt more.

The tuberose farmers lack improved technology and are deprived of fair prices for their produces due to various reasons that need to be explored. Based on the findings of the study, the following recommendations are put forward for the enhancement of tuberose cultivation at farm level. High yielding varieties tuberose seedlings (korom) should be made locally available to the farmers at the appropriate time. For this reason, government should encourage researcher and private seed companies for producing HYV seedling of tuberose. Usman et al. (2015) highlighted the contribution of timely availability of good quality seed from the market. The potential competitive use of land use, human labour and financial sourcing towards other similar cultivations of promising profitability, climatic flexibility, and exporting perspectives, are being antagonistic to the specific tuberose cultivation. Therefore, it is time to give attention to tuberose cultivation. Hossain et al. (2016) said that there is lack of scientific information of flower crop and other plants under protective covers or glass house or similar suitable environment controlled house in Bangladesh. This need more attention, particularly in view of strict quality requirements of cut flowers for export purpose, which is difficult to obtain during cultivation in the open. No significant progress has been made in the development of floriculture industry and research in Bangladesh.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Effects of cutting length, time and growing media on the sprouting of dormant semi-hardwood cuttings of pomegranate cv. Wonderful

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Scientific confirmation of the traditionally known health benefits of pomegranates caused a tremendous increase in the consumption and production of this highly valuable fruit tree. Reproduction of pomegranate plants with seeds shows great variability in tree vigour and fruit quality. Cuttings is one of the most successful and preferred methods for the propagation of pomegranates. It is easy, quick, economic and most the convenient method of obtaining true-to-type trees in considerably less time; however, it has a high rate of mortality. Therefore, the present study aimed to determine the effects of cutting length, collection time of cuttings and rooting media on the sprouting of dormant semi-hardwood cuttings collected from pomegranate cv. Wonderful. Studies conducted with the completely randomized block design with four replications, each replication containing 25 individual cuttings. According to the results, the highest sprouting percentage obtained from the 10 cm cuttings collected 43±3 days before sprouting (DBS) and grown in soil with 98%. Results showed that the sprouting percentage is higher at the shorter cuttings. It was also concluded from the results that, as time pass, the sprouting percentage of the cuttings decrease, and is important to collect cuttings about 40 days before sprouting. The growing media was found to significantly affect the sprouting percentage and soil was found to be better than perlite media.

Key words: Perlite, rooting, soil, sprouting, transplant.

INTRODUCTION

Pomegranate tree (*Punica granatum* L.) is among the first cultivated crops in the world. Although it is as long as history of cultivation, its production began to increase tremendously after 20th century, with the scientific confirmation of its health benefits (Jurenka, 2008). Furthermore, lots of research conducted about the health benefits of pomegranates and those research revealed the efficacy of pomegranate fruits, leaves, bark, juice etc. against cancer, heart disease, hypertension and some infectious diseases (Aviram et al., 2000; Lansky et al., 2005; Türk et al., 2008). Pomegranate tree originated in central Asia, but it is grown in variable geographic conditions due to its adaptability to wide range of climatic and soil conditions (Holland et al., 2009). The shrub-like

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trees of pomegranates are deciduous and known to be long-lived plants. Reproduction of pomegranate plants with seeds shows great variability in tree vigour and fruit quality. Therefore, the most desirable method for the propagation of pomegranate tree is vegetative propagation. In this respect, cuttings, air layering and tissue culture are successful (Karimi et al., 2012), but air layering is reported to adversely affect mother tree and is more expensive than cutting (Purohit, 1981). Reproduction of pomegranate plants from shoot cuttings is easy, quick, economic and most convenient method of obtaining true-to-type trees in considerably less time (Polat and Caliskan, 2006).

The most important problem for cuttings method is the high mortality rate (Sharma et al., 2009) which strongly varies among varieties (Owais, 2010), cutting length, time of the year (Sebastiania and Tognettib, 2004) and cultivation practices applied. It was previously reported that indole-3-butyric acid (IBA) (Sharma et al., 2009; Sarrou et al., 2014), ascorbic acid, gibberellic acid (GA3), hydrogen peroxide (H_2O_2) and melatonin (MEL) (Sarrou et al., 2014) have positive effects on the rooting, root number, root length and shoot length of pomegranate cuttings. The time of the year that shoot cuttings are being collected is so crucial for obtaining a high performance of sprouting in the cuttings. On the other hand, the length and diameter of the cutting is utmost important, in which it determined the physiological potential of the cuttings (Chandra and Babu, 2010). Generally, 6-12 mm thick stem cuttings provide optimum rooting (Dhillon and Sharma, 1992; Rajan and Markose, 2007) but no useful information exist for cutting length. The carbohydrate sink of the cuttings is important for sprouting, as it is required for the cuttings until it rooted (Chadha, 2001). Many studies have been conducted on the propagation of pomegranate (Polat and Caliskan, 2006; Sharma et al., 2009; Owais, 2010; Karimi et al., 2012; Sarrou et al., 2014; Chater et al., 2017), however no experiments are known to study the effects of cutting length, time and rooting media on the rooting of pomegranate cuttings. Thus, this present study aimed to determine the effects of cutting length, cuttings' collection time of the year and rooting media on the sprouting of dormant semi-hardwood cuttings collected from pomegranate (cv. Wonderful).

MATERIALS AND METHODS

Plant materials, experiments and growth conditions

Semi-hardwood cuttings from different time of the year (1st research parameter: time) with different lengths (2nd research parameter: cutting length) were planted in different growing media (3rd research parameter: rooting substrate). Dormant semi-hardwood cuttings of the present study were collected on 1st of February and 1st of March 2017, from 8 years old pomegranate tree (cv. Wonderful). Cuttings were taken from the above branches of the trees, not from the basal suckers. The thickness of the collected plant materials was uniform, equalling to 10±1 mm diameter as suggested by Rajan and Markose (2007). Since cutting length was a research parameter in present study, semi-hardwood cuttings were cut as 10, 20, 30 and 40 cm lengths, respectively. The other research parameter of present study was the growing media, which are soil (sandy loam) and perlite. Growing media was filled in plastic containers (30 × 50 cm), and one plastic container was used a replication for each treatment (cutting length). The experiments were designed according to the completely randomized block design with four replications, each replication containing 25 individual cuttings. Minimum two nodes from each cutting length were planted below ground (3 to 5 cm deep), where minimum two nodes were also left above ground. The plastic containers with shoot cuttings were then placed in a greenhouse with a temperature of 25 to 30°C. Cuttings were hand-watered every day for 4 weeks and then irrigated as needed. Sprouting of the cuttings began in Mid-March and studies continued until the end of May (119 days for cuttings was collected on 1st of February, and 91 days for cuttings collected on 1st of March). A foliar application of a nutrient solution including 200 ppm nitrogen, 80 ppm phosphorus and 60 ppm potassium was performed on 1st of April and 1st of May.

Data collection and statistical analysis

During the experiments, sprouting of the plants were checked regularly. At the end of the experiments, number of sprouted plants and length of the highest shoot of each cutting was recorded. The data was summarized using Office Excel and SPSS 20. Data was analysed with analysis of variance (ANOVA) and mean separations were done by Tukey's multiple range test at $P < 0.05$. Comparison of the different time of cutting collections was done with independent samples t-test at 1 and 5% level respectively.

RESULTS AND DISCUSSION

First sprouting of cuttings began on 16th of March 2017 (43±3 days after 1st of February and 15±3 days after 1st of March). Mean sprouting results of present study showed that since the cutting length increase, the sprouting percentage of the cuttings decrease (Table 1.). The highest sprouting percentage was measured as 98%, obtained from the 10 cm cuttings, collected 43±3 days before sprouting (DBS) and grown in soil. The same length of cuttings (each 10, each 20, each 30 or each 40 cm, respectively) from the same time (1st of February or 1st of March 2017), but grown in perlite showed 95% sprouting, and no statistical difference was obtained between them. The sprouting percentage of 40 cm cuttings collected 43±3 DBS was recorded to be 69% in soil and 59% in perlite. The lowest sprouting percentage (19%) was measured from the 40 cm cuttings collected 15±3 DBS and grown in perlite. The results showed that as time pass, the sprouting percentage of the cuttings decrease, and is important to collect cuttings about 40 days before sprouting. These results are consistent with other studies who reported that cutting length (Chadha, 2001; Sebastiania and Tognettib, 2004; Chater et al., 2017) and time of the year (Sebastiania and Tognettib, 2004; Chandra and Babu, 2010) are important factors for propagation of plants from cuttings. On the other hand, results of present study are differing from the results of

Table 1. Mean sprouting percentage of semi-hardwood pomegranate cuttings of different length obtained at different time of the year and planted in different growing media.

Treatments (cutting length cm)	Sprouting percentage					
	43±3 DBS		15±3 DBS		Average	
	Soil	Perlite	Soil	Perlite	43±3 DBS	15±3 DBS
10	98.00^{aA}	95.00^{aA}	95.00^{aA}	79.00^{aB}	96.50^{a}**	87.00^{a}**
20	94.00^{aA}	88.00^{aA}	87.00^{aA}	60.00^{bB}	91.00^{a}**	73.50^{a}**
30	80.00^{bA}	70.00^{bAB}	65.00^{bB}	30.00^{cC}	75.00^{b}**	47.50^{b}**
40	69.00^{cA}	59.00^{cB}	41.00^{cC}	19.00^{cD}	64.00^{c}**	30.00^{c}**

Values followed by the same small letter or letters ‘a, b, c’ within the same column; and same CAPITAL letter or letters ‘A, B, C’ within the same row are not significantly different at 5% level (Tukey’s HSD multiple range test). Average data were compared with independent samples t-test; and * used to show significant differences at 5% level, ** to show significant differences at 1% level, and ns represents non-significant at 5% level. DBS: days before sprouting.

Table 2. Mean shoot length of semi-hardwood pomegranate cuttings of different length obtained at different time of the year and planted in different growing media.

Treatments (cutting length cm)	Length of highest shoot (cm)					
	43±3 DBS		15±3 DBS		Average	
	Soil	Perlite	Soil	Perlite	43±3 DBS	15±3 DBS
10	11.74^{aAB}	10.42^{bC}	12.48^{aA}	11.36^{aB}	11.08^{b}ns	11.92^{a}ns
20	13.44^{bA}	11.80^{aB}	9.59^{bC}	9.41^{bC}	12.61^{a}**	9.50^{b}**
30	15.65^{aA}	11.69^{aB}	9.52^{bC}	5.82^{cD}	13.67^{a}**	7.67^{c}**
40	12.75^{bA}	11.92^{aA}	6.68^{cB}	5.42^{cC}	12.33^{ab}**	6.05^{d}**

Values followed by the same small letter or letters ‘a, b, c’ within the same column; and same CAPITAL letter or letters ‘A, B, C’ within the same row are not significantly different at 5% level (Tukey’s HSD multiple range test). Average data were compared with independent samples t-test; and ** used to show significant differences at 1% level, where ns represents non-significant difference at 5% level. DBS: days before sprouting.

Owais (2010) who recommended 20 cm cutting length for optimum propagation of pomegranate. Significantly, similar performance of 10 cm length cuttings with the cuttings of 20 cm length is a valuable result in which number of plants might be doubled by dividing the 20 cm cuttings into two; while at the same time the sprouting percentage of the cuttings would be better. Previously, Mehta et al. (2018) conducted a similar study with pomegranate cuttings collected in different periods of the year, which is end of December, mid-January and end of January. They reported that the rooting and sprouting percentage of the pomegranate cuttings are higher at the end of January. These results are supporting our findings, where highest sprouting percentage obtained from the cuttings collected in February. Kaur et al. (2016) conducted a similar study by collecting the pomegranate cuttings in August and in January; and they reported that the rooting percentages are higher in August. This study also supports present results in a case that: collecting time of the cuttings is crucial for success.

Contrary to the sprouting percentages, highest shoot length of the cuttings recorded from the 30 cm cuttings collected 43±3 DBS and grown in soil with 15.65 cm (Table 2). It was followed by 20 cm and 40 cm cutting lengths. Similar results were obtained at perlite growing media. The energy required for the cuttings is obtained from the carbohydrate source of the cuttings (Chadha, 2001) and these results are not surprising. The shooting length of the cuttings with 30 cm and 40 cm length obtained 15±3 DBS and grown in perlite were found to be shorter than the other shorter cuttings. These results are in agreement with the other results of present study and suggest that the cuttings should be collected at least 40 days before sprouting to reach success. The results of the present study are in agreement with the findings of Rajkumar et al. (2016) who reported that the rooting percentage of pomegranate cuttings is decreasing in perlite growing media while increasing in soil, or in the combination of soil with cocopeat. The low percentage or sprouting (and rooting) in perlite media seems to be due to its low water retention capacity. Previously many studies conducted to improve the rooting and sprouting percentage of the pomegranate cuttings, especially with indole-3-butyric acid (IBA) and gibberellic acid (GA3) (Ghosh et al., 1988; Sandhu et al., 1991; Rajan and Markose, 2007; Sharma et al., 2009; Sarrou et al., 2014;

Rajkumar et al., 2016; Mehta et al., 2018; Hakim et al., 2018). Auxins plays an important role in rooting and IBA promotes root development via promoting cell enlargement by affecting the synthesis of enzymes (Damar et al., 2014). However, the results of the present study are opposing to the idea that, pomegranate cuttings require auxins for initiations of rooting and sprouting (Singh, 2017); suggesting that correct time of collection of cuttings and correct length (mainly shorter lengths, average: 10 cm) provide high efficacy in rooting and sprouting without needing any rooting hormone.

Conclusion

The greatest percentage in sprouting was recorded in cuttings with short length (10 cm) which was collected 43±3 DBS. The growing media (soil or perlite) was also found to significantly affect the sprouting percentage and the soil was found to be better than perlite media. The results suggest that using shorter cutting lengths and collecting the propagation materials at the right time might double, even triple the number of rooted plants and improve economic performance of reproduction.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Preliminary survey of foliar maize diseases in North Western Ethiopia

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In Ethiopia, maize is the staple food and one of the main sources of calories particularly in the major maize producing-regions of the country. Survey was conducted to determine the prevalence, incidence and severity of foliar maize diseases in North Gondar Zone. A total of 150 farmers' fields were randomly sampled from five districts (Chilga, Gondar zuria, Takussa, Metema and Dembia) in North Gondar Zone of Amhara Region during cropping seasons of 2015 and 2016 years. Five quadrants were examined per farmers' field for estimation of maize foliar disease incidence and severity infestation. This was done followed by pathogen isolation and disease identification laboratory procedures. Both of two years surveyed data were collected, analyzed and expressed using simple percentage. Results indicated that the dominant maize diseases were caused by *Exserohilum turcicum, Puccinia sorghi* and *Cercospora zeae-maydis* pathogen. Maize disease incidence of *E. turcicum* ranged from 50 to 80%, *P. sorghi* 19 to 62% whereas that of *C. zeae-maydis* reached 42% on foliar maize disease. In addition 3-19% of disease incidence of maize streak virus was recorded from seemingly healthy maize plants. Among four identified diseases Turcicum leaf blight (TLB), Common leaf rust (CLR) and Gray leaf spot (GLS) were recorded as major disease, while maize streak virus (MSV) was as minor disease. The present study provides an indication of the incidence and severity of foliar diseases of maize on which management strategies could be derived to improve the maize production in the surveyed areas.

Key words: Disease, foliar, incidence, maize, prevalence, severity.

INTRODUCTION

Maize (*Zea mays* L.) is one of the popular crops grown in the world, ranking second to wheat and used as a staple food in the tropics and is a valuable source of raw material for many industrial products (Vasal, 2000; FAOSTAT, 2012). Maize is the most versatile crop, adapted to different agro-ecological and climatic conditions. Maize is among the leading cereal crops selected to achieve food self-sufficiency in Ethiopia (Benti and Ransom, 1993). Maize is widely grown in Ethiopia and ranks first in total production and yield per hectare, and next to teff in the area coverage of 1,77 ml ha with a production of 39 ml Qt (CSA, 2010). The major

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constraints to maize production in the country include both abiotic and biotic factors, such as a drought, nutrient deficiencies, weeds, diseases and insect pests (Ransom et al., 1993). Among the biotic stresses, diseases are one of the most important limiting factors in maize production. Diseases are one of the major constraints in realizing the potential yield of this crop. It suffers from a number of diseases but Turcicum leaf blight (*Exserohilum turcicum*), Southern rust (*Puccinia polysora*), are the important constraints ones in globe responsible for yield losses. Gray leaf spot (*Cercospora zeae-maydis)* disease causes yield losses from 5 up to 30% (Ward et al., 1999; Misgana, 2014).

Forty seven different types of diseases were found to affect maize production in Ethiopia (Assefa and Tewabech, 1993). The incidence of maize diseases may vary considerably with geographical location. The influence of agro-ecological zones on the severity of foliar diseases has also been suggested in Ethiopia, while severe systemic infection of maize in western Ethiopia, high incidence and severity of turcicum leaf blight (E. turcicum) were recorded at Omo Nada, Chena, Nedjo and Bure with 70-100% incidence for all localities and 50, 45, 35 and 32% severity, respectively an area characterized by diverse climate, physical geography, edaphic factors, and farming practices (Tewabech et al., 2001; Tilahun et al., 2001; Keno et al., 2018).

Although quantified data on yield losses due to disease are not available for the country, the importance of disease in maize production has been given due attention (Tewabech et al., 2001). Preliminary studies, which listed the constraints to maize production in North Gondar and subsequent reviews on maize diseases in the Amhara region, do not provide quantitative values of disease prevalence, incidence or severity, nor have any studies hitherto examined the relationship between disease severity and the agro-ecological location of the fields. Such knowledge gaps have hindered efforts to assess the true economic importance of diseases on Maize production in Amhara region. Indeed, few reports of estimated yield losses from diseases in Amhara region are based on visual estimates of individual diseases in spite of the fact that more than one disease is commonly observed in maize fields. These factors probably influence the distribution and severity of maize diseases, but detailed information on these effects is lacking. Obtaining of the data is a prerequisite for developing a reliable quantitative assessment of the economic impact of the diseases, which are most models are related to the yield of incorporating information on disease incidence and severity. Such information can be obtained on a District level through disease surveys (Benti and Ransom, 1993). Assessment of the prevalence and severity of foliar maize diseases is important to map the geographic distribution and determine the status of the disease in addition to providing baseline data to prioritize research problems (Rusuka et al., 1997). The aims of this study were to determine the prevalence, incidence, severity, and identification of major and minor maize foliar diseases in maize growing districts of Northern Gondar Zone, Ethiopia.

METHODOLOGY

Description of the field survey areas:

Foliar maize diseases were surveyed on five districts (Chilga, Gondar zuria, Takussa, Metema and Dembia) in the Northern Gondar Zone (Figure 1). Two surveys were carried out in during 2015 and 2016 cropping main seasons on farmer maize fields. The total fields at an altitude ranging from 580 to 2700 masl were assessed from all visited districts (BFED, 2013).

Survey and sample collection

Foliar disease prevalence, incidence and severity were recorded for maize crop. Maize fields were randomly selected at intervals of 5-10 km along the main and accessible rural roads except when there is no suitable field available, and then the next maize field was sampled. Within selected fields, five quadrants (2 × 3 m) diagonally spaced about 10 m apart were sampled. At each field site, a W-pattern was used to cover the whole field, making five stops and evaluated for incidence and severity of maize foliar diseases. Disease infected plants in each quadrant were collected for diagnostic use. A total of 150 fields were surveyed between silking and milking stage of the crop for both main cropping seasons. Eighty and seventy fields were assessed along the same route during the cropping seasons of 2015 and 2016 years, respectively. The survey transect was approximately 3120 km long both years and cover five different agro-ecological district fields. All sample fields belonged to small-holder farmers (Chemeda and Jonathan, 2001; Alemu et al., 2016).

Maize foliar disease identification

The maize disease infected leaves samples were collected from farmers' fields of five districts. The collected samples were taken for laboratory identification, using different media culture, microscopic observation and literature (Bock, 1974, Angelique et al., 2008). The leaf tissues obtained during the field survey were being used for isolation of pathogens to confirm those pathogens associated with symptoms observed in the field. The isolates obtained have been added to the collections of pathogens in each state. Each state has undertaken isolation and identification of fungal pathogens associated with foliar diseases. Semi-selective media were utilized to recover pathogens growth either present or maybe not. PDA media amended with streptomycin can be used to isolate fungal pathogens. The morphological and cultural characters were critically studied both visually and under high power magnification (40 X) from 15 days old pure cultures. Diagnosis is based on visual examination for symptoms and culturing onto artificial media. The diseases also identified based on CIMMYIT monograph on guide for identification of maize diseases edited by Carlos L (1984) and Singh et al. (2004).

Disease assessment

Disease severity, area of plant tissue disease was rated on 10 randomly selected plants using standard scales of 1-9 (CIAT, 1987) where 1 is no visible disease symptom and 9 is disease covering more than 25% of the foliar tissue. The severity grades were

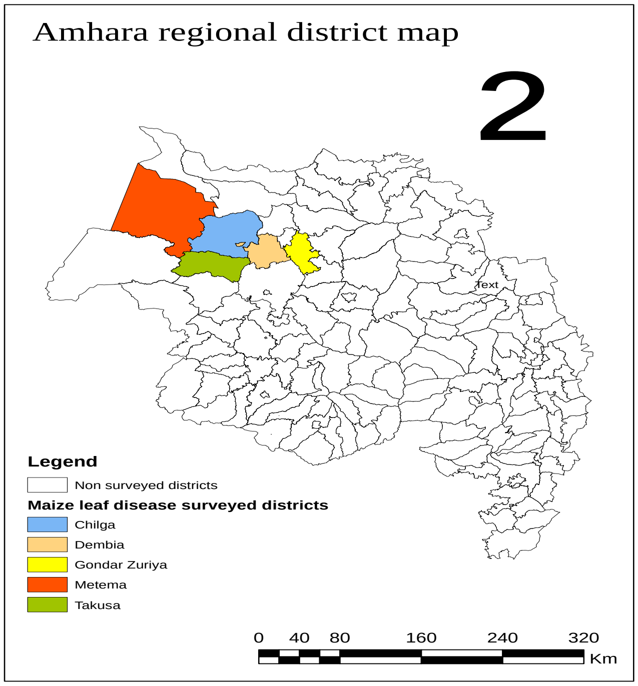

Figure 1. Map of maize foliar disease field surveyed districts in North Gondar Zone of Amhara Region.

converted in to percentage severity index (PSI) (Wheeler, 1969).

$$\text{PSI (\%)} = \frac{\text{Sum of numerical ratings} \times 100}{\text{No of plants scored} \times \text{maximum score on scale}}$$

Maize foliar disease incidence in each field was assessed as the proportion of plants showing symptoms in a field. In each field of 20 plants in the middle of each 1 m^2 areas were randomly selected and the number of plants having foliar disease symptoms on a whole plant basis counted and expressed as a percentage of the plant population. Any unknown disease samples were collected and put in paper bags for further inspection in the laboratory. To determine the incidence of maize disease at different farm fields used the following formula (Alemu et al., 2016).

$$\text{Disease incidence (\%)} = \frac{\text{Number of diseased plants observed}}{\text{Total number of plants examined}} \times 100$$

Disease prevalence was assessed by determining the number of fields where foliar maize diseases were recorded in relation to the number of fields sampled in surveyed districts.

Data collection

The mean rating for 50 plants of foliar disease was calculated for each field. Additional information was also collected on each assessed field, relative location; altitude (mt), type of cropping system was noted. Altitudes were recorded using a barometric altimeter. The plant population in each quadrant was counted and the mean plant population density was obtained by averaging the plant population in five quadrants farmers (Chemeda and Jonathan, 2001).

Data analysis

For each disease, prevalence and incidence or severity data was summarized. To obtain the variation between the surveyed diseases, descriptive statistics were used. The number of fields was in each severity category is expressed as a percentage of the total number of fields surveyed to obtain severity frequency distribution. In order to assess the relationship between agro-ecological zones and disease incidence, curves illustrating the frequency of fields with a given disease severity rating in each district were calculated. All statistical computing was used SAS software (SAS, 2002).

RESULTS

The present article reveals up to date information on maize diseases situation in North Gondar Zone districts. A total of 150 maize fields in North Gondar Zone were surveyed during 2015 and 2016 cropping seasons. Prevalence of most leaf diseases varies from field to field and year to year, depending on environmental conditions, tillage practices, cropping sequence, and hybrid susceptibility. Moderate temperatures and moisture in the form of rain and heavy dew usually favor development of foliar diseases and more than one type can be present on the individual plant. Four foliar diseases and their causal agents diagnosed on maize crop in five districts (Chilga, Dembia, Takussa, Gondar zuria and Metema) with an altitude ranged from 580 to 2700 masl are presented in Table 1. The incidence, severity and prevalence of diseases in maize were investigated during the 2015 and 2016 cropping season following a planned two time

Table 1. Major and minor of maize foliar diseases on five surveyed districts and its' diagnosed causal agents.

S/N	Maize foliar disease	Chilga (1850-2400 masl)	Dembia (1750-1900 masl)	Takussa (1600-1800 masl)	G/Zuria (1933-2700 masl)	Metema (580-1500 masl)
		D.I. (%)	D.I. (%)	D.I. (%)	D.I. (%)	D.I. (%)
1	Turcicum leaf blight (*Exserohilum turcicum*)	**	**	**	**	**
2	Common leaf rust (*Puccinia sorghi*)	*	**	**	*	**
3	Gray leaf spot (*Cercospora zeae-maydis*)	*	**	**	*	**
4	Maize Streak Virus	*	*	*	*	*

**=Major (≥25%); * = Minor (< 25%); D.I. =Disease Incidence
Numbers with bracket is altitude (masl=meter above sea level).

Table 2. Mean prevalence of maize foliar diseases for five districts during a survey of two cropping main seasons.

Maize foliar diseases	Chilga		Dembia		Takussa		G/Zuria		Metema		Total	
	N.F	%	N.F	%	N.F	%	N.F	%	N.F	%	N.F	%
Turcicum leaf blight	29	82.86	19	70.37	18	64.28	20	66.67	18	60.00	104	69.33
Common leaf rust	15	42.86	17	62.96	19	67.86	13	43.33	19	63.33	83	55.33
Gray leaf spot	11	31.43	9	33.33	9	32.14	7	23.33	9	30.00	45	30.00
Maize Streak Virus	4	11.43	5	18.52	6	21.43	4	13.33	7	23.33	26	17.33
Total assessed fields	35		27		28		30		30		150	

N.F. = Number of fields, where the disease samples were collected
% = Percentage of the disease that occurred from total sampled fields.

surveys across five districts in the North Gondar Zone. Totally the occurrence of the maize diseases was investigated in five districts of 80 and 70 farmers' fields, which were from the first and second year surveys respectively. The survey was carried out in major maize growing areas of Northern Gondar Zone by adopting roving survey methodology as mentioned in materials and methods. The present work was initiated with a survey to know the incidence, severity and distribution of foliar maize diseases in the surveyed districts. The survey was indicated that Turcicum leaf blight (*E. turcicum*), Common leaf rust (CLR) (*P. sorghi*) and Gray leaf spot (*C. zeae-maydis*) are major prevalent diseases in five districts of North Gondar zone with high severity and incidence. Maize streak virus is less important in areas with minor prevalent diseases (Table 1).

Foliar maize disease prevalence

A total of 150 maize fields were surveyed across five districts during 2015 and 2016 to document the occurrence and number of affected plants of foliar maize diseases. Turcicum leaf blight (*E. turcicum*) maize disease was the most widespread common foliar maize disease in the North Gondar surveyed districts. In both cropping seasons, the disease was detected in 150 (69%) of sampled fields. Turcicum leaf blight (TLB) was the most common disease and found from 104 fields out of 150 totals sampled maize fields on both surveyed years. The disease prevalence in two surveyed cropping seasons were found in major maize fields sampled in Chilga 82.86% and Dembia 70.37% compared to other districts (Table 2). During surveyed both cropping seasons TLB occurred less mean disease prevalent 60 and 64% in Metema followed by Takussa district respectively.

CLR of maize caused by *P. sorghi* was widely distributed throughout the surveyed major maize growing districts of North Gondar Zone. The occurrence was the second major disease found to be very high prevalence in North Gondar Zone, data indicated that higher prevalence 67.86 and 63.33% disease distribution occurred at Takussa and Metema districts respectively during two surveyed years (Table 2). However, the survey data indicated that the lowest prevalence was recorded on Chilga 42.86% followed by Gondar zuria with 43.33% CLR disease distribution on maize grown fields. The disease often appears relatively late in the growing season and seldom causes much loss in grain yield (Table 2). CLR disease in previous researcher was also indicated that one of the major diseases of maize growing areas in Ethiopia (Mengistu, 1982; Tarr, 1962).

Gray leaf spot caused by *C. zea-maydis* was one of the

Table 3. Maize foliar disease incidence in North Gondar Zone during 2015 and 2016 main cropping season.

Maize foliar disease	Chilga (D.I. %)		Dembia (D.I. %)		Takussa (D.I. %)		G/Zuria (D.I. %)		Metema (D.I. (%)	
	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
Turcicum leaf blight	70	80	65	63	60	70	65	73	50	54
Common leaf rust	19	23	44	50	54	62	23	25	40	38
Gray leaf spot	15	21	22	28	31	37	19	21	42	34
Maize Streak Virus	3	7	9	11	17	13	6	10	15	19

D.I. % = Disease Incidence percentage; G/Zuria = Gondar zuria.

important foliar maize diseases that occurred on five surveyed districts of North Gondar Zone. In two cropping season, the highest mean prevalence Gray leaf spot (GLS) maize disease was recorded from Dembia 33.33% followed by Takussa district 32.14%, while the lowest prevalence was occurred at Gondar zuria 23.33%.But the two years data mean prevalence of Gray leaf spot disease on maize field indicated that the disease occurrence from Demabia was higher than the Gondar zuria maize disease prevalence (Table 2). Foliar maize disease Maize streak virus (MSV) distribution has occurred in all surveyed districts, however the disease moderately prevalent than the rest of recorded foliar disease. In two years field survey mean data indicated that the highest MSV prevalence was recorded on lowlands of Metema followed by Takussa districts. But the lowest MSV prevalence was occurred at high land area of Chilga followed by Gondar zuria district. The disease prevalence during both cropping seasons was high in lowlands compared to the highlands. The overall sampled fields showed that the least mean prevalence disease 17.33% sample was recorded from MSV disease compared to other foliar maize disease (Table 2).

Disease incidence

The average percent diseases incidence was worked out based on the field observations. North Gondar Zone higher disease incidence of Turcicum leaf blight (*E. turcicum)* was recorded in all of the five surveyed districts. The result of survey conducted for 2015 year showed that Turcicum leaf blight (TLB) was widely distributed and caused greater disease incidence damage by 70% in Chilga and followed by 65% both in Gondar zuria and Dembia district on farmer maize fields, while the minimum disease incidence 50% was recorded on Metema district, where the lowest altitude was recorded. In 2016 cropping season the disease survey revealed that TLB was prevalent in major maize growing areas of the Northern Gondar Zone in low to severe form with the incidence of ranging from 54 to 80%, where the highest percent disease incidence was recorded from Chilga 80% and the minimum one was noticed in Metema district by54% disease incidence (Table 3). The TLB disease incidence infestation level across surveyed districts indicated that the maximum mean incidence 75% was recorded from Chilga where the altitude occurred more than 2000 m.a.s.l. But the minimum disease incidence 52% was recorded from Metema district, where less than 1500 m.a.s.l. altitude range area was occurred (Figure 2).

The maximum Common leaf rust (*P. sorghi)* disease incidence was recorded from Takussa 54% followed by Dembia 44%, while the minimum incidence of 19% in the Chilga district during 2015 maize growing season. In the second year cropping season, the maximum incidence 62% was also observed in the same surveyed district from Takussa and followed by Dembia with a 50% incidence (Table 3). (CLR) maize foliar disease in the different districts ranged from 21 to 58% mean incidence. To the period of 2015 cropping season the disease survey revealed that Gray leaf spot of maize was prevalent in all the maize growing surveyed districts in low to high form with the incidence ranging from 15 to 42%. Among the five districts in the first year survey, the maximum disease incidence was observed in Metema 42%, followed by Takussa 31% and the lower disease incidence by 15% in Chilga district. Survey in the second year indicated that the highest GLS incidence disease was observed in Takussa 37% followed by 34% in Metema district. However, the disease incidence was lower in Chilga than Gondar zuria district (Table 3).

During both main cropping seasons the Maize streak virus has occurred in five districts and recorded with a low incidence. Maximum disease incidence value was recorded from Takussa 17% followed by Metema 15% among surveyed districts of North Gondar Zone. But the minimum MSV incidence was obtained from Chilga and Gondar zuria assessed maize fields on the first surveyed year. The second year data indicated that the maximum disease incidence 19% followed by 13% was showed in Metema and Takussa district respectively (Table 3). The lowest TLB mean disease incidence 52% was obtained in Metema district, while the highest mean incidence 75% was recorded in higher altitude of Chilga district.

However, CLR foliar disease lowest mean incidence 21 and 24% was recorded in Chilga followed by the highest

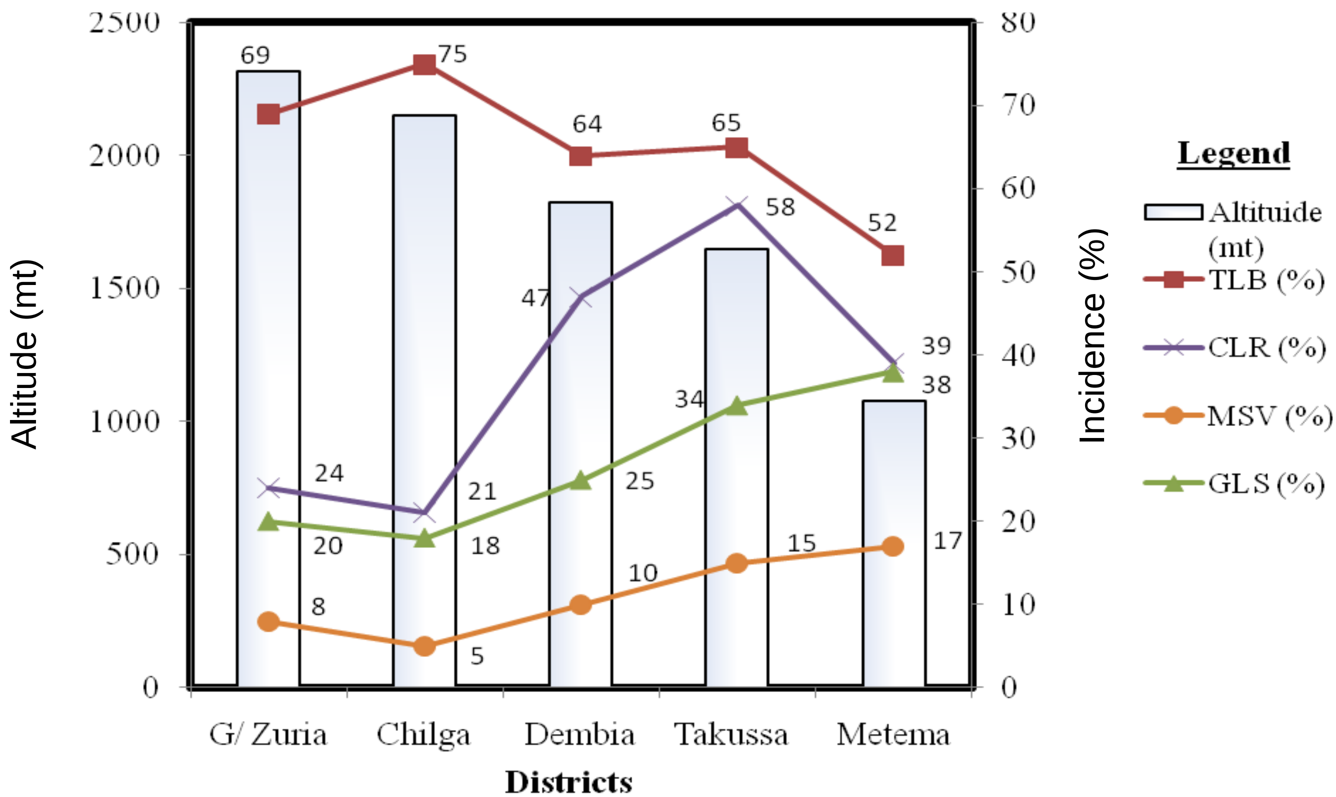

Figure 2. Two years mean maize foliar disease incidence across different district and altitude range.

altitude of Gondar zuria district respectively. The highest CLR mean incidence 58% was recorded in Takussa district where the lower altitude occurred. The overall mean incidence of the CLR disease was very high in the first and second of surveyed years and also the disease was more prevalent at flowering and grain filling stage in most of the surveyed areas. Two years foliar mean disease incidence showed that the lowest altitude of Metema district with the highest mean incidence 38% was recorded from GLS followed by MSV foliar disease incidence 17%, while the lowest incidence 18 and 5% was recorded on GLS and MSV foliar disease from higher altitude of Chilga district respectively (Figure 2).

Maize foliar disease severity

Survey on farmers' fields in major maize growing areas of Northern Gondar Zone revealed that, TLB severity varied from one locality to another, due to varied environmental conditions prevailing, cropping pattern and inoculums sources. During the 2015 cropping season among surveyed areas, the most affected fields were found in Gondar zuria with 28% disease severity, followed by Chilga district of 25% severity, but the minimum severity was noticed in Metema by 17%. In 2016 cropping season the disease survey revealed that TLB of maize was prevalent in major maize growing districts of the North Gondar Zone in low to severe form with the severity ranging from 17 to 35%,and the maximum disease severity 35% was observed in Chilga district with highland altitude areas of maize farm lands. But the minimum severity 17% was recorded from Takussa district (Table 4).

Among identified foliar maize disease Common leaf rust was the second widely distributed type of maize foliar disease, which documented severity next to TLB disease. The maximum CLR disease severity was observed in Metema 31% followed by Takussa 28% and the minimum severity was noticed in Gondar zuria by 16% during the first surveyed year. The disease severity in affected fields of Dembia and Chilga districts was 23 and 19% respectively during 2015 cropping year. CLR is one of the major foliar maize diseases having high disease severity that has been recorded in the surveyed districts. The infection was more serious during the second main cropping season in predominantly lowland areas were indicated the highest severity 33% in Metema and followed by 32% in Takussa district, and likewise in previous year the lowest severity 20% was recorded from Gondar zuria district (Table 4).

Gray leaf spot was also widely distributed throughout the major maize growing districts of North Gondar Zone. In the first cropping season, the occurrence of the

Table 4. Foliar maize disease percentage severity index in the surveyed districts during 2015 and 2016 main cropping season.

Maize disease	Chilga (PSI %)		Dembia (PSI %)		Takussa (PSI %)		G/Zuria (PSI %)		Metema (PSI %)	
	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
Turcicum leaf blight	25	35	18	22	21	17	28	22	17	21
Common leaf rust	19	23	23	25	28	32	16	20	31	33
Gray leaf spot	12	12	16	22	21	27	10	18	32	24
Maize Streak Virus	3	5	5	7	7	11	7	3	9	13

PSI =percentage severity index; G/Zuria = Gondar zuria.

disease was found to be very high severity ratings of 32, 21 and 16% at Metema, Takussa and Dembia districts respectively. To the period of 2015 cropping season the disease survey revealed that Gray leaf spot of maize was prevalent in all the maize growing surveyed districts in low to severe form with the severity ranging from 10 to 32%. The minimum severity 10% was noticed in Gondar zuria maize fields. During the second year, the highest severity 27% was indicated on Takussa and followed by severity of 24% on Metema district. Gray leaf spot maize was the third major maize disease of Northern Gondar Zone, which ranging from 12 to 27% severity (Table 4).

Among the five surveyed districts, the maximum MSV disease severity was observed in Metema 9%, followed by both Takussa and Gondar zuria districts having with the same value of 7%, however the minimum disease severity 3% was recorded from Chilga during the first cropping season. In the second surveyed year greater severity ratings were recorded by 13, 11 and 7% at Metema, Takussa and Dembia districts respectively. However, the occurrence of the disease was found to be very sporadic. The occurrence of maize streak virus foliar disease was observed in all surveyed districts with low infestation level ranging from 3 up to 13 percentages of severity index in both cropping season (Table 4).

Two years mean percentage disease severity indicated that the maximum TLB mean PSI 30% was observed in Chilga followed by 25% PSI in Gondar zuria district. But minimum PSI 19% value was showed by both Metema and Takussa lower altitude districts. In general TLB highest mean PSI was recorded on the higher altitude surveyed farm fields, while the lowest mean PSI was obtained from the lowest altitude district (Figure 3). In both cropping seasons 41 of 104 fields with TLB severity ratings ≥20% were recorded in the surveyed districts. Recently the area under maize was expanded in the improved varieties by replacing of the local varieties. The relation between the altitude and the mean maize foliar severity indicated that two years mean higher severity value of CLR, GLS and MSV foliar diseases were recorded in lowland areas of lower altitude districts, while the lowest severity of TLB foliar diseases was obtained from the same districts. It is apparent that among the surveyed districts the highest mean CLR mean PSI 32% was recorded from Metema, while the lowest was from G/zuria with 18% PSI, where the highest altitude occurred. The highest GLS mean PSI was recorded in Metema 28% followed by Takussa 24%, while the lowest in the Chilga district with 12% of PSI. The mean severity of MSV was ranged from 4 to 11%, the highest MSV mean PSI 11% was recorded from Metema, while the lowest PSI 4% was obtained from Chilga where the higher altitude range was recorded (Figure 3).

DISCUSSION

In addition to surveying for the occurrence and incidence of the above diseases, greater focused was placed on maize major leaf diseases of Turcicum leaf blight, Common leaf rust and Gray leaf spot and as a minor leaf disease of maize streak virus diseases were recorded in all five surveyed districts. The data indicated that the fields planted with local cultivar developed higher levels of TLB disease, while for improved cultivar was mainly affected with CLR disease. However, MSV diseases with lower incidence and severity infestation were observed in surveyed fields. Differences in disease severity in different fields planted with the same cultivar may have been caused by variations in levels of inoculums, plant maturity, nutritional status, local environmental conditions, and production methods. Several diseases attack maize crop during its growth stage. In Ethiopia 47 different types of diseases were recorded on maize, foliar and flower diseases being the most important once, which were mainly fungal diseases and among these the major foliar diseases were Turcicum leaf blight, Common leaf rust and Maize streak virus diseases (Tewabech et al., 2001).

The results of surveys conducted for two years showed that TLB was widely distributed and caused severe damage in Metema, Chilga, Gondar zuria, Dembia and Takussa districts. The highest incidence and percentage severity index of Turcicum leaf blight disease was recorded in Chilga and it might be attributed by favorable climatic conditions, high altitude, susceptible maize varieties grown and possibly disease pressure from the available patho-types of the pathogen. TLB disease has been reported throughout the world wherever maize is

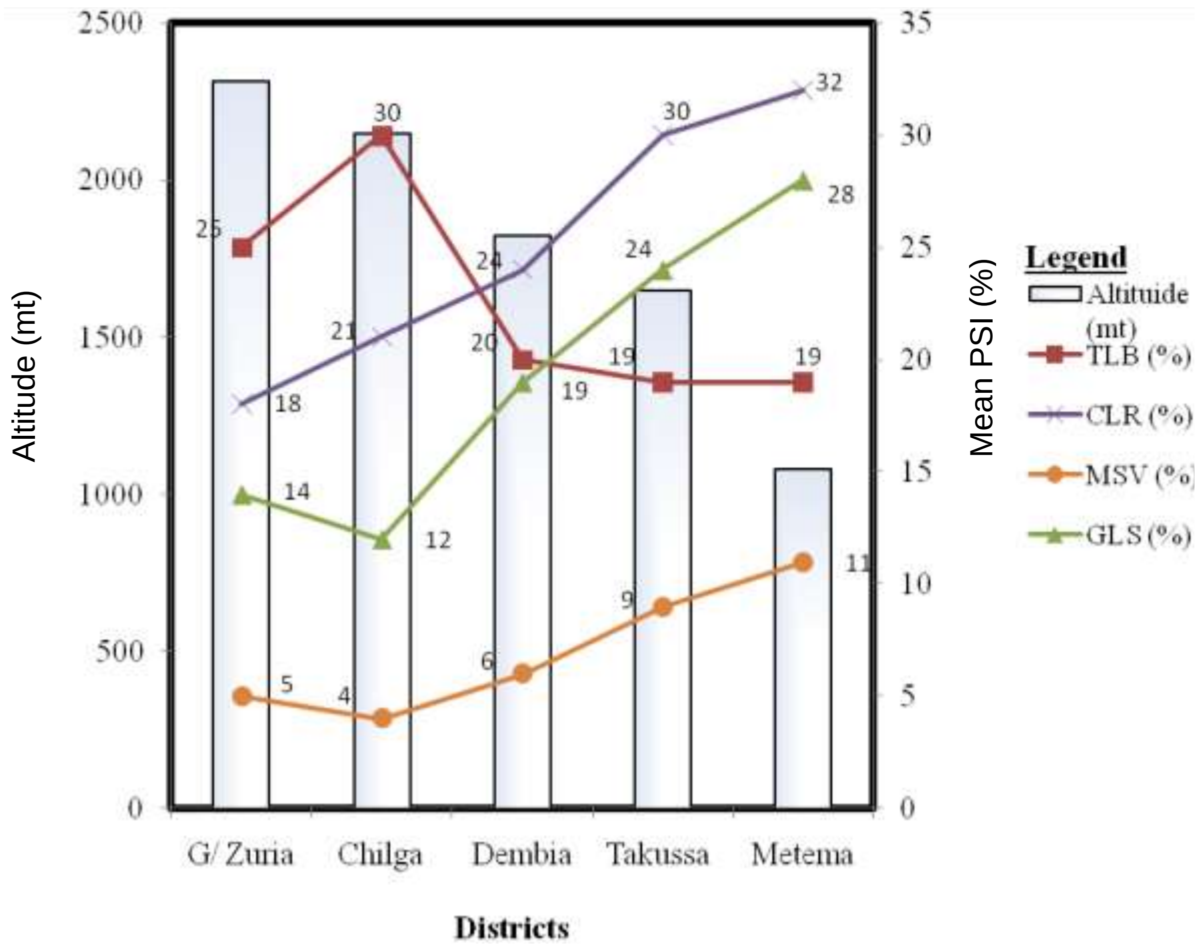

Figure 3. Two years mean maize foliar disease PSI in the surveyed. Districts across different altitude range (Mean = Two years severity average data, PSI = Percent severity index).

cultivated (Leonard et al., 1985; Adipala et al., 1993; Shiferaw et al., 2011). The high disease incidence and severity index in the highlands were obtained due to conducive climatic conditions. This result coincided with Nwanosike (2015) and showed that TLB disease incidence in highland district was significantly higher. Assefa (1999) also indicated that TLB infection is more serious during the main season in predominantly wet and humid areas. Generally TLB disease incidence and severity were severely affected the maize crop in the highlands as compared to relatively dry lowland districts. Similarly Adipala et al., (1993) and Ramathani et al (2011) reported that the prevalence of TLB in highlands and wetter areas of Kenya and Uganda was severely occurred. Previous reports showed that TLB is a serious disease in the highlands associated with cool, high relative humidity, mid-altitudes and cloudy weather conditions (Muriithi and Mutinda, 2001, Levic et al., 2008). Among surveyed districts the result indicated that Chilga was a hotspot area for TLB disease on maize crop. Particularly the disease incidence and severity were recorded on local and improved varieties, however the local variety was found to be lower incidence compared to the improved variety. TLB disease distribution indicated that the maximum mean disease incidence was recorded above 2000masl in Chilga followed by Gondar zuria district. But the minimum disease incidence was observed at 1825masl in Dembia district, where the relative humidity was not too much compared to the highlands. This disease is one of the major maize diseases having wide distribution and high economic importance in North Gondar zone districts. Two years surveyed data indicated that among foliar diseases the maximum mean disease incidence 75% was recorded on Turcicum leaf blight maize disease. This mean data was agreed with the previous study of Berhanu (2015) 100% incidence for all surveyed districts. The current survey study revealed TLB pressure in maize farms of Northwestern Ethiopia and needs to be designed in an efficient, inexpensive and sustainable management approaches against this disease. Tewabech et al. (2001) described that TLB is one of the major maize diseases having wider distribution and high economic importance in Ethiopia and the infection appears more during the main season in constantly wet and humid areas. Severe grain yield losses as high as 28 to 91% due to TLB have been reported in several parts of the world (Gowda et al., 1992; Harlapur et al*.,* 2000; Keno et al., 2018).

The second most important Common leaf rust disease was recorded in all surveyed districts and caused more intense damage in mid altitude areas of all improved varieties in both cropping seasons with higher mean disease incidence and percentage of severity index infestation level. The infection was more serious during the main season in predominantly wet and humid areas were indicated the incidence with very high severity. Keno et al. (2018) indicated that CLR is an important disease of maize in Ethiopia and widely distributed throughout the major maize growing regions of the country. However, the importance varies from place to place particularly during the survey it was more severe in mid-altitude than highland maize growing areas. CLR disease usually appears at the Knee-high stage or at tasseling. The survey results for both cropping seasons showed that CLR was widely distributed and caused more severe damage in Metema and Takussa with higher conducive temperature and sufficient rainfall conditions compared to other surveyed districts. Higher infestation incidence of 58% Common leaf rust in the surveyed area was due to the cultivation of improved susceptible variety and environmental condition. This study was coinciding with Chemeda and Jonathan (2001) previous work indicated that higher temperature and better rainfall during the survey seasons are often conducive factors for a rust epidemic and the hybrid maize varieties were highly susceptible to rust under Hararghe conditions compared to local adapted ones with the mean incidence ranged from 49-84%. In previous research work CLR on a susceptible cultivar was reduced grain yields by 23% from the western region of Ethiopia (Assefa and Tawabech, 1993).

Gray leaf spot caused by *C. zeae-maydis* was also an important major foliar disease, and widely distributed throughout the surveyed maize growing areas of North Gondar zone. Currently the disease is known to have widespread throughout the maize production areas of worldwide, including South America (Pozar et al., 2009), China (Zhang et al., 2012) and Ethiopia (Wegary et al., 2001). According to the report, GLS has become the principal maize disease since 1998 in Ethiopia. Recent survey in the country showed that GLS has increased in prevalence and severity in the major maize producing regions of western, southern and northwestern parts of Ethiopia (Alemu et al., 2016; Dagne et al., 2001). GLS is evident on plants as small spots first on lower leaves of plants at tassel initiation. The disease moves upwards and spots change into long characteristics lesions within a month turning plants into a diseased field. The disease is significant since it rapidly destroys foliage when the plant is near at grain maturity (Manandhar et al*.*, 2011). Observations showed that Gray leaf spot disease infestation was severe foliar disease in the surveyed districts. Although the disease intensity on maize production areas was varied due to weather, tillage system, and altitude range. It was occurred higher incidence and severity during the main season in predominantly warm temperature and high humidity maize production areas of low and mid altitude of Metema and Takussa districts. The study was agreed with Aschalew et al. (2012) previous work indicated that GLS disease is favored by humid and warm condition for its' development. Particularly in favorable climatic condition maize Grey leaf spot disease in South Africa is capable of reducing grain yields by 20-60% (Ward et al., 1997). To date, the disease is one of the most important threats to maize production in Ethiopia, causing yield losses as high as 29.10% and considerable yield loss in most maize growing areas of the country (Assefa, 1999; Wegary et al., 2004; Keno et al., 2018).

Maize foliar Maize streak virus disease observed from all surveyed districts, however this disease has occurred as a minor disease and the result was closer with Alemu et al. (1997) data at Bako maize planted in the off season was infected by MSV disease 15-20%. This study indicated that the maximum mean MSV foliar disease incidence was obtained from the lowest altitude Metema district, while the minimum incidence was recorded from the higher altitude of Chilga district. In Ethiopia, MSV is the most dominant viral disease of maize. Currently, MSV infestation has spread to the low, mid and highland areas of the country. But in recent years, the disease is becoming very important in the mid-altitude agroecology of Ethiopia and posing a significant threat to maize production in the country (Assefa, 1999; Keno et al., 2018). In Ethiopia condition, the main inoculums source of MSV disease was from grass family like *Digitaria* sp., *Eleusine indica* and *Panicum* species were found to serve as hosts to carry the disease over from season to season and further transmitted by several aphid species to maize crop (Alemu et al., 1997).

Conclusion

All the above identified diseases were destructive to maize production in North Gondar Zone, due to the fact that they occurred widespread in maize producing areas. From this study detected that foliar diseases are among the major production constraint that contributed yield losses in maize producing districts. During the survey maize growth period was also affected by drought, water logged and hail are highly observed as abiotic stresses that caused yield loss. Four important foliar maize diseases, three of them are as major and one is as minor diseases were identified and based on this study further the maize diseases management should be investigated.

It is anticipated that the disease will continue to threaten maize production until appropriate control methods are developed. Therefore it could be used for screening of germplasms for resistance against the diseases and urgent control measure should be taken especially on TLB, Common leaf rust and other foliar disease to save the crop. In general farmers do not use improved technologies. Hence, there is a need to introduce improved disease management techniques such as using resistant varieties, cultural and chemicals to be incorporated as integrated disease management practices.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Organic matter and maize straw influence on soil moisture in an Oxisol under different tillages

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Cultivation system is one of the main parameters inherent to soil water dynamics. Studies indicate that in soil conservation systems, such as no-tillage and crop-livestock integration, soil quality is maintained and even improved due to higher input of organic matter and straw kept on the soil surface. Thereby, the aim of this study was to analyze the influence of the spatial variation of maize straw and organic matter content on soil moisture of an Oxisol at the municipality of Jataí, southwest of Goiás. Nine soil-sampling points were used in 1 ha, allocated to three different types of soil management (No-Tillage, Crop-Livestock Integration and Conventional Tillage). For the study of organic matter, soil samples were collected in nine points and three depths (0 to 0.12 m, 0.12 to 0.24 m and 0.24 to 0.36 m) for each point. The dry mass of straw was determined by a stove at 65°C, after sampling in the area using a circle with internal area of 1.0 m², released randomly near the sampling point. Soil moisture was determined immediately after straw sampling at the same site, using a time domain reflectometry sensor. The results indicated good distribution of straw on both systems, but there was no correlation between straw and soil moisture. The organic matter content was highly correlated with soil moisture, especially in No-Tillage and Crop-Livestock Integration systems. The authors recommend the increase of organic matter of the soils for better maintenance of soil moisture.

Key words: Soil physical properties, soil water content, soil management systems, no-tillage, crop-livestock integration.

INTRODUCTION

Organic matter has been identified as a primary indicator of soil quality, since it acts directly and indirectly on the physical, chemical and biological attributes of the soil and on the plants (Doran, 2002; Payan et al., 2007). The straw deposited on the soil surface has also influenced these characteristics.

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During the whole development of the plant, it absorbs water and loses using the ground as a reservoir of water and nutrients. The spatial and temporal distribution of climate, the variety of culture and management of the agricultural system, modify the physical properties of the soil, which is directly related to storage of water (the main factor determining the productivity of crops), so, the amount of water consumed by a crop during its development varies with them (Moreti et al., 2007).

Carvalho et al. (2004) mention that soil has higher variability in their attributes, both vertically and horizontally, resulting from the interaction of the processes that govern the factors of their formation. Knowledge of the distribution of physical water soil attributes becomes a basic requirement when seeking to establish appropriate management practices for soil and crops, for failure to comply with these concepts will result in errors in sampling and soil management. This stems from the large spatial variation of soil attributes and meaning and direction of water flows (Iqbal et al., 2005).

Thus, it is observed that the cultivation system is one of the main parameters inherent in the dynamics of water in the soil. Therefore, several studies indicate that in conservation system, as no-tillage (NT) and crop-livestock integration (CLI), soil quality is maintained and/or even improved by greater input of organic matter, due to the straw kept on the soil surface (Tormena et al., 1998; Oliveira et al., 2004; Farinelli et al., 2006; Fontana et al., 2006; Salton et al., 2008; Carneiro et al., 2009).

Knowing the shape and magnitude of the changes in soil physical-water properties allows a better understanding of the relationships between the different management systems and the evaluation of their effects on associated processes, allowing the adjustment of indexes and generated models, for example in PC, adapting them to other types of management systems (Dalmago et al., 2009). In this sense, the aim of this work was to analyze the influence of the spatial variation of maize straw and organic matter content on moisture of an Oxisol.

MATERIALS AND METHODS

Experiment location

The experiment was carried out in open field at the Federal University of Goiás (UFG), Regional Jataí, in the southwest of the State of Goiás - Brazil (Figure 1), in a Savanah region. The location has 17° 52 '53 "S of latitude and 51° 42' 52" W of longitude, with 700 m of altitude, located in a climatic region type Cw, tropical savanna, mesothermal, with well-defined dry and rainy season, according to Kopen rating. The average annual rainfall varies around 1600 mm (Koetz et al. 2010). The studied soil was classified as a typical dystrophic red Oxisol, according to the classification of EMBRAPA (2013).

Experiment area

The experimental area was composed of three subareas of approximately 1 ha each, cultivated with soybean (*Glycine max*) in the first cultivation after the raining period began (season), with different soil management systems (Figure 1, b and c).

The subareas have been cultivated over the years with soybean in the first cultivation (season) and maize (*Zea mays*) or sorghum (*Sorghum bicolor*) in the second (off-season). Since the year 2008 for no-tillage (NT) (subarea 1), consorting soybean crops and pasture since 2009, for crop-livestock integration system (CLI) (subarea 2) and associated with the use of harrowing at the time of planting for conventional tillage (CT). Each subarea was divided into a regular grid containing nine cells measuring 30 × 30 m each (Figure 1, b, and c) considering the center of each cell as the reference point for sampling, similar to Andriotti (2013).

The nine sampling points were used to maintain the same experimental conditions for the treatments, with the same slope, even soil, and same environmental conditions.

Sampling and data collection

The determination and location of points for data collection was done by geo-reference, using a GPS system ("Global Positioning System"), GARMIN, MAP785 model.

On predetermined points, the maize straw was collected (crop residues deposited on the soil surface) using a circle with internal area of 1.0 m², released randomly near the sampling point, and dried in a forced ventilation stove at 65°C until constant mass (dry mass). Monitoring of soil moisture was done immediately after sampling the straw at each point. Moisture data were collected on nine points on each soil management system (NT, CLI and CT) at depths from 0 to 0.12, from 0.12 to 0.24 and 0.24 to 0.36 m, with instantaneous readings every 14 days, by means of an equipment type time domain reflectometry (TDR).

At the same nine sample points, deformed soil samples were collected to determine pH, percentage of organic matter (SOM), clay, silt and sand, and also undisturbed soil samples with the aid of metal cylinders (0.05 m high and 0.05 m in diameter) to estimate bulk density (*g cm*$^{-3}$), similar to Ferreira et al. (2003) and following recommendations of EMBRAPA (2011). For all sampling, four repetitions were made, collected randomly within a radius of 1 m from the reference point (center of each one of the nine cells measuring).

For calibration of the TDR sensor, a methodology similar to Abbas et al. (2011) simultaneously with sensor monitoring, 270 deformed soil samples were collected in five of the nine points of each management system, with 4 replicates per point (20 replicates per analyzed depth) were collected randomly within a radius of 1 m from the point, at three depths.

The study was conducted in an atypical year in which the rainy season began a little later than normal. Thus, the collections occurred between November 19, 2013 (end of the dry period of 2013) and January 15, 2014 (during the rainy season), which made it possible to obtain greater soil moisture variation due to greater variability between the months analyzed. These soil samples, duly collected and packed in hermetically sealed plastic bags, were then transported to the soil laboratory of the UFG, where the gravimetric moisture was determined by the stove method and subsequently converted to volumetric moisture (θ) using the density of the soil.

Statistical analysis

Soil characteristics were analyzed by the analysis of variance (ANOVA) and test *Scott-Knott* at 5% of probability (α = 0.05). The topography was analyzed using altimetric dimensions got from the GPS system previously mentioned and the data were worked on the SURFER® software for three-dimensional representation.

Spatial distribution of soil organic matter and straw were studied

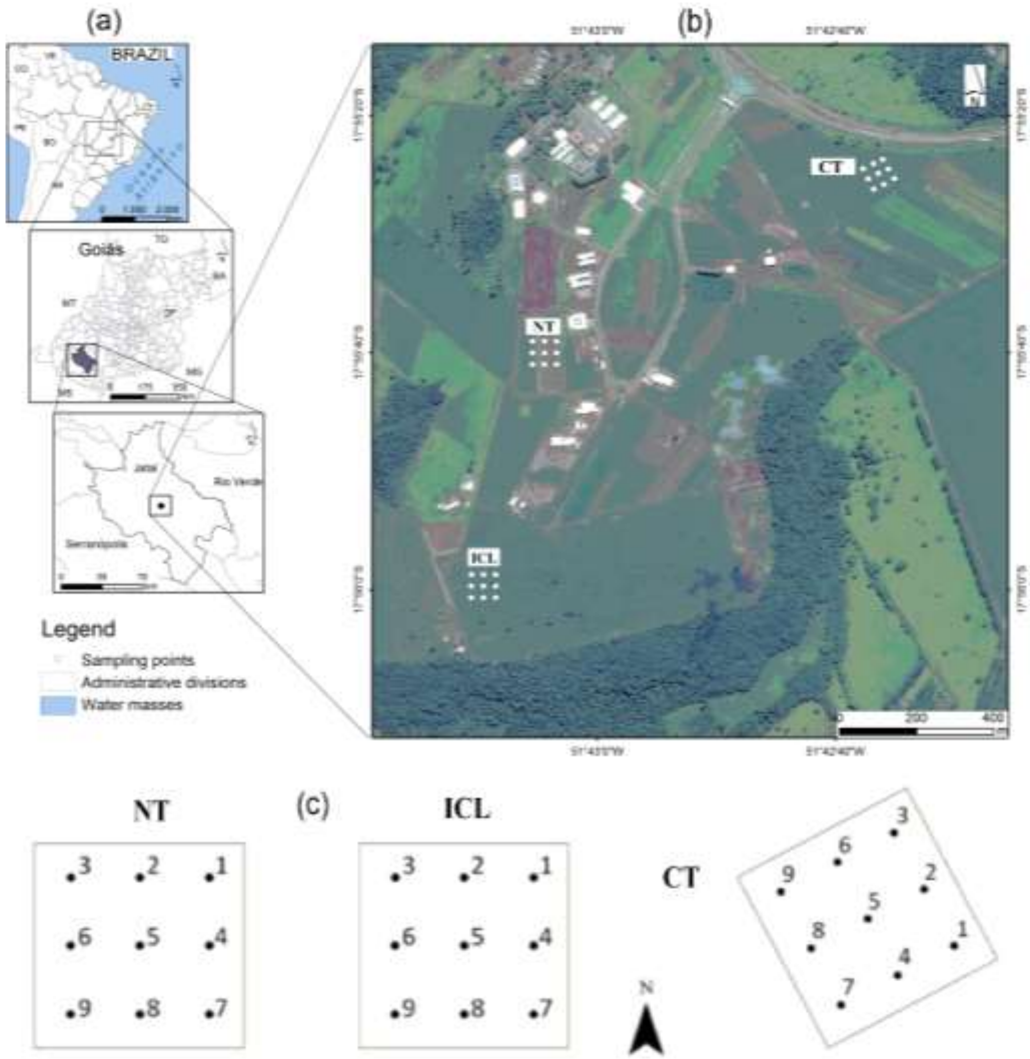

Figure 1. Experiment location (State System of Geoinformation of Goiás - SGEI, 2014) (a); Satellite image of the study area (© Google Earth, 20/04/2014) (b); Sketching and distribution of the sampling points in the study areas (c). NT: No-tillage; CLI: crop-livestock integration; CT: conventional tillage. Source: Guimarães et al. (2018).

on SURFER® software and the results showed by two-dimensional representation.

The correlation between soil moisture and variables investigated by the coefficient of Pearson, for the three types of management in different soil depths was also done similar to Gao and Shao (2012).

RESULTS AND DISCUSSION

Area characteristics

Although Schneider et al. (2008) mention that the continuing existence of the pattern of the water content in the soil can be influenced by the topography, the relief of the areas used in the study (the gently rolling plan) significantly reduces the interference of relief in the spatiotemporal pattern of moisture soil in these areas and significantly reduces the influence of the topography in the temporal analysis of soil moisture (Figure 2).

As the topography, vegetation also had little influence in the comparative analysis of soil moisture between the three types of soil management (NT, CLI and CT), since the plantation was carried out with the same crop (soybean) and on the same date for both.

Soil characteristics

The soil pH (H_2O) showed values close to 5.47 for all conditions, which is considered ideal for most crops (Novais et al., 2007).

After comparing soil attributes between depths for each system through analysis of variance and "Scott-Knott"

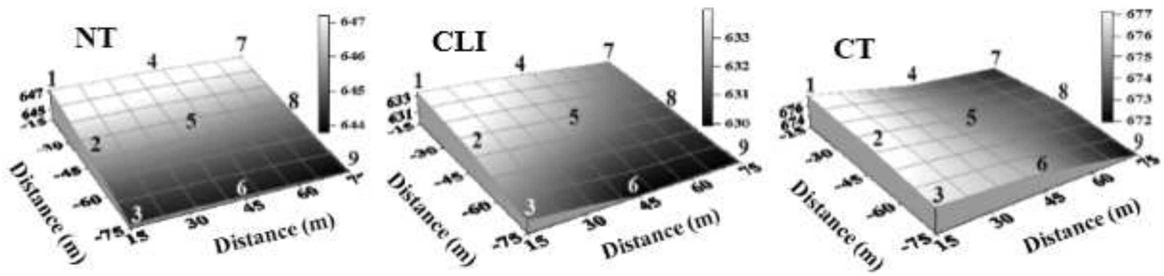

Figure 2. Topography in the study areas. NT: No-Tillage; CLI: Crop-Livestock Integration; CT: Conventional Tillage. Source: Guimarães et al. (2018).

Table 1. Characteristics of the studied Oxisol (ANOVA and test "Scott-Knott," α = 0.05).

Attribute	Parameter	NT-A	NT -B	NT -C	CLI-A	CLI-B	CLI-C	CT-A	CT-B	CT-C
	Average	1.26^{a}	1.19^{b}	1.17^{b}	1.32^{a}	1.22^{a}	1.14^{b}	1.24^{a}	1.26^{a}	1.27^{a}
	max.	1.44	1.37	1.32	1.38	1.37	1.25	1.37	1.39	1.38
Bulk Density (g cm^{-3})	min.	1.09	0.99	1.02	1.14	1.09	0.99	1.07	1.14	1.09
(n = 9)	SD	0.10	0.12	0.11	0.07	0.10	0.08	0.10	0.08	0.09
	CV	7.98	10.23	9.69	5.46	7.98	6.92	7.69	6.23	6.73
	SEM	0.03	0.04	0.04	0.02	0.03	0.03	0.03	0.03	0.03
	Average	3.1^{b}	2.7^{c}	2.2^{d}	3.4^{a}	3.3^{a}	2.6^{c}	3.5^{a}	3.4^{a}	2.7^{c}
	max.	3.61	3.59	2.97	4.10	4.04	3.56	4.05	3.61	3.25
Organic Matter (%)	min.	2.56	2.27	1.69	2.71	2.48	1.80	2.56	2.47	2.23
(n = 36)	SD	0.24	0.25	0.32	0.40	0.37	0.38	0.40	0.37	0.36
	CV	7.8	9.38	14.48	11.96	11.15	14.74	11.60	12.29	13.40
	SEM	0.04	0.04	0.05	0.07	0.06	0.06	0.13	0.12	0.12
	Average	45.1^{c}	44.3^{c}	48.2^{b}	43.1^{c}	46.8^{b}	48.3^{b}	57.6^{a}	58.0^{a}	58.9^{a}
	max.	49.32	50.16	52.74	48.59	50.48	53.12	64.85	63.19	63.84
Clay (%)	min.	42.19	36.31	44.21	35.12	39.96	44.19	52.06	47.83	54.16
(n = 9)	SD	2.83	4.64	2.74	4.42	3.36	3.10	4.87	5.37	3.78
	CV	6.27	10.46	5.68	10.25	7.17	6.43	8.45	9.25	6.42
	SEM	0.94	1.55	0.91	1.05	1.12	1.03	1.62	1.79	1.26
	Average	28.5^{a}	29.4^{a}	25.1^{b}	32.0^{a}	27.8^{a}	26.5^{b}	26.2^{b}	26.3^{b}	23.0^{b}
	max.	34.30	36.69	30.14	38.51	30.68	30.66	32.53	33.16	28.97
Silt (%)	min.	24.34	22.65	19.97	25.73	23.52	22.47	17.72	22.43	13.02
(n = 9)	SD	3.40	5.00	3.17	3.91	2.33	2.86	4.57	4.16	4.84
	CV	11.92	17.01	12.64	12.23	8.39	10.77	17.47	15.83	21.08
	SEM	1.13	1.67	1.06	1.30	0.78	0.95	1.52	1.39	1.61
	Average	26.4^{a}	26.3^{a}	26.7^{a}	25.0^{a}	25.4^{a}	25.2^{a}	16.3^{b}	15.7^{b}	18.1^{b}
	max.	29.11	29.35	29.76	26.86	29.36	25.93	18.76	19.23	25.18
Sand (%)	min.	21.38	20.92	24.55	17.25	22.00	24.26	14.18	13.07	14.61
(n = 9)	SD	2.27	2.99	1.68	2.96	2.62	0.57	1.50	1.95	2.99
	CV	8.60	11.35	6.26	11.85	10.33	2.27	9.22	12.40	16.51
	SEM	0.76	1.00	0.56	0.99	0.87	0.19	0.50	0.65	1.00

SD: Standard deviation; SEM: standard error of the mean; CV: coefficient of variation; n: sample size; S. Error: mean standard error; NT: no-tillage; CLI: crop-livestock integration; CT: conventional tillage; A: 0-0.12 m; B: 0.12-0.24 m; C: 0.24-0.36 m; Means followed by the same letter on the line, do not differ statistically.

test at 5% of significance, a statistical difference was noticed between the mean values of the soil attributes evaluated in this study. These differences are represented by different letters placed after the mean values of the attributes (Table 1).

Although the low CV values found for soil attributes

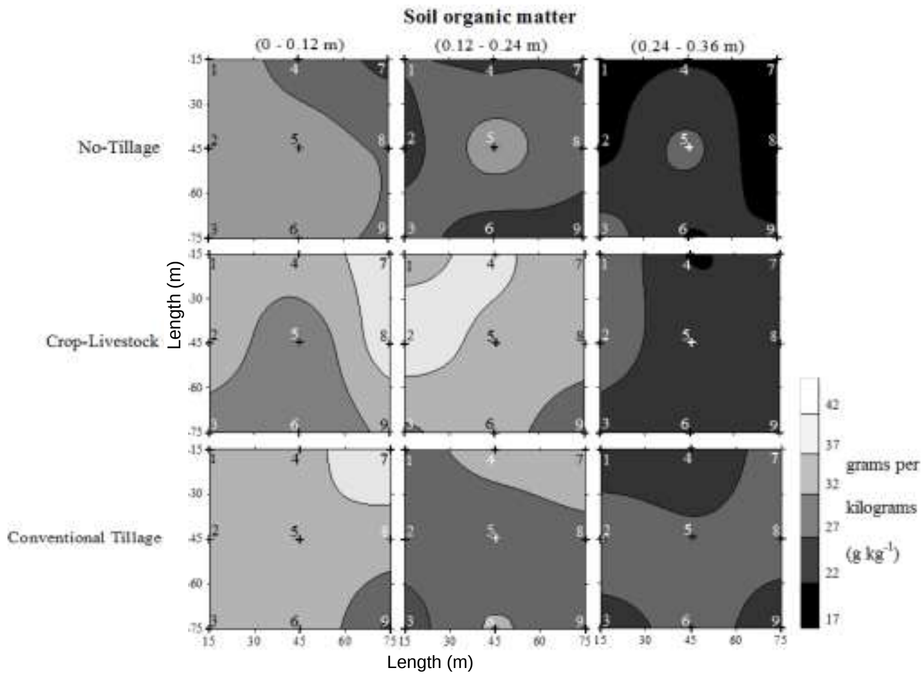

Figure 3. Spatial distribution of soil organic matter in three management systems and three depths.

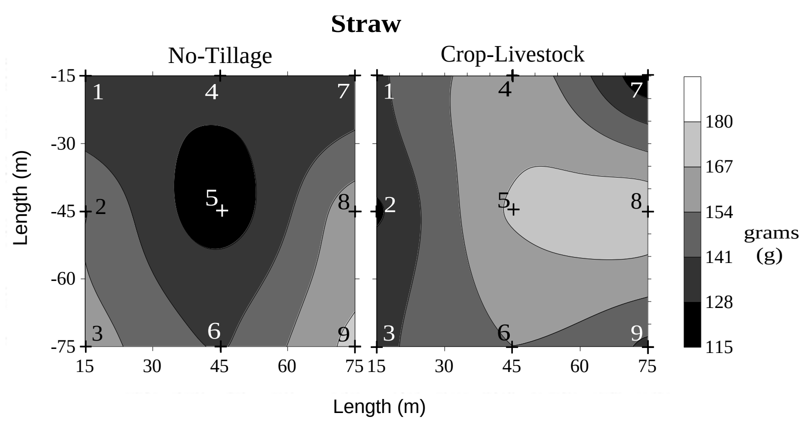

Figure 4. Spatial distribution of the dry mass of straw coming from remains of previous crops on the ground in three management systems and three depths

suggest reduced heterogeneity around the mean (Cruz et al., 2012), statistical difference between the meanings of soil characteristics was noted.

This heterogeneity may have occurred for different reasons, with the soil genesis and the slope that influences the distribution of the soil particles due to the drag (Bertoni and Lombardi Neto, 2008), but low slope of the studied area points to the genesis of soil as the main cause of this heterogeneity, associated with different cropping systems with their specific characteristics.

Different organic matter values were observed by analysis of variance (Table 1), but with a tendency to lower values with increasing the depth in all treatments (Table 1). This observation demonstrated a typical profile of soils in general, since the deposition of dry matter concentrated on the soil surface. The reduction of soil organic matter values was observed with soil depth, represented by gradually changing color pattern as shown in Figure 3; the lighter in the superficial layers to darker shades in deeper layers.

Difference was observed in spatial distribution of dry mass for maize straw between No-Tillage and Crop-Livestock Integration systems: higher values (lighter color) were concentrated at the ends on first system and they were concentrated in the central area on the second (Figure 4). These results corroborate some works that

Table 2. Descriptive statistics of soil moisture for the collection dates in the three management systems and three depths.

Date	Min (%)	Max (%)	Ȳ (%)	SD	CV (%)	Min (%)	Max (%)	Ȳ (%)	SD	CV (%)	Min (%)	Max (%)	Ȳ (%)	SD	CV (%)
	NT (0 - 0.12 m)					**NT (0.12 - 0.24 m)**					**NT (0.24 - 0.36 m)**				
9-Nov	27.7	33.2	30.2	1.7	5.7	27.2	30.4	28.8	1.1	4.0	26.4	32.1	29.2	1.6	5.4
23-Nov	24.9	32.8	28.5	2.5	8.7	19.6	27.7	23.5	2.6	11.0	20.6	25.2	22.8	1.4	6.2
7-Dec	34.0	37.1	35.5	1.1	3.1	27.9	33.9	30.9	2.1	6.8	29.4	32.9	31.4	1.1	3.5
21-Dec	35.4	40.3	38.0	1.7	4.4	30.1	36.2	33.3	2.0	6.0	29.0	36.2	32.6	2.4	7.5
18-Jan	26.7	32.5	28.3	1.8	6.3	22.4	27.2	24.3	1.4	5.9	22.7	27.8	24.3	1.5	6.2
1-Feb	29.8	35.4	33.2	1.8	5.3	27.9	32.5	29.8	1.7	5.6	27.3	31.5	29.0	1.3	4.6
15-Feb	33.1	39.7	35.7	2.4	6.7	27.9	33.5	30.8	1.9	6.0	24.7	31.5	28.0	2.1	7.5
	CLI (0 - 0.12 m)					**CLI (0.12 - 0.24 m)**					**CLI (0.24 - 0.36 m)**				
9-Nov	30.8	35.0	32.1	1.4	4.3	25.3	31.5	29.1	2.0	7.0	27.8	32.3	29.9	1.5	4.9
23-Nov	30.5	34.7	33.2	1.3	4.1	20.5	26.1	23.6	2.0	8.4	23.0	28.7	26.5	1.8	6.9
7-Dec	32.9	39.4	36.1	1.9	5.3	28.9	35.1	32.3	2.2	6.8	28.2	35.0	31.4	2.2	6.9
21-Dec	35.9	42.7	39.9	2.3	5.8	28.8	43.9	37.6	4.5	12.0	29.6	36.8	34.3	2.6	7.6
18-Jan	28.9	31.4	29.8	1.0	3.3	19.8	24.3	21.5	1.3	6.1	22.4	26.3	24.5	1.2	5.0
1-Feb	32.9	36.1	34.8	1.2	3.3	27.3	35.6	32.0	3.1	9.6	28.2	34.1	31.2	1.7	5.4
15-Feb	36.6	40.8	38.1	1.4	3.7	26.1	38.3	33.8	3.9	11.7	25.3	33.2	30.7	2.3	7.6
	CT (0 - 0.12 m)					**CT (0.12 - 0.24 m)**					**CT (0.24 - 0.36 m)**				
9-Nov	25.9	28.0	26.6	0.7	2.5	32.7	39.5	35.1	2.1	2.1	28.0	34.5	32.1	2.0	6.3
23-Nov	31.8	37.5	34.2	1.8	5.4	30.6	39.0	35.0	3.1	8.9	29.8	37.3	33.1	2.7	8.1
7-Dec	31.4	36.2	34.3	1.6	4.6	34.7	40.9	38.2	1.9	4.8	34.6	40.1	37.4	1.8	4.9
21-Dec	31.8	43.8	38.9	4.6	11.9	36.1	45.2	41.0	2.5	6.0	29.3	44.4	38.7	4.1	10.7
18-Jan	28.4	32.8	30.2	1.2	3.9	29.8	33.3	31.7	1.2	3.9	27.9	30.7	29.5	1.0	3.3
1-Feb	28.3	34.3	31.8	1.7	5.3	34.6	40.8	37.6	1.8	4.7	32.0	36.9	34.4	1.8	5.1
15-Feb	29.6	33.5	30.7	1.3	4.1	25.9	30.4	27.9	1.4	5.1	25.0	28.5	26.7	1.1	4.1

Min: Minimum; Max: maximum; Ȳ: average; SD: standard deviation; CV: coefficient of variation; NT: no-tillage; CLI: crop-livestock integration; CT: conventional tillage.

affirm the use of the soil in agricultural crops introduces new sources of heterogeneity. Among them is the irregular distribution of crop residues (Gomez and Gomez, 1984; Steel and Torri, 1997).

Considering average values of the moisture to all the campaigns, a variation between 22 and 41% is shown in Table 2, with a maximum value close to the field capacity (43%) determined by Koetz et al. (2010) in previous work in the same area of the experiment. The temporal profile was similar between the three systems, with highlights for NT and CLI, which were similar for the three depths, especially in relation to the more superficial layer that presented higher values than the other depths in both systems and in all campaigns.

This similarity between the systems was probably because they were very close to each other: approximately 500 m from NT to CLI and 800 m from NT to CT (Figure 1). Especially between NT and CLI, in addition to similar soil management, little soil rotation and high deposition of dry mass of the corn straw on the soil surface, preventing evaporation of the water.

Similar to Gao and Shao (2012), the correlation coefficient of "Pearson" was used to examine the dependence of the water content in the soil (WCS) in relation to the soil properties to NT, CLI and CT at three different depths (Table 3).

The results in Table 3 show that soil organic matter exerted greater influence on soil moisture in accordance with Gómez-Plaza et al. (2000), Zhao et al. (2010), and Biswas and Si (2011) in relation to straw. This behavior was characterized by the largest number of events with significant correlation coefficient values of "Pearson", which are greater than those found by Zhao et al. (2010) and Gao and Shao (2012). All soil organic matter significant events were positively correlated, that is, higher soil organic matter levels made possible the maintenance of the water content in the soil.

Conclusions

There was great influence of organic matter on soil moisture. The organic matter content showed a high correlation with soil moisture, especially in the more conservationist systems. In other words, the higher soil organic matter content, the higher the soil moisture

Table 3. Correlation coefficient of "Pearson" between soil moisture and variables investigated for the three types of management in different soil depths.

Management system	Depth	SOM	Straw
NT	A	0.68**	0.20
	B	0.10	0.29
	C	0.51**	0.51**
ICL	A	0.65**	0.22
	B	-0.33	0.13
	C	0.86**	-0.01
CT	A	0.21	-
	B	0.06	-
	C	0.18	-

**Significant to 1% (ANOVA); A: 0 to 0.12 m; B: 0.12 to 0.24 m; C: 0.24 to 0.36 m; SOM: soil organic matter; NT: no-tillage; CLI: crop-livestock integration; CT: conventional tillage.

content. There was a good distribution of maize straw on both systems, but there was no correlation with soil moisture, in other words, for this work, maize straw did not influence on soil moisture.

The authors recommend the increase of organic matter of the soils for better maintenance of soil moisture.

Future researches that compared other variables, such as soil density and texture, are encouraged by the authors and may provide different conclusions from those of this study.

CONFLICT OF INTERESTS

The authors have not declared any conflicts of interests.

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Influence of cytoplasmic genetic male sterility in the grain yield of maize hybrids

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One of the main barriers to the production of hybrid maize seeds consists of needing detasseling of female parent in order to avoid contamination with unwanted pollen. This activity is a laborious practice, which makes the production more expensive and promotes productive losses. Thus, the use of cytoplasmic genetic male sterility can facilitate the hybridization process, and consequently, the production of hybrid seeds. The objective of this study was compare grain yield in maize hybrids produced from the combination of two testers with five distinct lines of cytoplasm C "Charrua" versus the isogenic of fertile cytoplasm. Twenty common hybrids were evaluated in relation to grain crops and resistance to leaf diseases, white spot and gray leaf spot. The experiments were carried out during summer season of 2015/2016 and winter season of 2016. The use of lines with cytoplasm C was observed and it is promising since it did not affect the agronomic performance of hybrids, regardless of environment, crop season, parental and tester used. Therefore, cytoplasm C can be used as an excellent source of cytoplasmic genetic sterility in the production of hybrid seeds in seed companies that want to decrease the use of detasseling practice.

Key words: *Zea mays* L, production of seeds, detasseling.

INTRODUCTION

Maize is one of the most cultivated cereals in the world. In Brazil alone, more than 16 million hectares were sown during summer 2017/2018 and winter 2018 seasons. The average Brazilian corn yield is over 5 $kg.ha^{-1}$ (Conab,

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2018), and this is mainly because of hybrid seed adoption and management technologies. The simple hybrids ensure greater uniformity and enables high grain yield. However, the production process of hybrid seeds shows complications, such as the necessity of detasseling of female plants (Magalhães et al., 1999).

The manual corn detasseling is a seasonal and expensive practice. Consequently, this practice promotes an increase in production cost of hybrid seeds. Although there is equipment that enables the mechanization of this process, it is essentially manual repass. An alternative to eliminating labor in this process is the use of male sterile lines (Magalhães et al., 1999). The use of cytoplasmatic male sterility (CMS) and restoration fertility through nuclear genes permit the commercial exploitation of CMS systems for the production of hybrid seeds through the elimination of manual detasseling operation and generation assurance, F_1 fertility (Schnable and Wise, 1998). CMS is induced by the complementary action of nuclear and cytoplasmatic genes. These specific mutations in mitochondrial DNA make the plant produce no pollen or produce pollen that is not functional. Nevertheless, female fertility is not influenced by CMS, that is, male sterile plants can produce seeds if there is viable pollen available (Weider et al., 2009).

Basically, the CMS of maize can be divided into groups: CMS-T (Texas), which was practically eliminated in seed production due to the vulnerability to the fungus *Helminthosporium maydis* (Tatum, 1971; Duvick, 1973); CMS-S (USDA), which is unsteady and frequently shows fertility restoration; and CMS-C (Charrua), which has steady male sterility and a positive effect on grain yield (Weider et al., 2009; Stevanovic et al., 2016). Several types of research were realized in the area of molecular biology and cytogenetics with cytoplasm C (Lu et al., 2010; Yongming et al., 2016; Chen et al., 2016); however, information about the stability and productivity of materials in different environmental conditions is still very little disseminated (Weider et al., 2009), especially in tropical countries, such as Brazil. Considering this, the objective of this work is to evaluate agronomic performance of hybrids with cytoplasm C versus isogenic of normal cytoplasm from the main heterotic groups used by private breeding programs.

MATERIALS AND METHODS

The trials were carried out in the research stations of the company Dow AgroSciences in Brazil, in the summer season of 2015/2016 and in the winter season of 2016. During the summer season, the evaluations were carried out in four environments under irrigated conditions (Iguatama-MG, Indianópolis(1)-MG, Perdizes-MG and Varjão de Minas-MG) and seven non-irrigated conditions (Guarda Mor-MG, Indianópolis(2)-MG, Padre Olegário-MG, Uberaba-MG, Cascavel-PR, Castro-PR, and Itararé-SP). In the winter season, the trials were carried out in 13 environments under non-irrigated conditions (Montividiu-GO, Araguari-MG, Indianópolis-MG, Nova Mutum-MT, Primavera de Leste-MT, Sapezal-MT, Sorriso-MT, Cambé-PR, Cascavel-PR, Palotina-PR, Santa Terezinha do Itaipú-PR, Guaíra-SP, and Maracaí-SP).

The experimental design used was randomized blocks with two replicates. The plots were made up of four rows measuring four meters with spacing of 0.5 m between lines. At the moment of the sowing, NPK formulation used was 09-29-09 in doses of 450and 300 $kg.ha^{-1}$ for summer and winter seasons, respectively. The application of nitrogenous and potassic fertilizers were incorporated through sowing. In general, the dose of urea varied from 200 to 350 $kg.ha^{-1}$, and the dose of potassium chloride varied from 100 to 150 $kg.ha^{-1}$, distributed twice during the crop cycle. The crop management, such as pest control, weeds and diseases were made according to the recommendations for maize.

Twenty treatments were evaluated, being 10 simple sterile hybrids of cytoplasm C and their respective iso-hybrid of fertile cytoplasm, from the crossings of five sterile lines of cytoplasm C with two testers. The female genitor lines belonged to the heterotic groups Tuxpeno, Suwan and Cateto, while testers belonged to groups Cateto and Suwan. These testers, in combination with lines of cytoplasm C, produced hybrids partially and totally fertile, respectively. The seeds of 20 simple hybrids were produced by manual crossing in pollination fields of research station in Indianópolis during the winter season of 2015. After the flowering, the foliar diseases, white spot (*Phaeosphaeria maydis*/ *Pantoea ananas*) and gray leaf spot (*Cercospora zeae-maydis*), were evaluated through a diagrammatic scale proposed by Agroceres (1996). In this scale, score 1 represents plots with 100% foliar tissue undermined or premature death, and score 9 represents plots with no foliar spots. As the incidence of the pathogens is dependent on specific conditions, the environments chosen for the evaluations were those that presented greater occurrences of foliar diseases. The weight of grains in each plot was evaluated in the moment of harvest and submitted to the variance analysis according to the following statistical models (1):

$$y_{ijk} = \mu + b_i + h_j + l_k + hl_{jk} + e_{ijk} \quad (1)$$

y_{ijk}: value observed of the plot that received hybrid *i* of block *j* in the environment *k*;
μ: general average associated with all observations;
b_i: effect of *i*-th block, being b_i ~N $(0, \sigma_b^2)$ (*i* = 1 and 2);
h_j: effect of *j*-th hybrid, being h_j ~N $(0,\sigma_h^2)$ (*j* = 1, 2, 3, ... 10);
l_k: effect of *k*-th environment, being l_k ~N $(0,\sigma_l^2)$ (*k* = 1, 2, 3, … 11 for summer season; *k* = 1, 2, 3, … 13 for winter season; and *k* = 1, 2, 3, ... 24 for joint analysis);
hl_{jk}: effect of interaction between hybrids and environments;
e_{ijk}: average experimental error associated with plot *ij*, being e_{ij} ~N $(0, \sigma_e^2)$.

In the variance analysis, the decomposition of hybrid effect on sterile lines, fertile lines and testers was realized. The averages were compared through mean contrasts, using Scott-Knott test (Scott and Knott, 1974).The experimental accuracy (2) per environment was calculated according to Resende and Duarte (2007):

$$\hat{r}_{gg} = \left[\frac{1}{1+(\sigma_e^2/b)/\sigma_h^2}\right]^{1/2} \quad (2)$$

σ_h^2: variance between hybrids; σ_e^2: residual variance; *b:* number of blocks in the environment.
All statistical analyses were realized in the software R (R Core Team, 2017).

RESULTS AND DISCUSSION

The summary of joint variance analysis for grain yield of the 20 hybrids evaluated is shown in Table 1. The sources of variation hybrids, environments and interaction hybrids x environments differ between them at significance level of 0.01. With detasseling of hybrids effect, it was also possible to observe occurring significant differences ($p < 0.01$) for sterile lines, fertile lines, testers and the interaction between lines and testers. Moreover, all interaction unfolding between hybrids and environments were significant. Significant differences ($p \geq 0.05$) were not observed for the contrast between sterile and fertile lines and for the contrast between testers and lines. Thus, it is possible to conclude fertile lines do not have grain yield that is higher than those of sterile lines, independent of the tester used.

The focus of this work is on verifying the possible effect of cytoplasm Charrua or CMS-C in the productive performance of hybrids and if they differ between environments and/ or with the tester used. Although the effect of hybrids was significant ($p < 0.01$), no significant differences was determined in the contrast of average grain yield of hybrids for cytoplasm C and version of fertile cytoplasm with 9.68 and 9.71 $t.ha^{-1}$, respectively.

While having a key role in respiration and energy production, mitochondria is also related to cytoplasmatic male sterility. However, most parts of its performance and role arise from genetic information found in the cell nucleus. That is because the number of genes in the mitochondrial DNA is very small. Though the mitochondrial maize genome is one of the most complexes, there are 58 to 60 genes involved in different metabolic processes (Clifton et al., 2004; Chen and Liu, 2014).

Thus, in order for difference between hybrids on the basis of cytoplasm to occur, at first, there are two possibilities. One of them is that although mitochondria has a few genes, they could be influenced by genotype of the line used as recurring genitor or by some environmental factor, such as in the case of cytoplasm T and occurrence of *Bipolaris maydis*. The other possibility would be during backcrossing, substituting the nucleus of the donor line of cytoplasm for the nucleus of the commercial line. The transference would not occur in all crosses and, in this case, the donor line can cause differences. From the results obtained in this work, it is possible to infer that the cytoplasm C did not affect the agronomic performance of the hybrid, and the backcrossings realized for transferring the nucleus were efficient. However, as female fertility is not affected by the presence of cytoplam CMS, and male sterile plants can produce seeds if there is viable pollen available (Weider et al., 2009), it is expected yield does not change in plants that have cytoplasm CMS.

Robledo et al. (2013) evaluated the sterile and fertile versions of two maize hybrids submitted to four different management systems of detasseling in two different plant densities. The author determined there were no differences between fertile and male-sterile versions in grain yield. Sangoi and Salvador (1998) and Magalhães et al. (1999) did not observe differences in grains production between both versions of genotypes evaluated. Table 2 summarizes the individual variance analysis for grain yield in 24 environments during summer of 2015/2016, and the second winter of 2016. With the exception of Araguari – MG, the contrast between fertile and sterile lines, and Primavera do Leste – MT, for the interaction contrast between lines and testers, the environments showed p-value as non-significant ($p \geq 0.05$).

Considering all environments for both seasons, grain yield varied from 3.15 to 13.65 $t.ha^{-1}$. During summer of 2015/16, the average yield was 11.86 $t.ha^{-1}$; while the average was 7.76 $t.ha^{-1}$ during winter. The trial in Uberaba - MG had the greatest yield in summer; while the trial in Santa Terezinha do Itaipu - PR had the greatest yield in winter, exceeding 10 $t.ha^{-1}$. On the other hand, Presidente Olegário - MG and Primavera do Leste - MT were the least productive trials during summer and winter, respectively. The experimental accuracy ($\hat{r}_{gg}$) vary from 0.81 to 0.97 (Table 2), which shows high experimental quality (Resende and Duarte, 2007).

During summer of 2015/2016, the hybrids were classified into four distinct groups. Hybrids H 01, H 04, H 06, H 07 and H 09 were the most productive with average varying from 12.63 to 13.89 $t.ha^{-1}$. Hybrids H 02, H 03 and H 10 were in the intermediate groups, with averages varying from 10.6 to 12.3 $t.ha^{-1}$. Finally, hybrid H 08 was the least productive, with average varying from 4.8 to 5.1 $t.ha^{-1}$. Only hybrid H 05 showed significant statistical difference between the versions; being the version of fertile cytoplasm higher than version CMS-C, based on the Scott-Knott test at 5% of probability (Table 3).

In the winter season, hybrids were classified in five distinct groups. The hybrids H 01 and H 04 were the most productive with the average varying from 8.8 to 9.2 $t.ha^{-1}$ and were followed by the hybrids H 03, H 06 and H 07 whose averages vary from 8.2 to 8.7 $t.ha^{-1}$. In the third and fourth groups were the hybrids H 10 and H 02, respectively. Finally, the hybrid H 08 was the least productive with average varying from 2.61 to 2.67 $t.ha^{-1}$. Only the hybrids H 05 and H 09 showed significant statistical differences between the versions of fertile cytoplasm and CMS-C, according to the Scott-Knott test at 5% of probability (Table 3).

It should also be noted that the climate conditions in the summer were favorable for the development of the crop even with no irrigation. During winter, low rainfall rates were registered. There were productivity losses in a large part of the producing regions due to the shortage of rain, mainly in the Southeast and Central West part of the

Table 1. Summary of joint variance analysis for grains yield (t.ha^{-1}) of 20 simple maize hybrids evaluated in 24 environments. The degrees of freedom (DF), mean square (MS) and p-value are shown for each source of variation.

Sources of variation			DF	MS	p-value
Blocks/ Environments (B)			24	1.32	0.52
Environments (E)			23	281.18	0.00
Hybrids (H)			19	220.24	0.00
	Lines (L)		9	232.46	0.00
		Sterile Line (SL)	4	276.50	0.00
		Fertile Line (FL)	4	247.00	0.00
		SL vs FL	1	0.30	0.63
	Tester (T)		1	271.16	0.00
	T x L		9	202.38	0.00
		T x SL	4	247.50	0.00
		T x FL	4	207.25	0.00
		T x (SL vs FL)	1	2.70	0.16
E x H			426	2.95	0.00
	E x L		206	3.23	0.00
	E x T		23	7.25	0.00
	E x L x T		197	2.14	0.00
error			441	1.37	

Table 2. Summary of the mean square (MS) and p-value of the decomposition of the effect of hybrids in sterile line (SL), fertile line (FL) and tester (T), grain yield (t.ha^{-1}) and experimental accuracy ($\hat{r}_{gg}$) of each of the 24 evaluated environments and repective havest season, summer of 2015/2016 and winter of 2016.

Environment	Season	SL vs FL		T (SL vs FL)		Grain yield (t.ha^{-1})	$\hat{r}_{gg}$
		MS	p-value	MS	p-value		
Padre Olegário-MG	Summer	7.489	0.064	0.167	0.772	9.408	0.95
Guarda Mor-MG	Summer	0.720	0.524	0.940	0.465	10.685	0.95
Perdizes-MG	Summer	0.802	0.602	4.276	0.235	10.987	0.88
Indianápolis(1)-MG	Summer	0.267	0.699	0.227	0.721	11.446	0.94
Cascavel-PR	Summer	2.678	0.119	0.083	0.777	11.695	0.97
Indianápolis(2)-MG	Summer	1.508	0.408	0.374	0.678	11.847	0.92
Itararé-SP	Summer	0.280	0.542	0.000	0.956	11.962	0.96
Castro-PR	Summer	1.957	0.162	1.540	0.212	12.739	0.97
Iguatama-MG	Summer	0.920	0.604	0.540	0.691	12.901	0.93
Varjão de Minas-MG	Summer	0.886	0.445	1.622	0.304	13.240	0.94
Uberaba-MG	Summer	0.087	0.787	1.375	0.290	13.645	0.97
Primavera do Leste-MT	Winter	0.039	0.700	2.619	0.006	3.153	0.88
Araguari-MG	Winter	5.024	0.021	0.125	0.697	5.268	0.93
Nova Mutum-MT	Winter	1.466	0.099	0.272	0.461	6.017	0.95
Maracaí-SP	Winter	0.011	0.895	1.148	0.192	6.336	0.92
Indianópolis-MG	Winter	0.045	0.847	1.683	0.245	7.518	0.93
Cambé-PR	Winter	0.084	0.815	0.444	0.591	7.720	0.89
Sorriso-MT	Winter	0.088	0.661	0.700	0.224	7.848	0.96
Palotina-PR	Winter	0.271	0.608	0.041	0.840	8.092	0.96
Montividiu-GO	Winter	2.243	0.205	0.049	0.848	8.559	0.90
Sapezal-MT	Winter	4.223	0.151	5.998	0.091	8.996	0.81
Cascavel-PR	Winter	1.754	0.278	0.371	0.613	10.301	0.92
Guaíra-SP	Winter	3.089	0.204	0.030	0.899	10.482	0.95
Sta T. de Itaipu-PR	Winter	4.740	0.062	3.550	0.103	10.679	0.98

Table 3. Grain yield (t.ha^{-1}) of 20 simple hybrids from five fertile and five male-sterile lines crosses with two testers in the summer season of 2015/16 and in the winter season of 2016.

Hybrid	Female	Male (Tester)	Grain yield (t.ha^{-1})			
			Summer 2015/2016		Winter 2016	
H 01	F66PW	M51	13.607	A	9.017	A
H 01C	F66CPW	M51	13.894	A	9.103	A
H 02	F16PW	M51	11.305	C	6.832	D
H 02C	F16CPW	M51	11.505	C	6.809	D
H 03	F20PW	M51	12.375	B	8.515	B
H 03C	F20CPW	M51	12.093	B	8.268	B
H 04	F25PW	M51	13.289	A	8.813	A
H 04C	F25CPW	M51	12.960	A	9.144	A
H 05	F21PW	M51	12.638	A	8.335	B
H 05C	F21CPW	M51	11.927	B	7.920	C
H 06	F66PW	M52	13.398	A	8.523	B
H 06C	F66CPW	M52	13.162	A	8.689	B
H 07	F16PW	M52	12.903	A	8.336	B
H 07C	F16CPW	M52	13.604	A	8.747	B
H 08	F20PW	M52	5.151	D	2.675	E
H 08C	F20CPW	M52	4.851	D	2.610	E
H 09	F25PW	M52	13.438	A	8.721	B
H 09C	F25CPW	M52	13.629	A	9.203	A
H 10	F21PW	M52	10.619	C	7.688	C
H 10C	F21CPW	M52	10.972	C	7.870	C
Hybrids of Fertile Lines (PW)			11.872	A	7.746	A
Hybrids of Male-sterile Lines (CPW)			11.860	A	7.836	A

PW female fertile; CPW female sterile. Averages vertically followed by the same letter do not differ statistically by the Scott-Knott test at 5% of probability.

country. As a result of this, the yield of the trials varied from 3.15 to 10.6 t.ha^{-1}**.**

Table 4 shows the grain yield based on the parents. The results indicate there were significant differences between the hybrids obtained by each tester. The hybrids obtained from M51 were significantly higher, with an average yield of 10.28 t.ha^{-1}; while the hybrids of M52 produced 9.16 t.ha^{-1}. For crossings done with the tester M51, hybrids obtained from lines F25CPW, F25PW, F66CPW, and F66PW were the most productive and did not have significant differences between them. The least productive hybrids were obtained from lines F16CPW and F16PW with yield lower than 9 t.ha^{-1}. For crossings done with the tester M52, the most productive hybrids were those obtained from lines F16CPW, F25CPW and F25PW. The hybrids that had the worst performance were those obtained from crossing with lines F20CPW and F20PW.

According to the Scott-Knott test at 5% of probability, out of ten hybrids tested in both versions, only three showed significant differences in grain yield. Even so, F20PW/M51, F21PW/M51, and F16PW/M52 had their yields very close to the version CMS-C. Although some hybrids showed significant differences between versions of fertile cytoplasm and CMS-C, in the set of hybrids this difference is approximately 40 kg.ha^{-1}.

Historically, the use of male-sterile cytoplasm type T was highly criticized because of susceptibility to *B. maydis* (Tatum, 1971). Thus, the aim of this work was to evaluate the main diseases whose occurrence and severity are common in this region during summer and winter. Nevertheless, the occurrence of the disease depends on the presence of pathogens, a host that provides low genetic resistance and climate conditions favorable for the development of the pathogen; consequently, the study was limited to specific locals and conditions. The evaluations of foliar diseases white spot and gray leaf spot, according to Agroceres scale (1996) adapted, is in Table 5. The evaluations were realized in Irai de Minas – MG, during summer of 2015/2016; and in Indianópolis – MG, during winter of 2016, whose environments were more favorable to the evaluation due to the occurrence of pathogens. The use of fungicides associated with the usage of hybrids adapted that provide good tolerance to diseases did not permit the emergence and development of other pathogens, such as *Puccinia*

Table 4. Mean grain yield (t.ha^{-1}) based on the combinations between sterile lines (CPW at the end of the name) and fertile lines (PW at the end of the name) with each tester (M51 and M52).

Lines	Testers				
	M51		M52		Average
F16CPW	8.960	D	10.920	A	9.930
F16PW	8.880	D	10.430	B	9.660
F20CPW	10.020	C	3.660	E	6.870
F20PW	10.440	B	3.940	E	7.230
F21CPW	9.760	C	9.260	D	9.510
F21PW	10.310	B	9.150	D	9.760
F25CPW	10.970	A	11.230	A	11.100
F25PW	11.270	A	10.880	A	11.060
F66CPW	11.300	A	10.740	B	11.020
F66PW	11.120	A	10.760	B	10.940
Sterile lines (CPW)	10.190	a	9.180	b	9.690
Fertile lines (PW)	10.370	a	9.140	b	9.730

Averages vertically followed by the same capital letter and horizontally by lowercase do not differ between them by the Scott-Knott test at 5% of probability.

Table 5. Scores[1] of diseases for white spot (*P. maydis*/ *P. ananas*) and gray leaf spot (*C. zeae-maydis*) evaluated in 20 single hybrids in two locals, Irai de Minas - MG in summer season of 2015/16 and Indianópolis - MG in the winter season of 2016.

Hybrid	Female	Male (Tester)	White spot		Gray leaf spot	
H 01	F66PW	M51	5	MS	7	R
H 01C	F66CPW	M51	4	MS	8	R
H 02	F16PW	M51	4	MS	6	MR
H 02C	F16CPW	M51	5	MS	7	R
H 03	F20PW	M51	6	MR	6	MR
H 03C	F20CPW	M51	6	MR	8	R
H 04	F25PW	M51	5	MS	7	R
H 04C	F25CPW	M51	5	MS	8	R
H 05	F21PW	M51	5	MS	7	R
H 05C	F21CPW	M51	5	MS	7	R
H 06	F66PW	M52	5	MS	7	R
H 06C	F66CPW	M52	5	MS	7	R
H 07	F16PW	M52	4	MS	4	MS
H 07C	F16CPW	M52	4	MS	4	MS
H 08	F20PW	M52	5	MS	4	MS
H 08C	F20CPW	M52	4	MS	4	MS
H 09	F25PW	M52	5	MS	8	R
H 09C	F25CPW	M52	6	MR	8	R
H 10	F21PW	M52	6	MR	5	MS
H 10C	F21CPW	M52	6	MR	3	S
Hybrids of fertile lines			5	MS	6	MR
Hybrids of Male-sterile lines			5	MS	6	MR

[1]Scale of foliar damage: 9 (0%) AR = highly resistant; 8 (1%) R = resistant; 7 (10%) R = resistant; 6 (20%) MR = moderately resistant; 5 (30%) MS = moderately susceptible; 4 (40%) MS = moderately susceptible; 3 (60%) S = susceptible; 2 (80%) S = susceptible; 1 (>80%) AS = highly susceptible.

spp., Exserohilum turcicum, and *B. maydis*. This enabled a good characterization for white spot and gray leaf spot. The scores of white spot varied from 4 to 6, that is, they vary from moderately susceptible to moderately resistant. The hybrids H 03, H 03C, H 09C, H 10 and H 10C were moderately resistant, and the others were classified as moderately susceptible. Only hybrid H 09 obtained distinct classification in relation to its CMS-C isogenic.

The white spot is commonly associated with the fungus *P. maydis* and there are several molecules of fungicides registered to control this disease in maize crop in Brazil (Agrofit, 2018). However, Paccola-Meirelles et al. (2001) isolated the bacterium *P. ananas* with high frequency. Since then, this bacterium has been considered the etiologic agent of the white spot because it was the only pathogen to be successfully submitted to Koch's postulates (Manerba et al., 2013). Therefore, the genetic resistance as tool to control the white spot is the most effective and low-cost strategy (Carson, 2001; Schuelter et al., 2003; Lana et al., 2017). In this study, there was a small variation in the response of hybrids in relation to the tolerance to white spot. This brings a low variability of genotypes tested back, since no hybrid had full resistant behavior. Generically, hybrids CMS-C did not worsen or improve the level of tolerance to white spot when compared to their isogenic of fertile cytoplasm. For gray leaf spot, scores varied from 3 to 8, and the hybrids were classified between susceptible and resistant. Although many genotypes showed good tolerance to this disease, no hybrid was classified as highly resistant. The hybrids H 01, H 04, H 05, H 06 and H 09 were classified as resistant and did not differ from versions CMS-C. On the other hand, hybrids H 07 and H 08 were classified as moderately susceptible and did not show difference from their CMS-C isogenic. The hybrids H02 and H03 were classified as moderately resistant, while their CMS-C versions were classified as resistant. Finally, the version of fertile cytoplasm of hybrid H 10 was classification as moderately susceptible, higher than its CMS-C version, classified as susceptible. Although the hybrids show higher scores for gray leaf spot, male sterile cytoplasm did not contribute to this condition.

In general, hybrids of fertile cytoplasm obtained the same performance as hybrids CMS-C. Although studies such as that of Calugar et al. (2018) demonstrate that some agronomic characteristics of maize hybrids may be influenced by the alteration of the cytoplasm of the maternal genotype and the interaction between the cytoplasm and the testers, no significant changes were observed in the grain yield between fertile strains and CMS-C in the present study. Despite these results, the interaction among other lines may cause different reactions in the yield of grains or other agronomic characteristics. Stevanovic et al. (2016) observed higher yields of C and S cytoplasm maize lines in relation to normal cytoplasm lines. The authors state that a positive effect of CMS cytoplasm is expected because, as there is no pollen formation, there is less energy and nutrient consumption. In addition, Stevanovic et al. (2016) also observed higher grain yield in C cytoplasm genotypes than in S cytoplasm. Therefore, C type is somewhat more suitable for seed production.

Conclusion

The use of cytoplasm CMS-C to avoid manual detasseling is promising since it does not affect the agronomic performance of the hybrid. The productivity of hybrids CMS-C showed no considerable differences in relation to the fertile isogenic hybrids, independently of the environment, season, parental whose cytoplasm was incorporated and tester used. In addition, the hybrids CMS-C do not differ in tolerance to white spot and gray leaf spot when compared to isogenic fertile cytoplasm. The results constitute additional stimulus for companies who want to adopt cytoplasm C as an efficient and safe strategy to reduce the practice of manual detasseling.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ABBREVIATIONS

CMS, Cytoplasmic male sterility.

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