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Full Length Research Paper

Bioregulator and foliar calcium supplementation in soya (*Glycine max* L.)

Flávia Martins da Silva[1], Hamilton Cesar de Oliveira Charlo[2], José Luiz Rodrigues Torres[2], Victor Peçanha de Miranda Coelho[3], Adriane de Andrade Silva[4] and Ernane Miranda Lemes[5*]

[1]Crop Production, Instituto Federal de Educação Ciência e Tecnologia do Triângulo Mineiro (IFTM), Campus Uberaba, Brazil.
[2]Agronomy/Plant Production, IFTM, Campus Uberaba, Brazil.
[3]Botany, IFTM, Campus Uberaba, Brazil.
[4]Zootechny of the Universidade Federal de Uberlândia (UFU), Campus Monte Carmelo, Brazil.
[5]Instituto de CIências Agrárias, Universidade Federal de Uberlândia (UFU), Campus Glória, Uberlândia, Brazil.

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Flower and pod abortions in large quantities are commonly observed in soybean plants. In normal conditions of crop cultivation, flower abortion is commonly above 60%. Among the strategies that may promote great flower and pod set in soybean, there is the use of plant growth regulators and foliar applications of calcium. Thus, this study aimed to evaluate the effects of different doses of plant growth regulator and the calcium sprayed on the agronomic performance of soybean plants. Five doses of systemic bioregulator (cytokinin) and five doses of calcium carbonate ($CaCO_3$) were applied in a 5 x 5 factorial arrangement with four replications field implemented in randomized block design. Soybean plant biometrics, the calcium content in different plant parts and crop yield were recorded. None of the treatments had a significant effect on the characteristics observed. The result was probably due to adequate conditions that prevailed during the soybean cropping cycle. In fact, flower and pod abortions happen when plants experience water stress during the flowering period. This lack of any treatment effect indicated the importance of regular and trustful monitoring of crop important variables to prevent routine applications of growth regulator and calcium.

Key words: *Glycine max*, cytokinin, calcium carbonate, plant biometrics, crop yield.

INTRODUCTION

Currently, Brazil is the world's second-largest producer of soybeans, with an estimate of 215 million tones for the 2018/2019 harvest (CONAB, 2019), and there is perspective to overtake the USA soybean production in the next years becoming the largest soybean producer in the world. The grain yield for this crop is defined by components such as the number of pods per plant, or per area, the number of grains per pod and the weight of

*Corresponding author. E-mail: ernanefito@gmail.com.

grains and factors that are directly dependent on the number of flowers per plant (Caverzan et al., 2018). However, the abortion of large quantities of flowers and pods are commonly observed in soya.

Flowers set in *Glycine max* is controlled by relations among hormonal, anatomical and nutrition factors, as well as by the availability of photoassimilates (Liu et al., 2004). In normal conditions of cultivation, the flower abortion reaches up to 60-65% (He et al., 2019), and these losses are strongly induced by nutritional imbalances, water deficiency, extremes of temperature and light intensity. Fageria et al. (2006) also indicated that the period of up to three days after anthesis, when the initial processes of cell division are occurring, as the most sensitive period for soybean flower fixation. In this period, abiotic stresses, especially the water deficit, can significantly increase the number of flowers and pods aborted.

Plant nutrition is important to reduce the effects flower and pod abortion, especially the availability of nutrients such as calcium (Ca), which is very important to the process of cell wall synthesis, pollen germination and growth of the pollen tube (Fioreze et al., 2018). Its deficiency causes abortion of flowers and pods, directly influencing on yield components and on the soybean productivity (Faquim, 2005; Fageria et al., 2014). Also, Ca has low mobility in plant and hydric stress periods during flowering decreases the Ca availability to reproductive tissues increasing flower and pod abortion.

Another important factor is the supply of plant hormones, such as cytokinin (Basuchaudhuri, 2016). Cytokinin is a plant hormone that is directly involved in the photosynthetic metabolism and the processes of cell division and differentiation, being determinant for the proper development of the reproductive structures (Fiorenze, 2013). Bioregulators are products that present plant hormones and/or mineral nutrients have been used to inhibit, promote or modify morphophysiological processes in plants with the aim of increasing agricultural production.

Although there are studies on factors that can promote a greater fixation of the reproductive structures in plants, the information is scarce to support decision-making by use, concentration, specific environmental conditions and time of application of bioregulators in the soybean crop. Therefore, the objective of this work was to evaluate the effects of different doses of bioregulator including cytokinin and doses of foliar calcium carbonate on the agronomic performance of soybean crop.

MATERIALS AND METHODS

The present study was conducted in the experimental area of the Federal Institute of Education, Science and Technology of Triângulo Mineiro (Instituto Federal de Educação, Ciência e Tecnologia do Triângulo Mineiro - IFTM), Campus Uberaba, latitude 19° 39' 19" S and longitude 47° 57' 27" W, in the municipality of Uberaba, Minas Gerais state, Brazil. The soil of the area is characterized as dystrophic Red Latosol, with a medium texture. The climate of the region according to the classification of Köppen type is Aw (tropical, hot and humid summer, with cold dry winter) (Beck et al., 2018). The monthly rainfall recorded in the experimental area during the conduction of the experiment varied from 0 to more than 60 mm (Figure 1). In this period, total precipitation was about 1023.1 mm and average temperature about 23.4°C. The chemical analysis of the soil in the experimental area is presented in Table 1. At planting, 230 kg ha^{-1} of 08-28-16 fertilizer was applied in the planting furrow. The side-dress fertilization was performed ten days after sown by applying 400 ml ha^{-1} Supa Bor (10% B w/v) and 17 kg ha^{-1} of manganese sulfate. At 15 days after sown, 150 kg ha^{-1} of ammonium sulfate was applied (CFSEMG, 1999).

Seeds treatment consisted of 100 ml of Dermacor® (chlorantraniliprole - 625 g L^{-1}) for each 100 kg of seeds, plus graphite to facilitate the sowing. During the experiment applications of fungicide, insecticide and herbicide were performed. The products used were two applications of Elatus® (azoxystrobin - 300 g kg^{-1} + benzovindiflupir 150 g kg^{-1}) at the dose of 300 g ha^{-1}, 45 days after sown and 20 days after the first application. In the same fungicide spray, insecticide Connect® (imidacloprid 100 g L^{-1}) at the dose of 2 L ha^{-1}. The herbicide application was performed soon after sowing with Crucial® (isopropylamine salt of glyphosate 400.8 g L^{-1} + potassium salt of glyphosate 297.75 g L^{-1}) at a dose of 3 L ha^{-1}, plus Podium® (fenoxaprope-P-ethyl alcohol 110 g L^{-1}) at a dose of 0.8 L ha^{-1}.

A 5x5 factorial arrangement was adopted for the five doses of systemic bioregulator (0, 0.5, 1, 1.5, 2 L ha^{-1}) commercialized as Veritas®, and five doses of calcium carbonate ($CaCO_3$) (0, 1, 2, 3, 4 L ha^{-1}) commercial name Cal Super® (41% w/v), with four replications. Combinations were allocated to plots in a randomized block design. Veritas® is a systemic bioregulator of the diphenyl-urea chemical group (Bayer, 2018) that includes Zn (12 g L^{-1}), N (48 g L^{-1}), and Ca (60 g L^{-1}) readily available for plant absorption.

Each experimental unit was composed of 16 planting rows spaced by 0.5 m with 4 m long, totaling 32 m² in each portion of a total of 40 plots. The useful area consisted of the 14 central lines, being harvested the central 2 m of each line (280,000 plants per hectare). The sowing was mechanically performed with a sowing machine and tractor in the conventional system (tillage) along with the fertilizer. The cultivar sown was Monsoy 6410, which has indeterminate growth and medium cycle ranging between 108 to 135 days depending on the region. The application of Cal Super® and Veritas® was performed when the plants reached R1 phenological stage (beginning of flowering, one flower opened in the main stem), and were applied with the aid of a backpack sprayer, using a spray volume equivalent to 400 L ha^{-1}, according to manufacturer recommendations.

When plants reached R6 phenological stage (full seed, green seed fully filling the pod cavity in one of the four uppermost nodes), the height of first pod insertion (measured with a ruler with millimetric scale), number of grains per pod and number of pods per plant in 10 plants of each plot were recorded. The dry mass of plants (shoot), leaves, stems and pods were collected from the same 10 plants. Leaf samples were washed, dried in a forced air circulation oven at 65°C until constant weight, then ground for nitrogen, phosphorus, potassium, calcium, magnesium and sulfur contents analyses according to the methodology described by Bataglia et al. (1983).

Crop harvest was performed when plants reached R8 phenological stage that is when 95% of the pods are mature (brown color). Each plot was threshed using mechanical thresher, for subsequent grain weight determination and correction to 13% moisture. The average weight of 1000 grains and grain moisture were determined from three samples of 1000 grains taken from the total grain produced in the useful plot.

Grain ash was determined by calcination of the sample in the

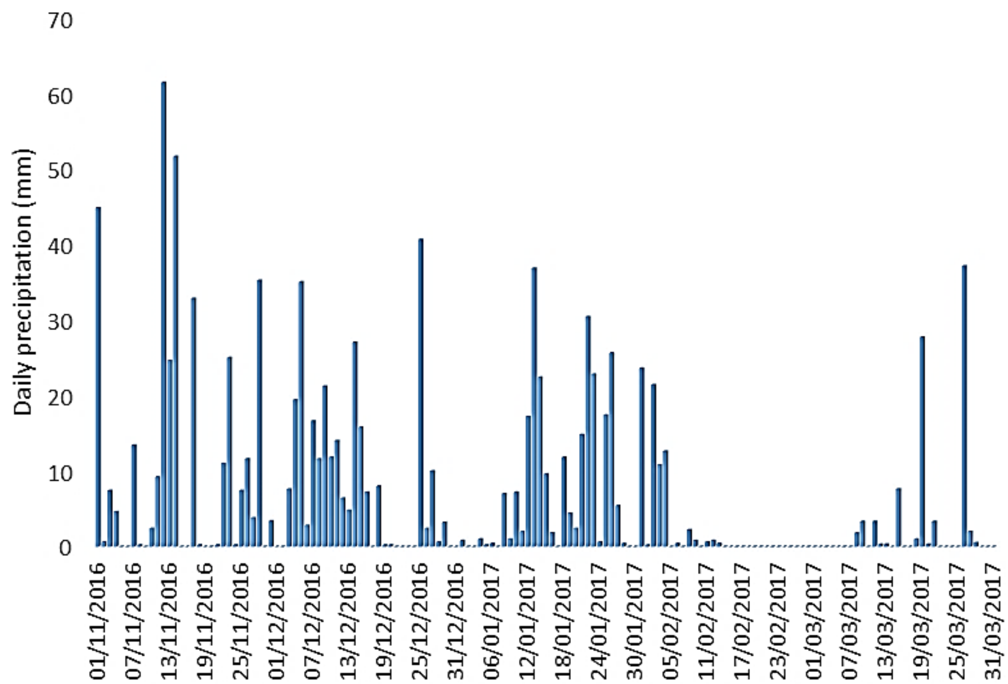

Figure 1. Dayly rain precipitation observed during field experiment.
Source: Meteorological station in the IFTM Campus Uberaba.

Table 1. Soil chemical characterization at 0 to 0.2 m soil depth.

pH (H_2O)	Ca	Mg	Al	P	K	S	H+Al	CEC	V	O.M.
---1:2.5---	-----$cmol_c$ dm^{-3}-----			------mg dm^{-3}------			-$cmol_c$ dm^{-3}-		%	g kg^{-1}
6.3	1.9	0.5	0	40.1	50.8	5	1.7	4.23	60	10

pH in H_2O, Ca, Mg, Al = KCl solution (1 mol L^{-1}); P, K = 0.05 mol L^{-1} HCl + H_2SO_4 0.0125 mol L^{-1}; S = 0.01 mol L^{-1} CaH_2PO_4; P available = extractor Mehlich-1; H + Al = SMP buffer solution (pH 7.5); CEC = cation exchange capacity at pH 7; V = saturation of bases; O.M. = soil organic matter (colorimetric method). Methodology source: Embrapa (2017).

oven at 550°C, until obtaining clear ash. Crude protein was obtained by the Kjeldahl method through the determination of the nitrogen in soybean following the methodology described by the Association of Official Analytical Chemistry (AOAC, 1990). Lipids content was determined using the Soxhlet gravimetric method - based on the quantity of material solubilized by ether - was used. Fibers were quantified by sample digestion (AOAC, 1990). Carbohydrate content was determined by the method proposed by Vannucchi (1990), using the formula: total carbohydrates = 100 - (% moisture + % ash + % protein + % lipids + % fiber).

The data collected underwent an analysis of variance for each of the parameters considered in the study, and multiple regression analysis was performed when significant differences were detected among treatments for a given variable.

RESULTS AND DISCUSSION

The summary of the analysis of variance for the agronomic characteristics presented no significant interaction between the application of Ca and bioregulator for any of the variables considered, as well as no effects of each Ca or bioregulator (Tables 2 and 3).

Klahold (2005) evaluated the effect of bioregulator (Stimulate®) applied in seed treatment, in foliar spraying and in combination of both and found grater averages of dry mass for leaf (11.68 g), stem (16.26 g), above-ground plant (shoot) (37.32 g) and whole plant (shoot + roots = 48.36 g). The lower averages of the variables reported in the present study - compared to Klahold (2005) study - can be related to cultivar differences between the studies and to the scarcity of rainfall during the initial phase of the experiment of the present study, despite the good rainfall distribution during the soybean flowering period.

Soybean reproductive stages, such as the beginning of the flowering stage (R1), the beginning of the filling of pods (R5), full-filling of pods (R6) and the germination phase are greatly affected by water deficiency (Pereira-

Table 2. Analysis of variance for agronomic characteristics recorded in function of doses of plant growth regulator and of calcium foliar-applied in soybean crop at R1 phenological stage (beginning of flowering).

Biorregulator	Dry mass (g)				First pod Insertion
($L\ ha^{-1}$) [B]	Plant	Leaf	Stem	Pod	(cm)
0	18.17	3.28	3.28	11.70	11.79
0.5	19.25	3.23	3.91	12.05	11.33
1.0	19.79	3.45	3.80	12.55	11.41
1.5	19.72	3.39	3.91	12.45	11.63
2.0	19.80	3.44	4.13	12.35	12.16
F value	1.069^{ns}	0.189^{ns}	1.635^{ns}	0.543^{ns}	1.520^{ns}
Calcium ($L\ ha^{-1}$) [C]					
0	18.53	3.18	3.70	11.65	11.54
1.0	19.33	3.11	3.89	12.35	11.87
2.0	19.10	3.38	3.95	11.80	11.98
3.0	19.87	3.63	3.63	12.75	11.51
4.0	19.90	3.48	3.86	12.55	11.42
F value	0.723^{ns}	0.882^{ns}	0.3^{ns}	1.032^{ns}	0.834^{ns}
Interaction B x C	1.52^{ns}	1.430^{ns}	0.78^{ns}	1.213^{ns}	0.779^{ns}
Mean	19.34	3.36	3.80	12.22	11.67
C.V. (%)	15.55	30.31	29.36	17.17	10.32

^{ns} = non-significant at 5% of probability level.

Table 3. Summary of the analysis of variance of agronomic characteristics evaluated in function of doses of plant growth regulator and calcium foliar-applied in the soybean plants.

Biorregulator ($L\ ha^{-1}$) [B]	Pods per plant	Grains per pod	Grain humidity (%)	100 mass (g)	Productivity ($Mg\ ha^{-1}$)
0	42.8	2.21	6.90	12.57	6.00
0.5	39.8	2.18	6.20	12.43	5.97
1.0	41.3	2.21	6.50	12.48	5.85
1.5	44.7	2.23	6.65	12.16	6.19
2.0	43.9	2.19	7.00	12.46	6.06
F teste	1.865^{ns}	0.246^{ns}	1.019^{ns}	1.347^{ns}	0.109^{ns}
Calcium ($L\ ha^{-1}$) [C]					
0	42.4	2.30	6.50	12.36	5.39
1.0	42.3	2.20	6.40	12.33	5.67
2.0	43.6	2.20	7.05	12.28	6.34
3.0	42.0	2.16	6.30	12.45	6.01
4.0	42.2	2.17	6.95	12.68	6.68
F teste	0.205^{ns}	1.731^{ns}	1.081^{ns}	1.434^{ns}	1.888^{ns}
Interaction B x C	0.829^{ns}	0.854^{ns}	0.780^{ns}	0.804^{ns}	1.233^{ns}
Average	42.49	2.20	6.65	12.4	6.02
C.V. (%)	15.22	8.41	21.33	4.83	27.95

^{ns} = non-significant at 5% of probability by the F test.

Flores and Justino, 2019). When hydric stress occurs at flowering triggers the abortion of flowers (Queiroz, 2014). In the present study, the good rain distribution during the flowering stage may prevent the occurrence of difference among the treatments for these variables.

In a controlled environment, soybean plants submitted to water deficit stress and shading at the flowering stage were treated with Ca, alone or in combination with cytokinin, and presented superior values of relative water content in soybean leaves at the end of the first water

Table 4. Calcium content and accumulation in different soybean parts as a function of doses of plant growth regulator and calcium foliar-applied in soybean plants.

Biorregulator (Lha^{-1}) [B]				Calcium accumulated			
	Leaf	Pod	Stem	Leaf	Pod	Stem	Total
	.............g kg^{-1}.............			.............mg pl^{-1}.............			
0	14.36	6,10	4.50	47.22	72.04	15.02	135.01
0.5	14.30	6.00	4.46	46.86	67.51	17.08	130.22
1.0	14.22	6.00	4.14	49.53	68.74	15.42	133.69
1.5	14.10	5.80	4.10	47.87	68.28	16.06	132.22
2.0	14.69	5.51	4.18	50.98	67.15	17.42	135.56
Teste F	0.263^{ns}	0.930^{ns}	0.409^{ns}	0.214^{ns}	0.329^{ns}	0.421^{ns}	0.102^{ns}
Calcium (L ha^{-1}) [C]							
0	13.84	5.70	4.53	44.47	66.27	16.97	128.39
1.0	14.01	6.20	4.35	44.35	73.53	16.58	134.46
2.0	14.25	5.64	4.69	48.00	67.86	18.07	133.93
3.0	14.64	5.99	3.89	53.34	68.27	14.18	135.81
4.0	14.96	5.86	3.93	52.59	67.50	15.21	133.97
F teste	1.115^{ns}	0.808^{ns}	1.427^{ns}	1.326^{ns}	0.705^{ns}	0.916^{ns}	0.314^{ns}
Interaction B x C	1.102^{ns}	1.383^{ns}	0.651^{ns}	1.334^{ns}	1.009^{ns}	0.588^{ns}	1.204^{ns}
Average	14.33	5.89	4.28	48.51	68.71	16.20	133.35
C.V. (%)	13.42	19.03	31.01	34.2	21.68	43.89	16.76

^{ns} = non-significant at 5% of probability by the F test.

deficiency cycle (p>0.05) (Fiorenze, 2013). The authors observed that the effects of Ca and cytokinin on the maintenance of water content seem to occur in an isolated manner and not jointly since the effect of the combined application was not superior to the effect of the application alone. The authors also reported an average number of pods of about 37.7, with the application of the bioregulator in R2 soybean stage. In the present study, the average number of pods per plant was 42.5 (Table 3).

The summary of the analysis of variance for the contents and accumulation of Ca is presented in Table 4. The variables of content and accumulation of Ca were similar among treatments and no significant effect was observed for the interaction between the factors (Ca or bioregulator). The Ca accumulation in leaf, pod and stem were 48.51, 68.71 and 16.20 mg $plant^{-1}$, respectively, summing 133.35 mg $plant^{-1}$ of Ca. Thus, the largest part of the Ca absorbed by the plant is accumulated in pods (51.52%), followed by leaves, which accumulated 36.37% of the total Ca in the plant.

Other studies found no beneficial results from the Ca application to soybean (Ben et al., 1993; Fioreze et al., 2018). However, it is noteworthy that the quantities of Ca applied in the present study are very small when compared to the nutritional requirement of the soybean plant, being expected only a flag effect of the application of Ca, since the quantities applied would probably not be able to change the leaf Ca content.

The control of the abscission of the reproductive structures in the soybean plant has sulfate to the availability of photoassimilates and nutrients, especially Ca, and the concentration of endogenous plant hormones, such as the cytokinin (Liu et al., 2004). These characteristics are also highly influenced by the environment of cultivation where the abortion of the reproductive structures can be observed in stressful conditions, such as water deficit, high temperatures or low light intensity, conditions hardly manipulated.

The summary of the analysis of variance for nutritional variables evaluated is presented in Table 5. There was no significant difference for the interaction between Ca foliar-applied and the bioregulator for none of the variables assessed, as there were no effects of the factors evaluated in isolation. However, Vieira and Castro (2001), evaluate the composition of soybean cultivars and found greater averages than those found in the present study for lipids (23%), proteins (40%) and carbohydrates (32%), and lower averages for ash (5.75%) and fiber (5.41%).

Considering the results obtained in the present study, it is possible to conclude that the factors not controlled in this field experiment, such as rainfall, temperature and luminosity must have created very adequate conditions for soybean cultivation. The reduced environmental stresses influenced the effectiveness of the bioregulator, as well as the application of Ca foliar. Therefore, further studies should be conducted, especially under controlled conditions, so the studied factors can be better understand under bioregulator and calcium influences. Also, the results found highlight the importance of careful

Table 5. Summary of the analysis of variance of nutritional compounds in soybean seeds.

Bioregulator (L ha^{-1}) [B]	Protein (%)	Ash (%)	Lipid (%)	Fiber (%)	Carbohydrate (%)
0	32.97	6.9	17.78	15.34	21.82
0.5	35.19	6.25	17.45	13.78	21.89
1.0	33.13	6.5	17.93	13.64	23.33
1.5	34.62	6.7	17.01	15.78	20.39
2.0	35.86	6.9	16.94	13.53	21.86
F teste	0.103^{ns}	0.604^{ns}	0.868^{ns}	0.697^{ns}	0.874^{ns}
Calcium (L ha^{-1}) [C]					
0	34.38	6.55	18.56	14.93	20.31
1.0	34.12	6.5	18.49	12.94	22.38
2.0	34.87	7.05	17.15	13	22.59
3.0	33.86	6.35	16	14.61	24.21
4.0	3.53	6.8	16.92	16.6	19.8
F teste	0.944^{ns}	0.608^{ns}	0.116^{ns}	0.345^{ns}	0.463^{ns}
Interaction B x C	0.268^{ns}	0.919^{ns}	0.487^{ns}	0.922^{ns}	0.572^{ns}
Average	34.35	6.65	17.42	14.41	21.86
C.V. (%)	11.68	22.5	20.28	44.2	38.57

^{ns} = non-significant at 5% of probability by the F test.

crop monitoring for appropriated decisions related to the application, or not, of inputs during the soybean crop cycle.

Conclusion

Under good conditions for soybean productions, no differences were observed among treatments including the application, or not, of bioregulator and/or Ca foliar. The lack of treatment effect indicated the importance of regular and trustful crop monitoring to prevent routine applications of growth regulator and calcium.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Influence of soil conservation practices on soil moisture and maize crop (*Zea mays* L.) productivity in Centre Benin

Akplo Tobi Moriaque[1*], Kouelo Alladassi Félix[1], Ahoglé Agassin Arcadius Martinien[1], Houngnandan Pascal[1], Azontondé Hessou Anastase[2], Benmansour Moncef[3,] Rabesiranana Naivo[4], Fulajtar Emil[5], Hounkonnou G. Joseph Jaurès [1], and Klotoé Dagbégnon Marc-André[1]

[1]Laboratory of Soil Microbiology and Microbial Ecology, University of Abomey-Calavi- National University of Agriculture Benin; Benin.
[2]Laboratory for Soil Science, Water and Environment, Agricultural Research Centre of Agonkanmey, National Institute for Agricultural Research of Benin; Benin.
[3]Soil-Water-Climat Division, National Centre of Energy, Nuclear Sciences and Technologies (CNESTEN), Rabat, Morocco.
[4]National Institute of Nuclear Sciences and Technologies (INSTN), Antananarivo, Madagascar.
[5]SWMCNS, Joint FAO/IAEA, Division of Nuclear Techniques in Food and Agriculture, Vienna-Seibersdorf, Austria.

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Soil quality constitutes a major factor for crops growth. This study aimed at determining the sustainable soil conservation practices that would increase maize growth as well as production and improve the soil moisture. Two experimental sites were chosen: Dan localized on Acrisol and Za-zounmè localized on ferralsol. Two cropping seasons were investigated: the long rain seasons of 2018 and 2019. The experimental design was split-plot with four replications. The main factor was tillage with three modalities: no-tillage (NT); ridging parallel to the slope (PR); Isohypse ridging (IR) and the second factor was mulching with four amounts: 0, 3, 5 and 7 t.ha^{-1}. Tillage, mulch amount and their interaction significantly influenced the soil moisture, maize growth and yield over the two investigated cropping seasons at both sites whereby the highest values were obtained under IR for tillage, 7 t.ha^{-1} (for mulch amount) and IR7M (for the interaction tillage x mulch amount). Overall, IR significantly increased the maize growth speed by 8% at Dan and by 16% at Za-zounmè; the maize grain yield by 33% at Dan and by 30% at Za-zounmè and the soil water content by 24% at Dan and 20% at Za-zounmè, in comparison with No-Tillage. An increasing effect of mulch amount was also observed. As far as mulching is concerned, the highest values (in average of LR2018 and LR2019) of growth speed (3.77 cm.day^{-1} at Dan and 4.08 cm.day^{-1} at Za-zounmè); grain yield (3003.03 at Dan and 3471.09 kg.ha^{-1} at Za-zounmè) and soil water content (26.89 mm at Dan and 20.44 mm at Za-zounmè) were observed. This suggests that isohypse ridging associated with an appropriate amount of organic mulch could be an option to mitigate dry spells and drought and improve local farmers' income in the area of low rainfall in sub-Sahara Africa.

Key words: Crop residues, tillage practices, conservation agriculture, food security, watershed of Zou.

INTRODUCTION

Food security, soil and water conservation and climate change mitigation are the most important challenges that are facing developing countries including countries of sub-Saharan Africa (SSA) (Clover, 2003; Kiboi et al., 2019). Current cropping systems need therefore to be transformed for meeting these challenges. In Benin, agriculture is rainfed-based and small-scale farmers provide more than 80% of food production. In most of agro-ecological zones in Benin, continuous land use, burning of crop residues, and deep tillage have led to soil degradation (Saïdou et al., 2012). In addition, the erratic distribution of rainfall seriously compromises agricultural production. It is therefore necessary to adopt alternative practices to ensure food security for a growing population. For several decades, organic farming has been identified as an alternative form of farming to ensure food security and reduce the impact of agriculture on the environment (Badgley et al., 2007). Among all developed practices for tillage reducing and soil coverage improvement, retaining crop residue as soil mulch or soil cover is one of the highly beneficial practices of good soil management (Vincent-Caboud et al., 2019). Besides poor soil nutrient status, water is also a limiting factor of food production under rain fed conditions, and thus water and nutrients alternate within a particular season as key factors limiting crop production. According to its role on agricultural production in a tropical environment, the purpose of tillage is to ensure the well establishment of crops, improve water and air circulation of in the soil, promote warming, limit water runoff and weed infestations (Kurothe et al., 2014; Kiboi et al., 2019). Despite all the benefits mentioned above, many problems arise from continuous, yearly intensive tillage of agricultural soils (Idowu et al., 2019). Intensive disturbances of the soil may decrease soil quality (e.g., reducing organic matter, increasing soil erosion, etc.) (Idowu et al., 2017). Labreuche et al. (2007) consider that intensive tillage is generally considered to be an unfavorable factor for carbon storage and therefore unfavorable for soil organic matter. The main effect seems to be a lifting of the physical protection of organic matter by tillage. Under certain soil, climate and management conditions, No-tillage (NT) may have potential advantages over tillage. Reduction of runoff and erosion, increase in soil organic carbon (SOC), increase of root length density and soil water conservation are some of the main outcomes of NT practices (Lal, 2004; Martínez et al., 2008; Kolb et al., 2012; Soane et al., 2012; Fiorini et al., 2018). NT practices are considered as Conservation Agriculture practices (FAO, 2011) and reaching up to 70% of the total cultivated area in South America. However, various soil types react differently to the same tillage method with respect to some selected soil properties, and the effects of tillage method on crop yield vary with the crop species (Sharma and Abrol, 2005). In Africa and Europe, NT practices are not widespread and a decrease in crop yield and an increase in runoff and soil loss during its establishment has been reported (Akplo et al., 2019a; Basch et al., 2008; Pittelkow et al., 2015). However, there is lack of a clear understanding of their effects on soil conditions and crop yield for different soil, crop and climate condition (Singh and Malhi, 2006). In addition, in Benin as well as in several countries in Sub-Saharan Africa, tillage is made using manual hoe. Tillage systems that can enhance crop productivity under smallholder farming systems are therefore desirable. Contour ridges are regarded as water harvesting methods in semi-arid regions. It transforms the land into small pockets called tied ridges or soil bund called furrows and is very useful to stabilize yield (SUSTAINET, 2010; Uwizeyimana et al., 2018).The advantages of mulch are widely recognized (Araya et al., 2015; Toom et al., 2019). Crop residues as mulch at the soil surface acts as shade, protects the soil surface against raindrops and limit surface runoff (Bashagaluke et al., 2018), increased carbon sequestration (Balesdent et al., 2000), maintains soil moisture and maintains high soil biological activity (Douzet et al., 2010; Mazarei and Ahangar, 2013). Keeping this in view, this study aims at determining the effect of different tillage systems in combination with different crop residues amounts used as mulch on rain-fed maize growth and yield component and soil moisture.

METHODOLOGY

Site description

Field trials were carried out on an Acrisol at Dan (7°21'35" N; 002°05'09" E) and on a Ferralsol at Za-zounmè (7°12'50" N; 002°15'40" E) on the watershed of Zou (Figure 1). At Dan, the soil had Sandy-clay-loamy in texture and characterized by moderate organic matter content (1.37%); exchangeable Potassium (0.33 meq/100g of soil), available Phosphorus (12.6 ppm), pH (5.63) and high total nitrogen content (0.088%). The C: N ratio of 9.03 indicated a good decomposition of organic matter by microorganisms in the soil. Water infiltration was very slow (41 cm.day^{-1}) and the slope is 5%. At Za-zounmè, the soil is Sandy-loamy with moderate contents of organic matter (1.24%); exchangeable Potassium (0.36 meq/100 g of soil), high available Phosphorus (18.12 ppm), and total nitrogen content (0.069%) while the pH of the soil (6.40) was neutral. An average C: N ratio of 10.42 indicates good decomposition of organic matter. Water infiltration was very slow (120 cm.day^{-1}) and the slope is 4.6%. The rainfall pattern is bimodal in the two sites: Long Rain season (LR) from April to July and Short Rain season from September to November. For both sites, long time annual average rainfall ranged from 1100 to 1300 mm (Figure 1).

*Corresponding author. E-mail:moriaqueakplo@gmail.com.

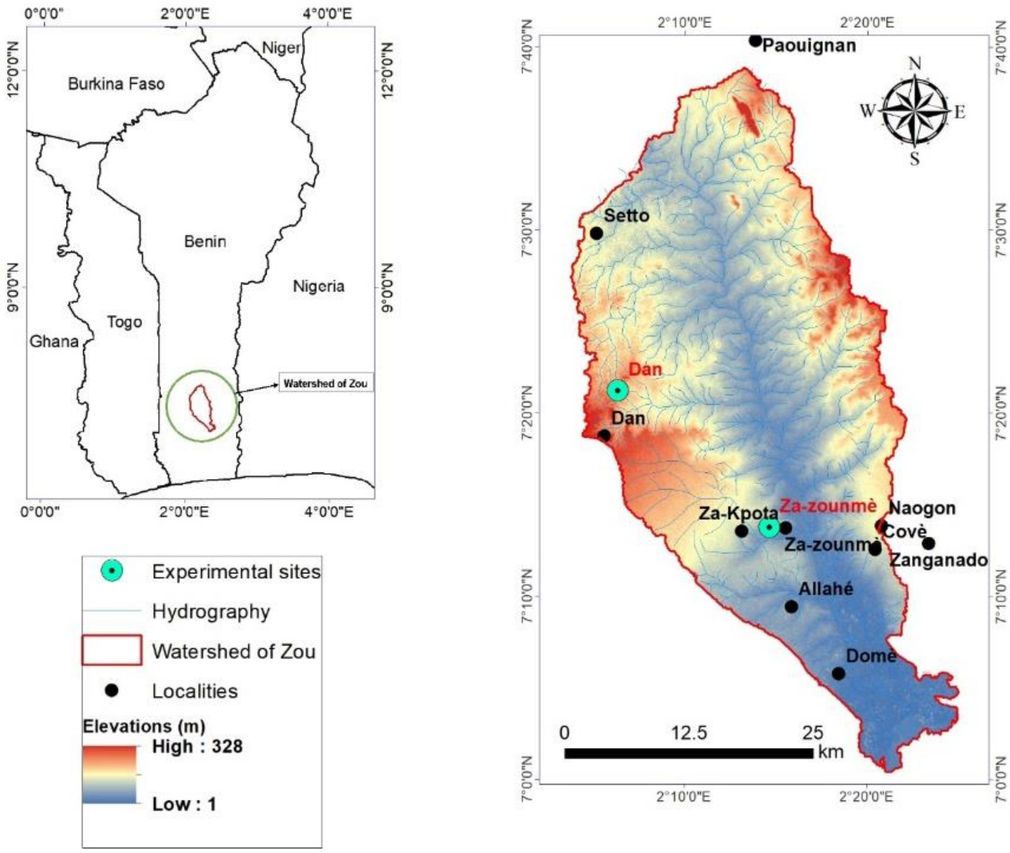

Figure 1. Map of the study area.

Table 1. Treatment factorial combinations.

Tillage practices	Mulch amounts (t. ha^{-1})	Abbreviations
No tillage	0	NT0M
No tillage	3	NT3M
No tillage	5	NT5M
No tillage	7	NT7M
Ridging parallel to the slope	0	PR0M
Ridging parallel to the slope	3	PR3M
Ridging parallel to the slope	5	PR5M
Ridging parallel to the slope	7	PR7M
Isohypse ridging	0	IR0M
Isohypse ridging	3	IR3M
Isohypse ridging	5	IR5M
Isohypse ridging	7	IR7M

Experimental layout, treatments and management

The experiment was conducted for two long rains seasons, 2018 (LR2018) and 2019 (LR2019) at Dan and Za-zounmè. The both experimental sites were under fallow since 2000. The experiment was laid out in split-plot design. Three tillage practices (NT (no-tillage); PR (ridging parallel to the slope); IR (Isohypse ridging) were considered as main treatment while Four mulch amounts (0; 3; 5 and 7 t.ha^{-1}) were assigned in sub-plots. The treatments (Table 1) were replicated four times at each site. The plot sizes were 17 m x 6 m and 6 m by 3.5 m at Dan and Za-zounmè. The Ridging was done using a hand hoe to 30 cm depth. The Ridging parallel to the slope is a type of tillage whose seedling lines are oriented parallel to the slope direction. Isohypse ridging designates a type of ridging perpendicular to the slope. The no-tillage as made in this study consists in directly sowing without any actual soil work. The mulching was made at sowing with maize crop residue. The seedling poops were made with a machete or hoe on a depth proportional to the size of the seed. Being the dominant annual crop in the watershed of Zou, maize (*Zea mays* L.) variety DMR-AK94 was the test crop. The planting density was 62500 plants. ha^{-1}. Weeds management was done twice using a hand hoe on no-mulched plots while under mulched plots, it was by hand pulling. The harvest of maize was done 105 days after sowing.

Data collection

Rainfall measurement and soil moisture assessment

The rainfall amount was recorded daily over the two investigated cropping seasons. The device used was installed on each of the experimental site by the Direction of Agrometeorology of Benin. In each investigated season, gravimetric soil moisture was determined in the 20 top centimetres of soil two weekly. The soil samples were oven dried at 105°C for 48 h before determining gravimetric water content. For bulk density determination, soil samples were collected by stainless steel cores of 100 cm^3 in volume. Soil samples for bulk density determination were collected before planting from outside the plots but within the same field. Gravimetric water content for each soil layer were calculated using the procedure outlined by Anderson and Ingram (1993). Soil water content in percentage was converted in millimetres by multiplying volumetric water content by 20 cm and by the bulk density.

Growth parameters

Chlorophyll concentration of maize leaves and plant height were collected as maize growth index during the experiment. Height of adjacent plants on the middle row per plot were sampled (non-destructive sampling). Relative Chlorophyll content of maize leaves was taken at 30 Days After Sowing (6^{th} leaf stage) and 60 Days After Sowing (10^{th} leaf stage) using a Soil Plant Analysis Development SPAD-502Plus®meter. The measurements were made at middle of four leaves per plant and the mean values were recorded. Plant height was measured on the 15^{th}, 30^{th}, 45^{th} and 60^{th} Days after sowing using a ruler as the distance from the base of the plant to the uppermost extended leaf tip. Maize growth speed was estimated fitting linearly maize plant height in function of the time (15^{th}, 30^{th}, 45^{th} and 60^{th}). Growth speed rate was represented by the coefficient (“a”) of the regression equation.

Yield components

Maize was harvested at maturity from a net area of 15 m² in each plot at both sites. At the harvest, the cobs in each plot were separated from the stover and fresh weight of both grain and stover was determined. Also, the fresh weight of the straw was recorded. The cobs and straw were oven dried at 65°C until constant weight and weighed. The cobs were then hand shelled and the grains weighed. Grain yield (kg DM ha^{-1}) was estimated as follow (Saïdou et al., 2012):

$$\text{Grain yield} = \frac{10000 \times P \times DM \times n}{NA}$$

Where: DM denotes the Dry matter factor = dry weight of sample / wet weight of sample; n denote the Shelling factor = dry weight of grain / dry weight of cob; P denotes total wet weight and NA is the harvested net area.

The Straw yield (kg DM ha^{-1}) of maize were determined as follow:

$$X = \frac{10000 \times P \times DM}{NA}$$

Where: X denotes Straw; Husk and raffle yield.

The Harvest Index was calculated as follow:

$$HI = \frac{\text{Grain Yield}}{\text{Grain Yield} + \text{Straw Yield}}$$

Data analyses

Data were analyzed by year and by site due to variable weather of the LR2018 and LR2019 and growing conditions at Dan and Za-zounmè. The data collected from the experimental trials were submitted to a three-way analysis of variance (ANOVA) using PROC MIXED procedure. Tillage and mulching were assigned as fixed factor while block was considered as random factor. When F-test is significant for any fixed effects, a subsequent mean separation was performed using Post hoc Tukey’s Honestly Significant Difference at 5% significance level. Statistical analyses were carried out using SAS (Statistical Analysis System) software, (version 9.2).

RESULTS

Rainfall amount and distribution

The 2018 (LR2018) and 2019 (LR2019) long rain cropping seasons were characterized by different rainfall patterns at the both sites. At Dan, LR2019 was more rained than LR2018 (Figure 2a). Cumulatively, 443 and 355.88 mm were recorded in rainfall during respectively in LR2019 and LR2018 cropping seasons. Conversely, LR2018 was more rained than LR2019 at Za-zounmè where 620.6 and 541.1 mm were recorded over the LR2018 and LR2019 (Figure 2b). For the both sites, the most of the rain fell from 1 May to 30 June during the two year. This period closed with the vegetative stage of the maize variety grown at both sites. Total of 213.66 and 397.5 mm were recorded during the LR2018 whereas 250.3 and 388.6 mm were recorded during the LR2019 respectively at Dan and at Za-zounmè at the vegetative stage of the maize. During the LR2018, a meteorological drought (defined as the absence of rainfall for a period above twenty-eight days during the growing season) was observed from the 76^{th} to 103^{th} days after sowing at Za-zounmè while dry spells (absence of rainfall in periods ranging between 10–28 days during crop growing season) was observed at Dan from 89^{th} to 110^{th} days after sowing. Dry spells were observed at Dan from the 100^{th} and 110^{th} days after sowing in LR2019. At Za-zounmè, two dry spells were observed. They appeared during the vegetative stage (from 33^{th} to 44^{th} days after

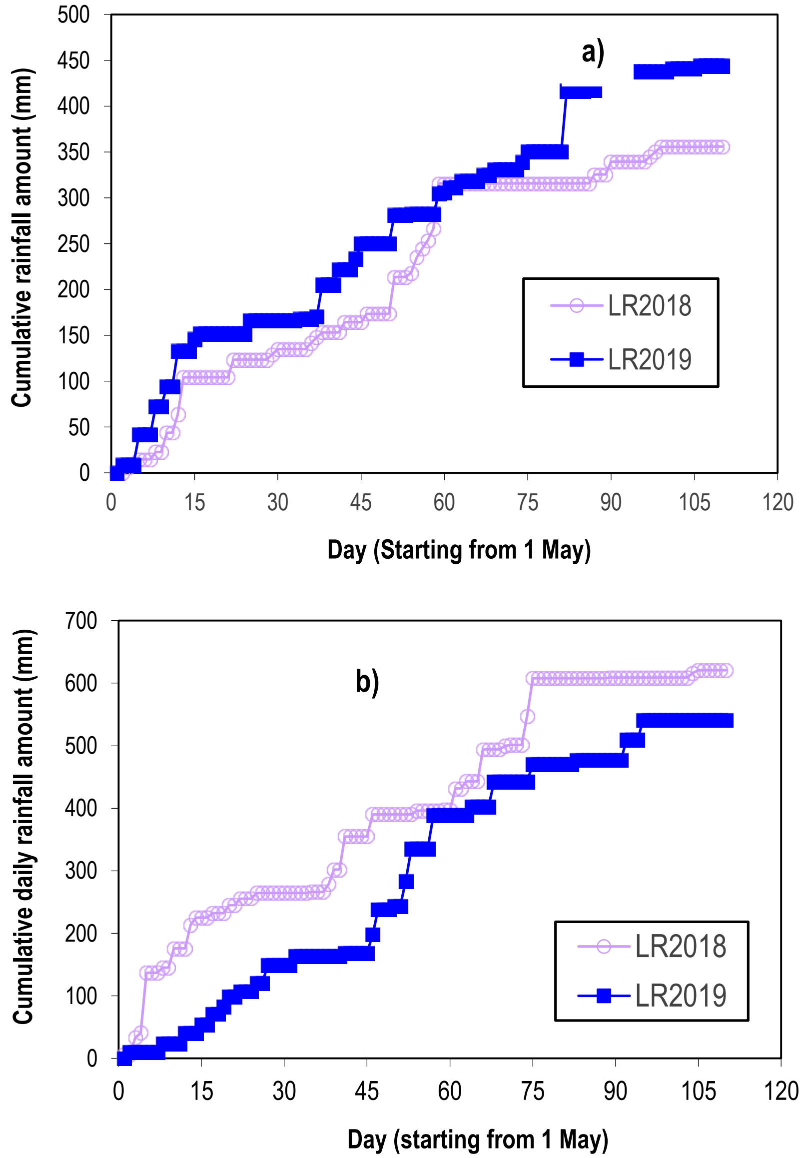

Figure 2. Rainfall at Za-zounmè between 1 May and 31 August during 2018 and 2019 Long Rain seasons at a) Dan and b) Za-zounmè.

sowing) and during the ripening stage (from 93th to 110th days after sowing).

Average soil water content at 0-20cm during the maize growing season

Average soil water content at 0-20 cm depth was significantly affected by tillage, mulching and the interaction (Tillage*mulching) in the both sites during the two investigated seasons (LR18 and LR19) (Table 2). IR yielded the highest soil water content at 0-20cm on the both sites. PR was not significantly different from NT in LR2018 at Dan and in LR2019 at Za-zounmè. The soil water content significantly increased ($p<0.05$) with an increase in mulch amount whereby the highest soil water content was obtained with 7 t ha^{-1} mulch. However, during LR2018, significant difference was not obtained between 3 and 5 t ha^{-1} mulch at Dan while the difference was not significant between 0, 3 and 5 t ha^{-1} mulch at Za-zounmè. During LR2019, no significant difference was not found between 0 and 3 t ha^{-1} mulch at Dan whereas 7; 5 and 3 t ha^{-1} significantly increased respectively by 39, 31 and 21% compared with 0 t ha^{-1}. As far as the interaction (Tillage*Mulching) is concerned, the highest water content was found with the treatment IR7M while the treatment NT0M led to the lowest water content at 0-20 cm (Figure 3a and b). Overall, for each tillage

Table 2. Simple effects of tillage and mulching on the average soil water content (mm) at Dan and Za-zounmè during LR2018 and LR2019 seasons.

Treatments	LR2018		LR2019	
	Dan	Za-zounmè	Dan	Za-zounmè
Tillage[1]				
NT	20.58[b]	17.71[c]	21.08[c]	15.36[b]
PR	23.79[b]	18.99[b]	24.16[b]	15.80[a]
IR	25.51[a]	20.87[a]	26.20[a]	18.78[b]
Mulching[2]				
0M	19.85[c]	18.69[b]	20.32[c]	12.44[d]
3M	22.41[b]	18.93[b]	22.87[c]	15.83[c]
5M	24.21[b]	18.65[b]	24.99[ab]	17.92[b]
7M	26.71[a]	20.48[a]	27.08[a]	20.39[a]

[1]Tillage: NT= No-tillage; PR= Ridging in the slope direction; IR= Isohypse Ridging
[2] Mulching: 0M= 0 t ha^{-1} of mulch; 3M= 3 t ha^{-1} of mulch; 5M= 5 t ha^{-1} of mulch; 7M= 7 t ha^{-1} of mulch

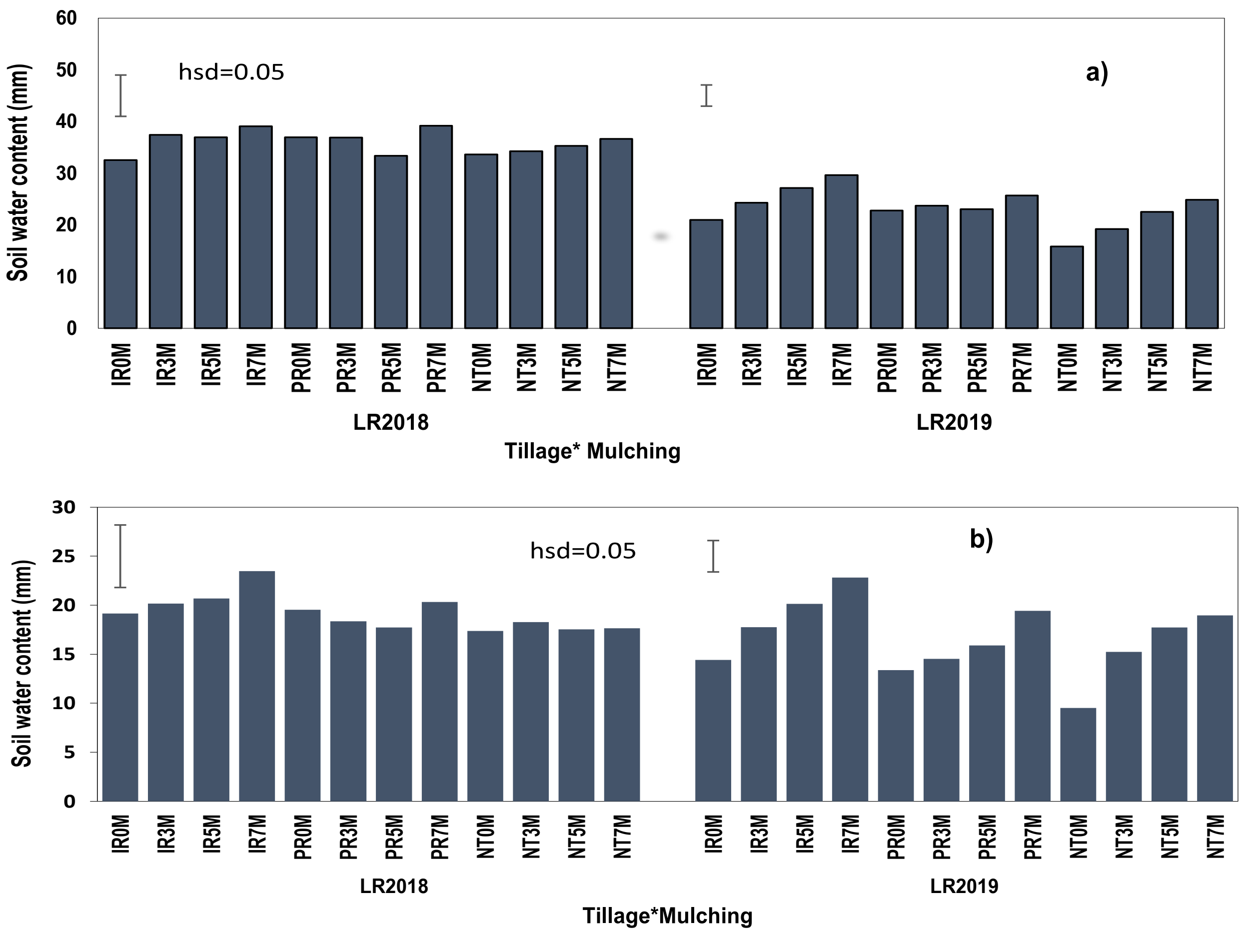

Figure 3. Interactive effect of tillage and mulching on soil water content during LR2018 and LR2019 seasons at a) Dan and b) Za-zounmè. Significant differences of the post hoc Tukey's HSD test performed in case of effects if the model were significant (p≤0.05). Error bars indicate the Honestly Significant Difference value.
IR0M= Isohypse ridging+0 t ha^{-1} of mulch; IR3M= Isohypse ridging+3 t ha^{-1} of mulch; IR5M= Isohypse ridging+5 t ha^{-1} of mulch; IR7M= Isohypse ridging+7 t ha^{-1} of mulch; PR0M= Ridging in the slope direction +0 t ha^{-1} of mulch; PR3M Ridging in the slope direction +3 t ha^{-1} of mulch; PR5M= Ridging in the slope direction+5 t ha^{-1} of mulch; PR7M= Ridging in the slope direction +7 t ha^{-1} of mulch; NT0M= No-tillage+0 t ha^{-1} of mulch; NT3M= No-tillage+3 t ha^{-1} of mulch; NT5M= No-tillage+5 t ha^{-1} of mulch; NT7M= No-tillage+7 t ha^{-1} of mulch.

practice, the water content increased with the mulch amount. At Dan, no significant difference was obtained between the treatments IR3M; PR0M; PR3M; PR5M; PR7M and NT5M in LR2018 whereby an average soil water content of 23 mm was obtained. During LR2019, the water content was lower than LR2018 and no significant difference was found between NT0M; NT3M; NT5M and NT7M. At Za-zounmè, the water content obtained with the treatments IR0M; PR0M (13.38 mm); PR3M (14.52 mm); PR5M (15.89 mm) and NT3M (15.23 mm) was statistically equal during LR2018. In opposite to Dan, the water content was high in LR2019 than LR2018 at Za-zounmè. During LR2019, IR3M (24.29 mm); PR3M (23.72 mm); PR5M (23.00 mm); NT5M (22.49 mm); NT7M (22.82 mm) were statistically equal (Figure 3).

Variation of the soil water content during the growing season of maize

There was some fluctuation in the soil water content during the maize growing season at the both studied sites. In general, the significant effect of tillage, mulch amounts and tillage x mulch amounts were associated with the low soil water content level. The effect of tillage was significant on the 15th day during the LR2018 and from 15th to the 90th days after sowing during the LR2019 at Dan (Figure 4a). At Za-zounmè, the tillage effect was significant from the 15th to 45th while it was from 75th to 105th days after sowing (DAS) in LR2018 season and from 15th to the 60th and on the 105th DAS in the LR2019 season (Figure 4b). The soil water content was slightly higher on the IR and PR compared with the No-Tillage on the vegetative stage (from 0th the 45th days after sowing) and on the grain formation and ripening phase of the maize (from the 45th to the 105th DAS). As far as mulching effect is concerned, it was found out that a significant effect on the 15th, 30th and 45th DAS in the LR2018 season and on the 15th, 30th, 45th, 75th and 90th DAS in the LR2019 season at Dan (Figure 5a). Comparatively higher soil water contents were obtained on the mulch applied plot than the 0 t ha^{-1} mulch. However, on the days with significant effect of the mulch amounts, the water content recorded on the plot with 5 and 7 t ha^{-1} were not significantly different. On the site of Za-zounmè, the mulch amounts significantly influenced the soil water content on the 15th, 30th, 45th, 75th and 90th days after sowing in the LR2018 and on the 15th, 15th, 30th, 45th, 60th, 75th 90th and 105th days after sowing in the LR2019 season (Figure 5b). In contrast with of the trend observed at Dan, the soil water content significantly increased with the mulch amounts and significant difference was observed between the water content recorded on the plot with 3, 5 and 7 t ha^{-1}. The interactive effect of tillage and mulching (Tillage x mulching) was significant of the soil water content on the 15th, 45th, 60th and the 90th days after sowing in the LR2018 and on the 15th, 30th, 45th, 75th, 90th and 105th days after in the LR2019 season at Dan (Figure 6a). However, at Za-zounmè, the effect was significant on the 30th, 45th, 75th, and 90th in the LR2018 season and on the 15th, 30th, 45th, 60th, 75th, 90th and 105th days after sowing in the LR2019 (Figure 6b). During the both studied growing seasons of maize, the highest soil water contents were recorded with IR7M and IR5M at Dan and with IR7M at Za-zounmè.

Influence of soil tillage mulching on maize growth

Growth speed

Tillage and mulching have significantly influenced the growth speed of maize during the both Long rain seasons (LR2018 and LR2019). The highest maize growth speed was observed with Isohypse Ridging (IR) during the two trial seasons in the both sites (Table 3). At Dan, the growth speed was significantly increased by 7% under IR and PR compared with the NT during both LR2018 and LR2019 seasons. At Za-zounmè, IR significantly increased growth speed by 8% during LR2018 season and by 29% during LR2019 season compared with NT. The influence of the mulching was significant on the growth speed of maize at the both sites during the two trial seasons. At Dan and Za-zounmè, it was observed an increasing effect of the mulch amount on the growth speed of the maize. The highest maize growth speed was obtained when 7 t.ha^{-1} mulch were applied and the lowest growth speed was obtained on the plot with 0 t.ha^{-1} mulch. The interactions' (Tillage x Mulching) had no significant effect on the maize growth speed in LR2019 season (Table 4). Conversely, its influence was significant on maize growth speed on the both sites in LR2018 season. During the both trial seasons, the highest growth speed was obtained with the IR7M treatment. In LR2018 season, it was found out that at Dan there is no-statistical difference between the treatments IR0M (2.72 cm.day^{-1}); NT0M (2.46 cm.day^{-1}) and PR0M (2.62 cm.day^{-1}) which led to the lowest growth speed. Also, the difference between the treatments IR3M (3.17 cm.day^{-1}); IR5M (3.31 cm.day^{-1}); NT3M (3.17 cm.day^{-1}); NT5M (3.24 cm.day^{-1}) and PR3M (3.06 cm.day^{-1}) was no-significant. However, treatment IR7M lead to the highest growth speed (4.05 cm.day^{-1}) at Dan. Similarly, at Za-zounmè, the lowest maize speed growth was observed with the treatment NT0M (3.43 cm.day^{-1}) and the highest maize speed growth was observed with the treatment IR7M (4.85 cm.day^{-1}). The treatments IR0M; IR3M; NT3M; NT5M and PR3M were found to be statistically equal and led to 3.60 cm.day^{-1} in average.

Relative chlorophyll content

Tillage and mulching have significantly influenced maize

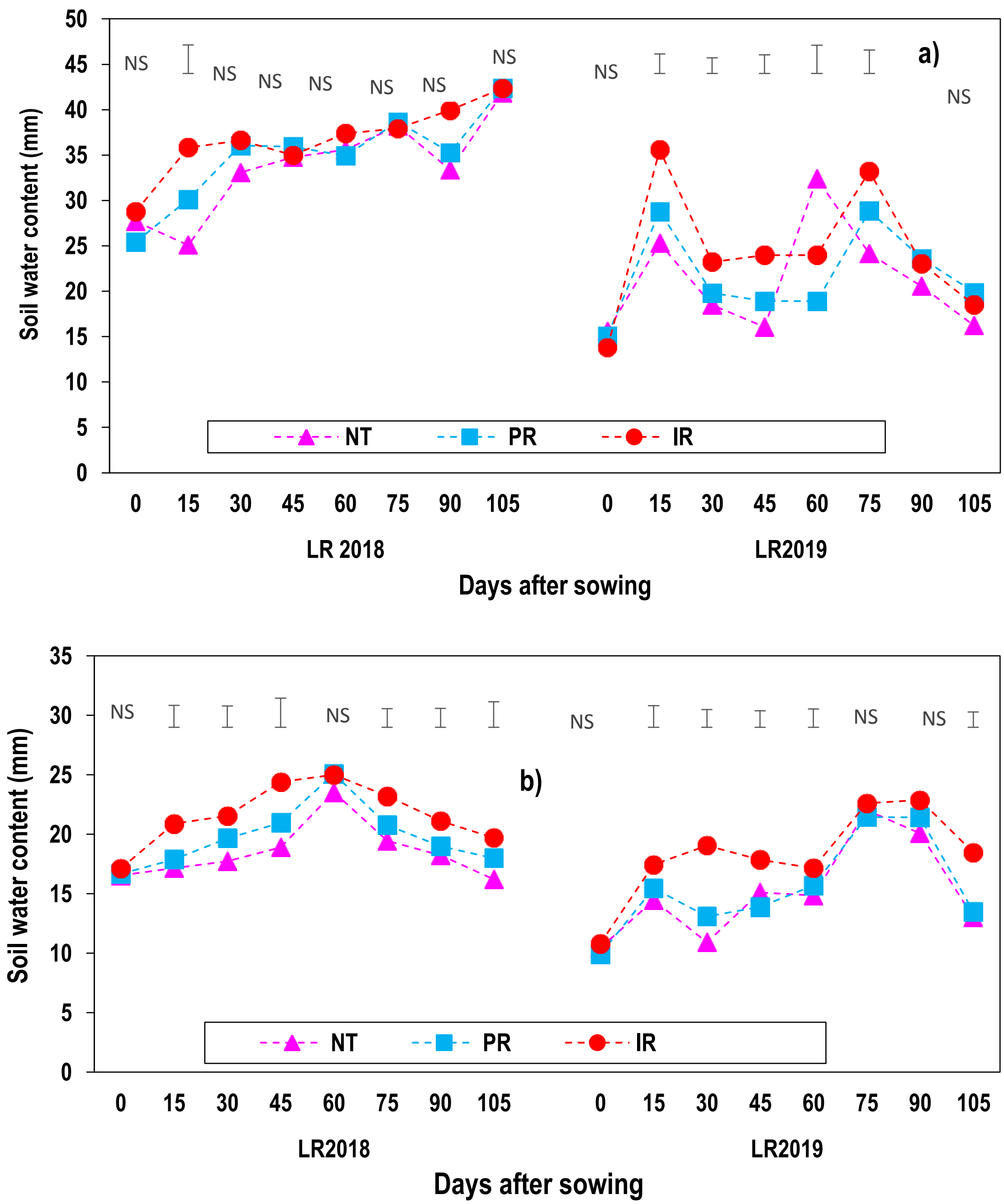

Figure 4. Effect of tillage on soil water content distribution during LR2018 and LR2019 seasons at a) Dan and b) Za-zounmè. NT= No-tillage; PR= Ridging in the slope direction; IR= Isohypse Ridging. Significant differences of the post hoc Tukey's HSD test performed in case of effect if the model was significant ($p < 0.05$). Error bars indicate the Minimum Significant Difference value. NS= not significant.

leaves chlorophyll content (Chl) on the 30th Days After Sowing (6th leaf stage) and 60th Days After Sowing (10th leaf stage) at both sites during the LR2018 season. During the LR2019 season, tillage significantly influenced chlorophyll levels in maize leaves at the 6th leaf stage at Dan and at the 10th leaf stage at Za-zounmè (Table 3). The effect of mulching was significant at the 6th leaf stage at Za-zounmè at the 10th leaf stage at Dan and Za-zounmè. At the 6th leaf stage, Chl content was significantly higher under IR and PR of 26% compared to NT at Dan and 17 and 7% compared with NT at Za-zounmè during the LR2018 season. During the LR2019 season, Chl content was also significantly higher under IR and PR of 18 and 13% compared to NT at Dan and 19 and 11%

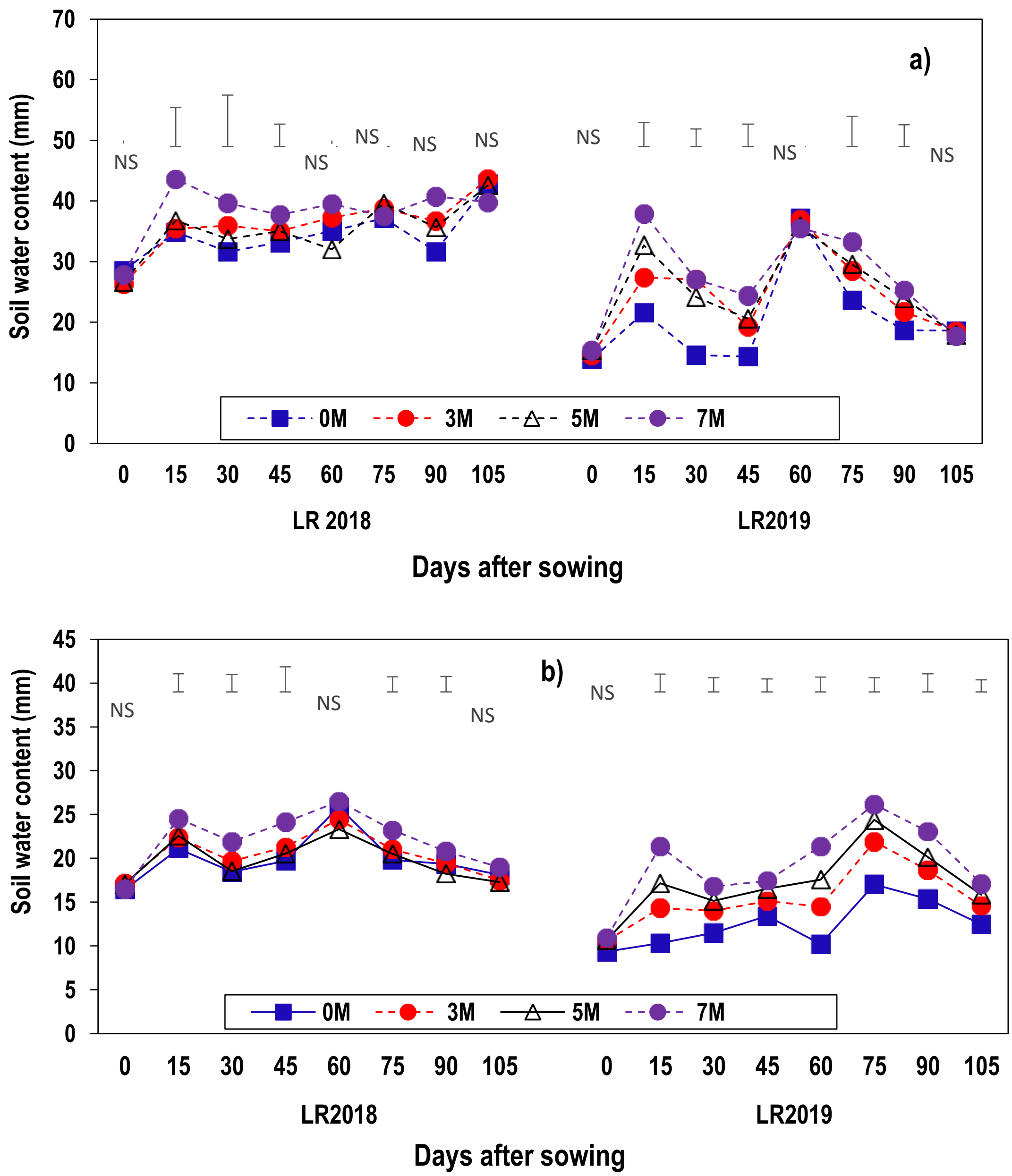

Figure 5. Effect of Mulching on soil water content distribution during LR2018 and LR2019 seasons at a) Dan and b) Za-zounmè. 0M= 0 t ha^{-1} of mulch; 3M= 3 t ha^{-1} of mulch; 5M= 5 t ha^{-1} of mulch; 7M= 7 t ha^{-1} of mulch. Significant differences of the post hoc Tukey's HSD test performed in case of effect if the model was significant ($p < 0.05$). Error bars indicate the Minimum Significant Difference value. NS= not significant.

compared to NT at Za-zounmè. For mulching, the strongest Chl content was observed with 7 t.ha^{-1} mulch except at Za-zounmè where the strongest Chl content was obtained with 5M during the LR2018 season. Compared with the plot without mulch (0 t.ha^{-1} mulch), 7, 5, and 3 t.ha^{-1} mulch significantly increased content Chl by 23%, 17% and 8% at Dan and by 3, 15 and 3% at Za-zounmè during the LR2018 season. During the LR2019 season, Chl content grew significantly by 22, 16, and 13% under 7; 5 and 3 t.ha^{-1} mulch compared with 0 t.ha^{-1} mulch at Dan and 24, 20 and 8% under 7; 5 and 3 t.ha^{-1} mulch compared with 0 t.ha^{-1} mulch at Za-zounmè. The interactive effect (Tillage x Mulching) was significant only at Za-zounmè during the LR2018 season (Table 4). Isohypse ridging associated with 7 t.ha^{-1} mulch (IR7M) yielded the highest Chl content in the 6^{th} leaf stage of maize leaves. As for the 10^{th} leaf stage, the content was significantly higher under IR and PR compared to NT at

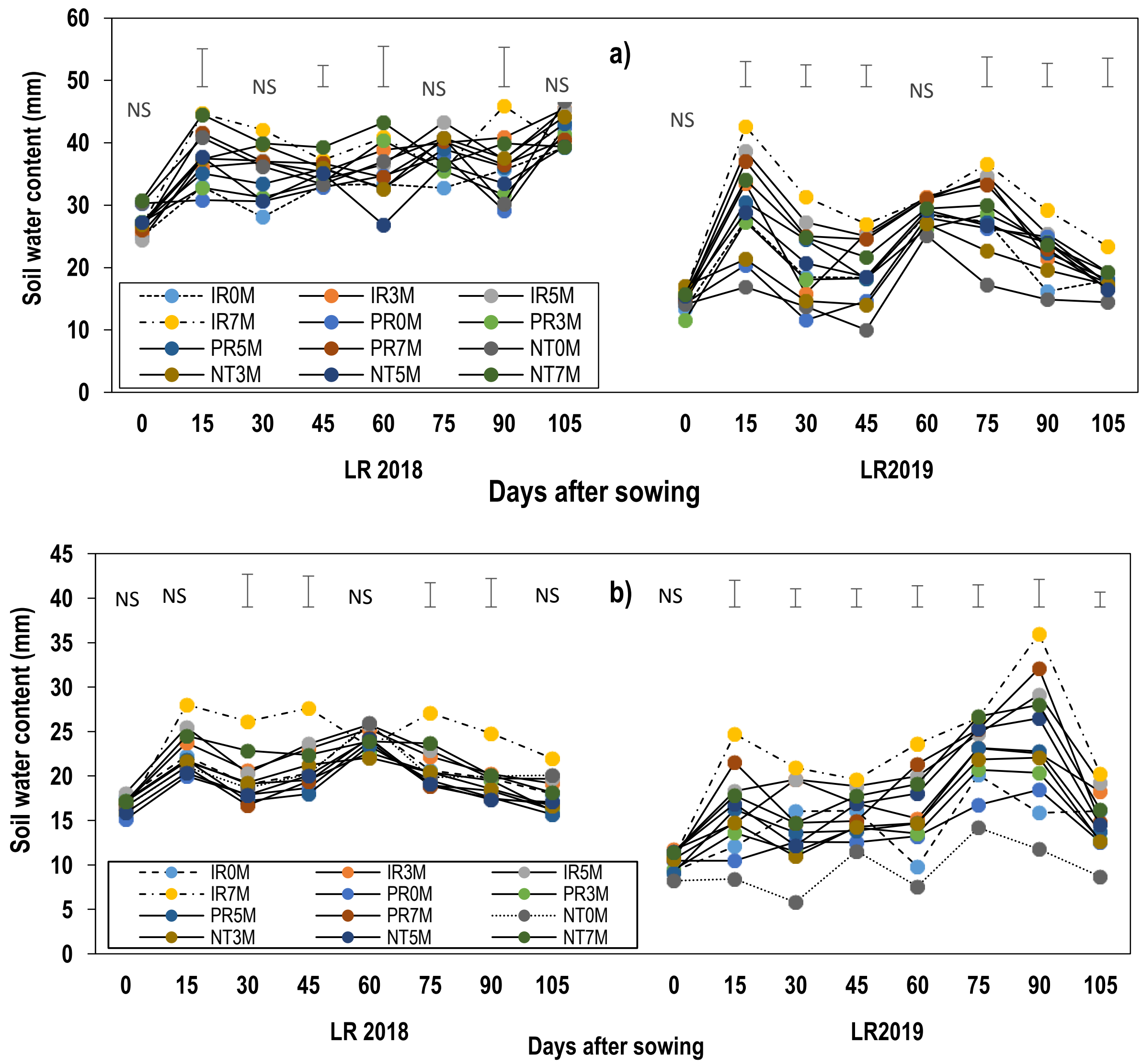

Figure 6. Interactive effect of tillage and mulching on soil water content distribution during LR2018 and LR2019 seasons at a) Dan and b) Za-zounmè. IR0M= Isohypse ridging+0 t ha^{-1} of mulch; IR3M= Isohypse ridging+3 t ha^{-1} of mulch; IR5M= Isohypse ridging+5 t ha^{-1} of mulch; IR7M= Isohypse ridging +7 t ha^{-1} of mulch; PR0M= Ridging in the slope direction +0 t ha^{-1} of mulch; PR3M Ridging in the slope direction + 3 t ha^{-1} of mulch; PR5M= Ridging in the slope direction+5 t ha^{-1} of mulch; PR7M= Ridging in the slope direction +7 t ha^{-1} of mulch; NT0M= No-tillage+0 t ha^{-1} of mulch; NT3M= No-tillage+3 t ha^{-1} of mulch; NT5M= No-tillage + 5 t ha^{-1} of mulch; NT7M= No-tillage+7 t ha^{-1} of mulch . Significant differences of the post hoc Tukey's HSD test performed in case of effect if the model was significant ($p < 0.05$). Error bars indicate the Minimum Significant Difference value. NS= not significant.

both sites for both seasons. The highest Chl content was obtained with isohypse ridging (31.52 and 34.79 respectively at Dan and Za-zounmè). Regarding mulching, the highest value was obtained under 7 t.ha^{-1} mulch at Dan during the two seasons and at Za-zounmè during the LR2019 season. During the LR2018 season, the highest value of Chl content at the 10th leaf stage was observed under 5M. The interactive effect (Tillage x Mulching) was not significant on the Chl content at the 10th leaf stage at both Dan and Za-zounmè sites and during both seasons.

Influence of soil tillage and mulching on maize yield

Grain yield

Tillage, mulch amount and their interaction (Tillage x mulching) significantly influenced the grains yield of maize at the both investigated sites. During the both LR2018 and LR2019 seasons, Isohypse Ridging recorded the highest grain yield (Table 3). PR increased in average the grain yield by 4% in LR2018 and by 22% in LR2019, compared with NT. However, the difference between the

Table 3. Simple effects of tillage and mulching on maize growth and yield at Dan and Za-zounmè during LR2018 and LR2019 seasons.

Modalities	LR2018						LR2019					
	Growth speed (cm. day^{-1})	Relative chlorophyll content at 30 after sowing	Relative chlorophyll content at 60 after sowing	Grains Yield (kg.ha^{-1} Dry matter)	Straw Yield (kg .ha^{-1} Dry matter)	Harv est index	Growth speed (cm. day^{-1})	Relative chlorophyll content at 30 after sowing	Relative chlorophyll content at 60 after sowing	Grains Yield (kg. ha^{-1} Dry matter)	Straw Yield (kg.ha^{-1} Dry matter)	Harvest index
Dan												
Tillage[1]												
NT	3.05^{b}	26.26^{b}	34.50^{b}	2040.25^{b}	2219.47^{b}	0.48^{a}	3.11^{b}	27.26^{b}	32.53^{c}	2289.42^{c}	2594.67^{b}	0.45^{b}
PR	3.29^{a}	30.67^{a}	36.73^{a}	2145.71^{b}	2797.85^{a}	0.43^{b}	3.35^{ab}	30.81^{a}	35.97^{b}	2781.43^{b}	2805.54^{b}	0.52^{a}
IR	3.31^{a}	31.96^{a}	37.04^{a}	2569.27^{a}	2771.62^{a}	0.47^{a}	3.52^{a}	32.28^{a}	38.65^{a}	3185.50^{a}	3304.42^{a}	0.48^{ab}
Mulching[2]												
0M	2.59^{d}	26.48^{b}	34.23^{b}	1880.50^{c}	2047.28^{d}	0.49^{a}	2.94^{b}	26.67^{c}	31.55^{c}	2055.72^{b}	2403.81^{c}	0.46^{b}
3M	3.13^{c}	28.63^{ab}	35.34^{b}	2178.65^{b}	2334.26^{c}	0.47^{ab}	3.01^{b}	30.20^{b}	34.16^{b}	2394.12^{b}	2563.14^{bc}	0.48^{ab}
5M	3.51^{b}	30.88^{a}	39.25^{a}	2343.17^{b}	2686.07^{b}	0.47^{ab}	3.62^{a}	31.05^{ab}	37.98^{a}	3157.43^{a}	2942.83^{b}	0.52^{a}
7M	3.81^{a}	32.52^{a}	35.34^{b}	2604.84^{a}	3317.65^{a}	0.44^{b}	3.73^{a}	32.55^{a}	39.18^{a}	3401.21^{a}	3695.02^{a}	0.48^{ab}
Za-zounmè												
Tillage[1]												
NT	3.72^{b}	34.50^{b}	30.87^{b}	2791.30^{b}	3086.1^{c}	0.48^{a}	3.02^{c}	32.53^{c}	27.98^{c}	2121.27^{b}	2392.75^{b}	0.45^{a}
PR	3.80^{b}	36.73^{a}	32.06^{b}	2891.95^{b}	3482.04^{b}	0.46^{ab}	3.53^{b}	35.97^{b}	30.62^{b}	2472.38^{b}	2939.43^{ab}	0.47^{a}
IR	4.02^{a}	37.04^{a}	34.79^{a}	3174.12^{a}	4331.54^{a}	0.42^{b}	3.89^{a}	38.65^{a}	35.08^{a}	3108.51^{a}	3644.13^{a}	0.46^{a}
Mulching[2]												
0M	3.53^{c}	34.23^{b}	30.20^{a}	2514.52^{d}	2926.1^{c}	0.46^{a}	3.19^{b}	31.55^{c}	28.62^{b}	1808.06^{c}	2611.70^{c}	0.42^{a}
3M	3.67^{bc}	35.34^{b}	33.02^{ab}	2775.34^{c}	3687.07^{b}	0.43^{b}	3.37^{b}	34.16^{b}	31.24^{ab}	2230.81^{bc}	2569.55^{c}	0.52^{a}
5M	3.88^{b}	39.25^{a}	34.28^{a}	2953.39^{b}	4100.50^{a}	0.43^{b}	3.50^{ab}	37.98^{a}	32.03^{a}	2788.12^{ab}	3029.65^{b}	0.48^{a}
7M	4.33^{a}	35.34^{b}	32.79^{ab}	3566.57^{a}	4003.28^{a}	0.47^{a}	3.83^{a}	39.18^{a}	33.00^{a}	3375.62^{a}	4217.11^{a}	0.44^{a}

[1]**Tillage: NT**: no-tillage; **PR**: ridging parallel to the slope; **IR**: Isohypse ridging; [2] **Mulching: 0M**: 0 t ha^{-1} of Mulch; **3M:** 3 t ha^{-1} of Mulch; **5M**: 5 t ha^{-1} of Mulch; **7M**: 7 t ha^{-1} of Mulch. For each factor, same superscript letters denote no significant difference between the means at a given site. Different letters indicate significant differences of the post hoc Tukey's HSD test performed in case effects if the model were significant (p≤0.05).

grain yield obtained with ridging parallel to the slope (PR) and those obtained on No-Tillage (NT) were not statistically significant in LR2018. Conversely, the difference was significant in LR2019. Mulch amount significantly increased the maize grains yield. For both studied sites, the highest grains yield was obtained when 7 t.ha^{-1} mulch was applied while the least mean values was obtained with 0 t.ha^{-1} mulch. But it is statistically remarkable that the difference between 3, 5 and 7 t.ha^{-1} mulch was not significant at Dan contrarily to Za-zounmè where the difference between the mulch amounts was significant. The means of grain yield obtained under the interactive effect of tillage and mulching (treatments) are presented in Table 4. As it can be seen, the treatments IR7M gave the highest grains yield at Dan (2991.15 and 4475.31 kg.ha^{-1} DM respectively in LR2018 and LR2019 seasons) and Za-zounmè (3648.74 and 4722.62 kg.ha^{-1} DM respectively in LR2018 and LR2019 seasons) whereas, the smallest maize grains yield were obtained with the treatments PR0M and NT0M at the both site throughout the study period. In general,

Table 4. Interactive effect of tillage and mulch amounts on maize growth and yield at Dan and Za-zounmè during LR2018 and LR2019 seasons.

Treatments	LR2018						LR2019					
	Growth speed (cm.day^{-1})	Chlorophyll content at 30 after sowing	Chlorophyll content at 60 after sowing	Grains yield (kg.ha^{-1} Dry matter)	Straw Yield (kg.ha^{-1} Dry matter)	Harvest index	Growth speed (cm.day^{-1})	Chlorophyll content at 30 after sowing	Chlorophyll content at 60 after sowing	Grains Yield (kg.ha^{-1} Dry matter)	Straw yield (kg.ha^{-1} dry matter)	Harvest index
							Dan					
IR0M	2.72^{e}	26.78^{bc}	24.29^{d}	2102.36^{def}	2258.58^{gh}	0.48^{b}	2.91^{a}	26.76^{a}	26.66^{a}	2052.34^{de}	2696.16^{bcd}	0.43^{b}
IR3M	3.17^{d}	30.66^{abc}	29.58^{abc}	2540.04^{bc}	2373.43^{fg}	0.51^{ab}	3.52^{a}	27.83^{a}	28.41^{a}	2526.61^{cd}	2790.51^{bcd}	0.48^{ab}
IR5M	3.31^{d}	33.31^{ab}	34.94^{ab}	2643.53^{ac}	2828.27^{cd}	0.48^{b}	3.70^{a}	27.02^{a}	28.85^{a}	3687.92^{b}	3167.68^{bc}	0.54^{ab}
IR7M	4.06^{a}	37.09^{a}	37.28^{a}	2991.15^{a}	3626.21^{a}	0.45^{c}	3.83^{a}	33.30^{a}	33.69^{a}	4475.31^{a}	4563.15^{a}	0.50^{ab}
PR0M	2.62^{e}	30.16^{abc}	27.96^{abc}	1780.96^{f}	2505.18^{efg}	0.41^{e}	3.03^{a}	31.22^{a}	28.60^{a}	2493.62^{cde}	2729.93^{bcd}	0.48^{ab}
PR3M	3.06^{d}	28.97^{abc}	29.63^{abc}	2078.02^{def}	2580.57^{de}	0.45^{c}	3.25^{a}	33.58^{a}	31.85^{a}	2602.^{cd}	2413.6^{cd}	0.52^{ab}
PR5M	3.67^{bc}	29.53^{abc}	30.33^{abc}	2278.57^{cde}	2817.99^{cd}	0.46^{c}	3.33^{a}	30.41^{a}	31.75^{a}	3165.62^{bc}	2379.08^{cd}	0.59^{a}
PR7M	4.00^{ab}	34.00^{ab}	37.42^{a}	2445.31^{bcd}	3287.66^{b}	0.43^{d}	3.73^{a}	33.14^{a}	34.43^{a}	2863.8^{bcd}	2856.29^{bcd}	0.50^{ab}
NT0M	2.46^{e}	22.5^{ac}	21.40^{d}	1758.11^{f}	1378.07^{i}	0.56^{a}	2.90^{a}	28.88^{a}	27.84^{a}	1621.2^{e}	1785.32^{d}	0.47^{ab}
NT3M	3.17^{d}	26.27^{cd}	25.97^{abc}	1917.9^{ef}	2048.78^{h}	0.48^{b}	2.99	28.10^{a}	28.7^{a}	2053.17^{de}	2485.32^{bcd}	0.45^{b}
NT5M	3.24^{d}	29.81^{abc}	33.61^{ab}	2107.16^{efd}	2411.94^{fg}	0.47^{b}	3.36^{a}	31.68^{a}	31.48^{a}	2618.75^{cd}	3281.73^{bc}	0.44^{b}
NT7M	3.36^{cd}	26.45^{bc}	32.03^{abc}	2377.81^{bcd}	3039.08^{bc}	0.44^{d}	3.31^{a}	30.25^{a}	33.6^{a}	2864.57^{bcd}	3665.63^{ab}	0.44^{b}
							Za-zounmè					
IR0M	3.65^{bcd}	35.23^{abc}	30.81^{ab}	2674.2^{e}	3421.13^{cd}	0.44^{cdef}	2.86^{a}	33.61^{a}	31.22^{a}	1926.33^{c}	2520.78^{bc}	0.45^{a}
IR3M	3.67^{bcd}	34.67^{abc}	34.54^{ab}	3101.18^{cd}	4514.22^{a}	0.41^{f}	3.45^{a}	34.97^{a}	32.69^{a}	2397.10^{bc}	2776.81^{bc}	0.46^{a}
IR5M	3.94^{bc}	37.90^{abc}	37.75^{a}	3272.36^{bc}	4520.62^{a}	0.40^{f}	3.71^{a}	34.19^{a}	31.16^{a}	3087.07^{b}	3433.65^{bc}	0.47^{a}
IR7M	4.85^{a}	40.37^{ab}	36.09^{ab}	3648.74^{a}	4870.19^{a}	0.45^{bcde}	4.02^{a}	37.93^{a}	34.51^{a}	4722.62^{a}	5511.84^{a}	0.46^{a}
PR0M	3.52^{cd}	34.58^{abc}	28.59^{b}	2765.05^{e}	3067de	0.47^{abcd}	2.79	31.59^{a}	29.85^{a}	1800.31^{c}	3076.55^{bc}	0.49^{a}
PR3M	3.67^{bcd}	36.37^{abc}	33.16^{ab}	2484.7^{e}	3248.35^{cd}	0.40^{f}	3.38^{a}	35.02^{a}	33.87^{a}	2442.44^{bc}	2869.21^{bc}	0.47^{a}
PR5M	3.95^{bc}	41.68^{a}	32.76^{ab}	2803.2^{de}	3800.30^{b}	0.46^{abcd}	3.48^{a}	34.63^{a}	32.94^{a}	2313.11^{bc}	3082.39^{bc}	0.46^{a}
PR7M	4.08^{b}	34.28^{abc}	33.74^{ab}	3514.83^{ab}	3812.52^{b}	0.48^{abc}	3.77^{a}	36.16^{a}	33.79^{a}	3083.72^{b}	3729.58^{b}	0.47^{a}
NT0M	3.43^{d}	32.87^{bc}	31.23^{ab}	2104.31^{f}	2290.15^{f}	0.48^{abcd}	2.63^{a}	29.44^{a}	24.84^{b}	1700.22^{c}	2082.80^{bc}	0.46^{a}
NT3M	3.66^{bcd}	34.97^{abc}	31.36^{ab}	2740.15^{e}	2746.67^{e}	0.50^{a}	3.39^{a}	34.68^{a}	30.08^{a}	1727.02^{c}	1893.71^{c}	0.47^{a}
NT5M	3.77^{bcd}	38.18^{abc}	32.34^{ab}	2784.61^{e}	3630.86^{b}	0.43^{efd}	3.67^{a}	36.82^{a}	29.4^{a}	3122.32^{b}	2553.73^{bc}	0.45^{a}
NT7M	4.04^{b}	31.97^{c}	28.55^{b}	3536.12^{ab}	3676.71^{b}	0.49^{ab}	3.66^{a}	37.56^{a}	33.02^{a}	1968.92^{c}	3140.77^{bc}	0.46^{a}

NT0M: No tillage + 0 t ha^{-1} of mulch; **NT3M**: No tillage + 3 t ha^{-1} of mulch; **NT5M:** No tillage + 5 t ha^{-1} of mulch; **NT7M:** No tillage + 7 t ha^{-1} of mulch; **PR0M:** Ridging parallel to the slope + 0 t ha^{-1}; **PR3M :** Ridging parallel to the slope + 3 t ha^{-1} of mulch; **PR5M** : Ridging parallel to the slope + 5 t ha^{-1} of mulch; **PR7M** : Ridging parallel to the slope + 7 t ha^{-1} of mulch; **IR0M :** Isohypse ridging + 0 t ha^{-1} of mulch; **IR3M :** Isohypse ridging + 3 t ha^{-1} of mulch; **IR5M:** Isohypse ridging + 5 t ha^{-1} of mulch; **IR7M**: Isohypse ridging + 7 t ha^{-1} of mulch.

the grain yield was greater in LR2019 season than in LR2018 season at Dan while LR2018 yielded to great grains compared with LR2019 at Za-zounmè.

Straw yield

Mean straw yield of maize under the different tillage practice, mulch amount and their interactions are shown in Tables 3 and 4. Tillage showed significant ($p < 0.05$) difference in straw yield of maize at Dan and Za-zounmè with the lowest values of 2407.07 and 2739.43 kg. ha^{-1}, respectively, being observed under No-Tillage (NT) whereas the largest values of 2978.56 and 3987.84 $kg.ha^{-1}$, respectively, were obtained Isohypse ridging (IR) in average over the two investigated season. There were significant variations ($p < 0.05$) among the different mulch amount. The greatest maize straw yield was observed under the plot with 7 $t.ha^{-1}$ mulch. On the other hand, smallest yield of maize straw was observed under 0 $t.ha^{-1}$ mulch throughout the LR2018 and LR2019 seasons. Tillage and mulch amount interacted to influence significantly the maize straw yield over the both investigated cropping seasons. The largest value was obtained under isohypse ridging with 7 $t.ha^{-1}$ mulch (4094.68 $kg.ha^{-1}$ in average at Dan and 5191.02 $kg.ha^{-1}$ in average at Za-zounmè over LR2018 and LR2019). Similar to the grain yield, the straw yield was greater in LR2019 season than in LR2018 season at Dan while LR2018 yielded to great straw compared with LR2019 at Za-zounmè.

Harvest index

According to the ANOVA results, the maize harvest index was significantly ($p < 0.05$) affected by tillage, mulch amount and their interaction at Dan throughout the both investigated seasons. The harvest index was higher under No-tillage (NT) and Isohypse Ridging (IR) in LR2018 (0.48 and 0.47 respectively) whereas the highest values were observed under ridging parallel to the slope (PR) and Isohypse Ridging (IR) in LR2019 (0.52 and 0.48 respectively) (Table 3). As far as the effect of mulch amount is concerned, the smallest harvest index was obtained under 7 $t.ha^{-1}$ mulch in LR2018 season and under 0 $t.ha^{-1}$ mulch in LR2019 season. Tillage and mulch amount interacted to influence significantly the maize straw yield at Dan over the investigated cropping seasons.

No-tillage associated with 0 $t.ha^{-1}$ (0.56) in LR2018 season and ridging parallel to the slope associated with 5 $t.ha^{-1}$ (0.59) in LR2019 season yielded the highest harvest index. At Za-zounmè, the effects of the different factors under study were significant on the maize harvest index only in LR2018 season. The harvest index was highest under No-tillage (0.48) and lowest under Isohypse Ridging (0.42) in LR2018 at Za-zounmè. For the mulching treatments, 0 $t.ha^{-1}$ and 7 $t.ha^{-1}$ mulch yielded the great harvest index. Similarly to the site of Dan, the interactive effect of tillage and mulching was significant in the LR2018 season (Table 4). Isohypse Ridging associated with 5 $t.ha^{-1}$ mulch (IR5M); Isohypse Ridging associated with 3 $t.ha^{-1}$ mulch (IR3M) and ridging parallel to the slope associated with 3 $t.ha^{-1}$ (PR3M) yielded the lowest harvest index.

DISCUSSION

Water constitutes a key factor for crops growth and yield (Mansouri et al., 2010). Over the two investigated cropping seasons, the rainfall patterns were quite different at both sites. At Dan, total seasonal rainfall for both LR2018 and LR2019 was below the long-term average of 572.34 mm. Also, total seasonal rainfall recorded was low than the long-term average of 670.12 mm at Za-zounmè. From the sowing date to the vegetative stage of the maize, 213.66 and 397.5 mm were recorded during the LR2018 whereas 250.3 and 388.6 mm were recorded during the LR2019 respectively at Dan and at Za-zounmè. These rainfall values are below the 450 mm needed for optimal maize growth. This implies that maize mays need supplementary water input as water control plant phenological, physiological and morphological (Pandey et al., 2000).

The results of this study show that tillage and mulching significantly influence water content of soil, maize growth as well as its yield components. The highest soil moisture values were obtained under Isohypse ridging whereas the lowest were obtained under No-tillage at both sites during the two investigated cropping seasons. Consequently, IR allowed the highest maize growth, grain and straw yield compared with Ridging parallel (PR) to the slope and No-Tillage (NT). This may be due to the fact that in Isohypse Ridging system, ridges are made following the contour lines and acts as obstacle for runoff by reducing the velocity of water and maintain it onsite. Contour ridges constitute water conservation and erosion control practice used to increase surface run-off storage near the cropped area, in contrast to the flat tillage and ridging parallel to the slope over some area in Africa (Uwizeyimana et al., 2018; Akplo et al., 2019b). It may also due to the fact that NT practices are used at first time on the experimental sites. These findings are similar to those of Basch et al. (2008) and Pittelkow et al. (2015) which notified a decrease in crop yield and an increase in runoff and soil loss during the establishment of NT. Mekhlouf et al. (2011) found that there is no significant difference between different tillage practices on grain and straw yields in wheat. Hountongninou (2016) reported that tillage does not significantly affect maize grain yield and justified this by the sandy-clayey texture of its study site, which provides a similar environment under plowing. Although NT is recognized as one of the Conservation Agriculture practices (Lal, 2004; Reicosky and Saxton, 2007; Martínez et al., 2008; Tabaglio et al., 2009; Kolb et al., 2012; Soane et al., 2012; Fiorini et al., 2018), its adoption requires a transition phase (on average 7-8 years), characterized by higher annual weed and disease pressures, slow rebuilding aggregates in soil, and lower and variable yields (Knowler and Bradshaw, 2007; Pagnani et al., 2019). However, as outlined in Sharma and Abrol (2005), soil types react differently to the same tillage method. That certainly why the grain yield had significantly decreased by 708.8 $kg.ha^{-1}$ under no-tillage at Za-zounmè, whilst it increased by 262.8 $kg.ha^{-1}$ at Dan under no-tillage between 2018 and 2019. In addition, Results of this study revealed that significant effect of tillage on the soil moisture is associated with the low rainfall period where low soil contents were recorded. On other hand in case of high rainfall period, tillage effect was not significant. This demonstrates that IR can be used for drought and dry spells.

The mulching treatments resulted in a significant improvement in soil water content, maize growth and yield over the investigated cropping seasons at both experimental sites. Soil water content consistently increased with increase in surface cover across the three tillage practices. On other hand, the lowest values of soil moisture, growth speed, relative chlorophyll content, grain yield, straw yield and harvest index were obtained with 0 $t.ha^{-1}$ mulch whereas the highest values were recorded with 5 and 7 $t.ha^{-1}$ of mulch. One of the major roles played by mulch cover was probably reducing soil evaporation (Mupangwa et al., 2007). Hatfield et al. (2001) reported a reduction of 34-50% in soil water evaporation under residue mulching. Under the high rainfall of LR2018, the soil water contents under 0, 3 and 5 $t.ha^{-1}$ mulch were not significantly different at Za-zounmè while significant difference was found in LR2019 where low rainfall were recorded. Our results are consistent with those of many researchers (Mupangwa et al., 2007; Barthès et al., 2010; Badou et al., 2013, Uwizeyimana et al., 2018; Akplo et al., 2019a). Over the two targeted cropping seasons, the highest water contents of soil are associated with a great maize growth and yield. Researchers agree on the advantages of the mulching practice. Mulch increases soil moisture and nutrients availability to plant roots in turn, leading to higher plant growth and yield (Muhammad et al., 2015; Qi et al., 2016). Also, the vegetation cover itself constitutes a source of organic matter which, after decomposition, increases the cation exchange capacity and the water retention capacity of the soil, which improves the nutrient supply of the soil (Pervaiz et al., 2009). Tillage and mulching significantly interacted on soil moisture, maize growth and yield. The interactive effect of tillage and mulch was significant on the 15^{th}, 45^{th}, 60^{th} and the 90^{th} days after sowing in the LR2018 and on the 15^{th}, 30^{th}, 45^{th}, 75^{th}, 90^{th} and 105^{th} days after in the LR2019 season at Dan while at Za-zounmè, significant effect was observed on the 30^{th}, 45^{th}, 75^{th} and 90^{th} during the LR2018 season and on the 15^{th}, 30^{th}, 45^{th}, 60^{th}, 75^{th}, 90^{th} and 105^{th} days after sowing during the LR2019. In general, the periods with significant effect were associated with prolonged period of short precipitation resulting water deficiencies and lack of soil moisture. Overall, for each tillage practice, the water content increased with the mulch amount. Similar trends were observed with maize growth and yield. Mulch enhanced the effect of tillage whereby the highest values were found with the treatments Isohypse Ridging with at least 3 $t.ha^{-1}$ mulch (IR3M, IR5M; IR7M), Ridging Parallel to the slope + 7 $t.ha^{-1}$ mulch (PR7M) and No-tillage + 7 $t.ha^{-1}$ mulch (NT7M) and while the treatment No-tillage + 0 $t.ha^{-1}$ mulch NT0M load to the lowest values.

Conclusion

Water conservation constitutes a key challenge in crop productivity stabilization in rain-fed cropping systems in Sub-Saharan Africa. This study explored the effect of different tillage practices in combination with different crop residues amounts used as mulch on rain-fed maize growth and yield component and soil moisture. Findings from this study revealed that Isohypse Ridging saved water and improved maize growth and yield in the drought conditions comparatively to Ridging Parallel to the slope and No-Tillage over the investigated cropping seasons. Also a significant increase of water content of soil, maize growth and yield with an increased in mulch cover was observed whereby the highest values were obtained under 5 and 7 $t.ha^{-1}$ mulch. Tillage and mulch amounts interacted significantly on water content of soil, maize growth and yield. Findings of this study showed the high values under isohypse ridging + 7 $t.ha^{-1}$ mulch. This suggests that isohypse ridging associated with organic mulching could be at short time, an option to mitigate dry spells and drought and to improve local farmers' income in the area of low rainfall.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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Grain morphological characterization and protein content of sixty-eight local rice (*Oryza sativa* L) cultivars from Cameroon

Marlyn Njimeni Tchuisse[1,2], Eddy Leonard Magaptche Ngonkeu[3,4], Dorothy K. Malaa[3], Honoré Tekeu[4], Thomas Mballa[4], Joseph Hubert Yamdeu Galani[5], Akindeh Nji[2] and Thaddée Boudjeko[1,2]*

[1]Laboratory of Phytoprotection and Plant Valorisation, Biotechnology Centre, Yaoundé, Cameroon.
[2]Department of Biochemistry, Faculty of Science, University of Yaoundé I, P. O. Box 812, Yaoundé, Cameroon.
[3]Institute of Agricultural Research for Development (IRAD), P. O. Box 2123, Yaoundé, Cameroon.
[4]Department of Plant Physiology, Faculty of Science, University of Yaoundé I, P. O. Box 812, Yaoundé, Cameroon.
[5]School of Food Science and Nutrition, University of Leeds, Leeds, LS2 9JT, UK.

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Rice (*Oryza sativa* L.) cultivated in Cameroon is appreciated by consumers for its nutritive quality and good taste. Diversity of 68 local rice cultivars was investigated via grain morphology and protein content characterization. The size and shape of grains were determined and used with yield parameters to classify the cultivars and perform Principal Component Analysis (PCA). Total protein content and glutelin content of eight selected cultivars (CMRGNd, CMRGDn, CMRGTï, CMRTBa, CMRDWb, CMRDTc3, CMRDTx5 and CMRDTx6) were evaluated by Bradford assay and correlation analysis of all the parameters studied was performed. Long size grains (42) were predominant over extra-long (16), medium (9) and short (1) grains. Slender shaped grains (36) were distinguished as well as medium (28) and bold (4) grains. The 68 cultivars were grouped into four clusters independent of their origins. PCA revealed three principal components accounting for 74.4% of total variation. Highest total protein content was observed in CMRGNd (14.3%) and highest glutelin content in CMRGDn (10.1 mgEqvBSA/g DW). Pearson correlation of the different variables revealed no significant correlation between total protein and glutelin contents with the agro-morphological parameters evaluated in this study. This suggests that none of these parameters could be descriptor for protein content. Positive correlation between grain length and yield (r = 0.7) suggests grain length as yield descriptor.

Key words: Rice, diversity, grain morphology, protein, glutelin.

INTRODUCTION

Rice (*Oryza sativa* L.) is the second most consumed cereal in Cameroon after maize and is presently a staple food for both rural and urban populations. However, the national production estimated at about 360000 tons in 2017 (FAO, 2019), remains far below consumption that is estimated at about 635,000 tons (USDA, 2017). The deficit between demand and production results from several factors among which a non-effective distribution of high impact technologies and the low competitiveness of local rice. Domestic rice though, is dominated by imported rice and fails to compete both quantity and quality-wise with imported rice.

However, rice produced in Cameroon is appreciated by many; 90% of the national produce is exported to Nigeria in paddy form. While trying to identify the physico-chemical characteristics that determine the consumption preferences of local and imported rice in the North-west region, Fon and Fonchi (2016) obtained 83.9% of respondents confirming that locally produced rice tastes better than imported rice. Consumer demand for fine rice varieties is high due to good nutritional quality, palatability, aroma and taste. But quality can as well be considered from the view point of size, shape and appearance of grain (Cruz and Khush, 2000). IRRI classifies brown rice grain length into extra-long (>7.50 mm); long (6.61-7.50 mm); medium (5.51-6.60 mm); and short (≤5.50 mm). IRRI's Standard Evaluation System (SES) equally classifies shape as slender (length–width ratio >3.0), medium (ratio 2.1–3.0), bold (ratio 1.1–2.0) and round (ratio <1.1) (IRRI, 1996).

Scientific studies are indispensable to effectively demonstrate the quality of the local rice. In this light, Odenigbo et al. (2014) studied the gelatinization properties and amylose content of some local rice varieties and provided information on the physical, gelatinization, cooking and textural properties of TOX 3145, an improved rice variety cultivated in Cameroon. These studies focused on the cooking and eating quality and did not report the nutritional quality of the local rice. Protein content and composition are crucial to rice grain quality and nutritional value (Lin et al., 2005) but the genetic base of rice seed proteins in Cameroon's locally cultivated rice is relatively narrow.

The rice grain is composed of 12% water, 75–80% starch and 7% protein with a full complement of amino acids (Verma and Srivastav, 2017). Its high protein digestibility and excellent biological value make it an important part of consumers' daily nutrient intake. Yang et al. (2011) reported the digestibility of rice protein as being a major factor that influences cholesterol metabolism through the inhibition of cholesterol absorption. Rice protein hydrolysates are equally known to possess antioxidative and blood pressure regulating properties (Zhao et al., 2012; Phongthai et al., 2017). These physiological functions make rice suitable to prevent life style-related diseases such as malnutrition, obesity and high blood pressure which are adult risk factors. Besides, the enhancement of rice seed storage proteins to improve rice nutritive value has lately and gradually become an important target for rice quality breeding (Jiang et al., 2014). Data on characterization of grain and proteins of local Cameroon rice cultivars, which will be very useful in upscaling the potential of its production, is scanty. This study therefore explored Cameroon's locally cultivated rice to determine the quality of the cultivars cropped and provide additional rice diversity information.

MATERIALS AND METHODS

Establishment of an inventory of local rice cultivars

Sixty-eight cultivars from three agro-ecological zones of the country were used in this study (Table 1): sudano-sahelian zone (Garoua), humid forest with bimodal rainfall zone (Yaoundé) and western highlands zone (Dschang and Tonga). The cultivars collected in Yaoundé had recently been introduced from Benin while those from Garoua, Dschang and Tonga had been cultivated for long by local farmers over several campaigns.

Grain morphological characterization of cultivars

The morphology of grains was studied by measuring their dimensions (length and width) and determining the length/width ratio, which were used to characterise them following IRRI's standard evaluation system for rice (2013). They classify brown rice grain as extra-long (more than 7.5 mm), long (6.6 to 7.5 mm), medium (5.51 to 6.6 mm) and short (5.5mm or less) for size; and slender (over 3.0), medium (2.1 to 3.0), bold (1.1 to 2.0) and round (less than 1.1) for shape (length/width ratio) (IRRI, 1996).

Measurement of grain dimensions

Paddy samples were dehulled with a Satake rice dehuller machine (Satake, USA) and cleaned to eliminate dirt and husks. Length and width of three representative paddy and kernel grains from each sample were determined using a Vernier calliper. The grain shapes were then determined using the following equation:

Length to width ratio = Average grain length (mm)/Average grain width (mm).

Protein content evaluation

Samples were ground with a blender and sieved through a 5 mm mesh sieve to obtain fine powder. The powder was then stored at room temperature (25°C) in airtight bottles untill analysis.

Total protein extraction

Total proteins were extracted by mixing 0.1 g of rice powder with 1 mL of Tris urea buffer (0.05 M Tris-HCL, 5 M Urea, 2 % SDS, 1 % β-mercaptoethanol, and pH 8.0) and centrifuging at 10,000 rpm for 15 min (Tanaka et al., 2016). The supernatant was collected and the pellet rinsed with another 1 mL of Tris urea buffer. The new supernatant was collected into the previous and the proteins were quantified by Bradford assay (Bradford, 1976).

*Corresponding author. E-mail: boudjeko@yahoo.com.

Table 1. Rice cultivars used in this study.

Code	Variety name	Origin (locality)	Cycle (days)	N° tillers	Yield (Kg/h)	1000 GWt at 14% moisture
CMRYOr1	B1 ORYLUX 1	Yaoundé	119	12.4	7689.2504	24.0262
CMRYOr3	B2 ORYLUX 3	Yaoundé	119	15	7471.7740	22.3744
CMRYOr4	B3 ORYLUX 4	Yaoundé	119	12.6	10327.8066	23.1682
CMRYOr5	B4 ORYLUX 5	Yaoundé	131	14.2	7580.3924	22.7685
CMRYOr6	B5 ORYLUX 6	Yaoundé	105	24.8	4664.6814	22.8311
CMRYAr1	B6 ARICA 1	Yaoundé	133	20.4	6465.9251	23.7566
CMRYAr2	B7 ARICA 2	Yaoundé	111	7	8819.1999	23.2846
CMRYAr3	B8 ARICA 3	Yaoundé	111	11.6	9065.1237	23.9182
CMRYAr4	B9 ARICA 4	Yaoundé	105	6.4	2884.3836	20.3011
CMRYAr7	B12 ARICA 7	Yaoundé	112	5.6	3441.2896	22.5624
CMRYAr8	B13 ARICA 8	Yaoundé	133	13.2	6669.0641	22.2713
CMRYAr9	B 14 ARICA 9	Yaoundé	119	8.4	6628.4652	22.8311
CMRYAr10	B15 ARICA 10	Yaoundé	111	15.8	4886.5829	20.1414
CMRYAr11	B16 ARICA 11	Yaoundé	111	12	4977.8014	22.98023
CMRYAc1	B17 ARC-39-155-L-2	Yaoundé	119	11.6	5857.4306	24.6205
CMRYAc2	B18 ARC-37-16-1-5-G	Yaoundé	119	19	7798.2232	23.3664
CMRYAc3	B19 ARC-36-2-P-2	Yaoundé	119	11	6583.9304	23.7080
CMRYAc4	B20 ARC-39-135-VL-5	Yaoundé	112	13.6	6495.7222	23.6442
CMRYAc5	B21 ARC-39-130-EP-4	Yaoundé	112	13.4	3063.6420	22.0008
CMRYAc6	B22 ARC-39-145-EP-3	Yaoundé	112	8.2	6588.3679	22.1286
CMRYFl	B24 FL478-1-53	Yaoundé	112	6.4	5275.0568	23.5972
CMRYGc	B25 GOLD COAST FINGO	Yaoundé	119	9.8	6364.5332	21.7439
CMRYIr1	B26 IR4630-22-2	Yaoundé	133	24.4	5741.6468	22.3521
CMRYNl23	B28 NERICA-L-23	Yaoundé	111	11.6	6944.9369	22.6136
CMRYNl24	B29 NERICA-L-24	Yaoundé	111	10.4	6391.1240	22.3101
CMRYNl27	B30 NERICA-L-27	Yaoundé	112	10.8	4976.9713	23.1682
CMRYNl9	B31 NERICA-L-9	Yaoundé	119	12.8	7184.6219	23.0962
CMRYIr2	B32 IR64-SUBI	Yaoundé	112	5.8	3630.5247	22.1446
CMRDTx1	D13 TOX 3145-34-2-3	Dschang	119	8.6	7111.4402	25.9163
CMRDTx2	D14 TOX 3145-34-2-3-1	Dschang	119	20.6	12621.0426	24.6890
CMRDTx3	D15 TOX 3440-151-2-3	Dschang	113	13.2	6427.3875	23.3579
CMRDTx4	D16 TOX 3440-151-2-3	Dschang	107	9	7598.6249	26.0926
CMRDTx5	D17 TOX 3887-6-2-3	Dschang	107	9.2	4657.1479	25.6578
CMRDTx6	D18 TOX 40094-4-3	Dschang	115	16.8	15755.5428	24.7880
CMRDTc1	D20 TOC 2N 14-2	Dschang	114	14.4	4221.6066	26.1110
CMRDIr2	D26 IR 7167-33-2-3	Dschang	77	11	7978.0822	27.2030
CMRDIr3	D27 IR 155-79-135-3	Dschang	114	13.2	5847.0000	24.1402
CMRDWt	D28 WAT 311, WAS 7083-5-11	Dschang	114	10	5450.6507	24.2009
CMRDIt306	D30 ITA 306	Dschang	110	13.4	7513.4600	24.8605
CMRDFk60	D31 FKR 60	Dschang	114	10.6	6315.0885	23.3981
CMRDWb	D32 WAB	Dschang	114	15.8	13848.4079	22.9255
CMRDIb23	D35 IB 23	Dschang	114	8.8	6346.2671	22.5624
CMRDRv5	D36 RV 5	Dschang	112	9.2	5068.1764	24.3010
CMRTM16	T2 M 16	Tonga	115	13.2	8320.7283	23.3981
CMRTNl56	T3 NERICA-L-56	Tonga	115	13.8	7618.5760	25.5708
CMRTNl59	T4 NERICA-L-59	Tonga	115	10	8176.6444	24.2481
CMRTBa	T9 Bankou	Tonga	115	9.2	5393.0761	23.8159
CMRGIr46b	G14 IR 46	Garoua	114	10.4	7644.5798	23.5520
CMRGNe3	G3 NERICA 3	Garoua	110	3.2	1809.9551	23.2877
CMRGNl42	G19 NERICA-L-42	Garoua	114	13.8	7383.2482	20.7660

Table 1. Contd.

CMRGR48	G22 R48	Garoua	110	10.6	7432.5723	24.5873
CMRGTï	G25 TAÏTCHINGA	Garoua	127	15	11072.6567	22.8612
CMGNl60	G27 NERICA-L-60	Garoua	115	15.2	6509.0001	22.8928
CMRGRw	G28 RWISI	Garoua	115	21.8	5410.8288	24.1886
CMRGNe3	G18 NERICA 3	Garoua	110	6.8	3767.1202	23.70808
CMRGRw2	G32 RWISI (2)	Garoua	110	11.2	4010.5932	23.0137
CMRGDj	G33 DJOUNGA	Garoua	110	12	6513.3394	23.1225
CMRGNd	G35 NDOUNGOURI SAMORI	Garoua	115	12.6	11291.5231	23.6932
CMRGLa	G36 LASSIRI	Garoua	110	9.8	5700.4089	23.4834
CMRGDn	G38 DOUNGOURI SANTA	Garoua	107	10.6	4580.6372	24.2481
CMRGTx	G41 TOX	Garoua	93	17.8	8873.4296	24.3932
CMRG13	G13 Variété M	Garoua	114	14.6	10558.2449	21.1733

B, Benin; D, Dschang; G, Garoua; T, Tonga.

Glutelin extraction

Glutelins were extracted with 0.2 N acetic acid after elimination of albumins, globulins and prolamins with 35 mM KPi buffer (pH 7.6) (Nasri and Triki, 2007; Tanaka et al., 2016). One millilitre of inorganic potassium phosphate (KPi) buffer was added to 0.1 g of sample in the Eppendorf tube and mixed. The mixture was then placed on a rotating wheel at 34 rotations per minute (rpm) for 2 h 30 min for homogenization, and extraction was performed at 10,000 rpm for 15 min and the supernatant discarded. The pellet was then mixed with 1 mL of 0.2 N acetic acid and homogenized on the rotating wheel (34 rpm for 2 h 30 min) before centrifugation (10,000 rpm for 15 min). The supernatant was then collected into a new Eppendorf tube and quantified by Bradford assay. Glutelin content was expressed as milligrams equivalent of Bovine Serum Albumin per dry weight (mgEqvBSA/g DW).

Protein and glutelin data were analysed with SPSS software version 20. One way ANOVA test and Pearson correlation analysis were performed. The differences were considered as significant if $P < 0.05$, and highly significant if $P < 0.01$.

Statistical analyses

The data collected were subjected to multivariate analyses. The construction of the phylogenetic tree was done using software R. The Ward D method was used for calculating Euclidean distance. Principal component analysis (PCA) was performed using the SPAD V5 software.

RESULTS

Grain dimensions

Kernel sizes ranged from extra-long to short (Figure 1), with 16 extra-long cultivars, 42 long cultivars, 9 medium cultivars and 1 short cultivar. Kernel shapes were slender (36 cultivars), medium (28) and bold (4). It appeared that majority of the varieties from each of the localities were of long size (Yaoundé-56.6%, Dschang-57.9%, Garoua-60% and Tonga-75%), and slender shape (Yaoundé-66.7%, Dschang-52.6%, Garoua-53.3% and Tonga-50%).

Cluster and PCA analysis

Cultivars were grouped into four main clusters (Figure 2). Cluster 1 had six cultivars relatively low yielding (1810 to 3767 t/h) with varying sizes and shapes from extra-long and slender to long and medium. Cluster 2 had 34 cultivars with relatively high yields (6315 to 9065 t/h) and mainly slender and medium shaped grains, as well as many extra-long and long size grains with a few medium size and one short size grain cultivar. Cluster 3 had 8 cultivars with the highest yields (4752 to 15755 t/h) and mainly extra-long size and medium shape grains. Cluster 4 had 20 cultivars with low yields (4221,6 to 5858,2 t/h), mainly slender and medium in shape with one bold grain cultivar and many long size grain cultivars with a few extra-long and medium size grain cultivars.

PCA analysis demonstrated three components with Eigen value greater than 1 (Table 2): PC1, PC2 and PC3 which all contributed to 74.41 % of total variation. PC1 involved grain dimensions, PC2 involved agronomy parameters while PC3 involved yield components (Figure 3). Grain dimensions (paddy and kernel length and length/width ratio) which are associated to PC1 demonstrated a better distinction of the cultivars than other variables such as paddy and kernel width, number of tillers, thousand grain weight, cycle and yield.

Eight cultivars were selected for protein evaluation; four which were taught to be traditional and four improved cultivars which had PCA values similar to those of the traditional for comparison. Table 3 shows the eight selected varieties with their localities of origin and ecologies, and Figure 4 shows images of the paddy and kernels of these cultivars.

Total protein extraction and quantification

Protein contents were appreciably high, greater than the reported range of 7 to 10% (Figure 5). Highest protein

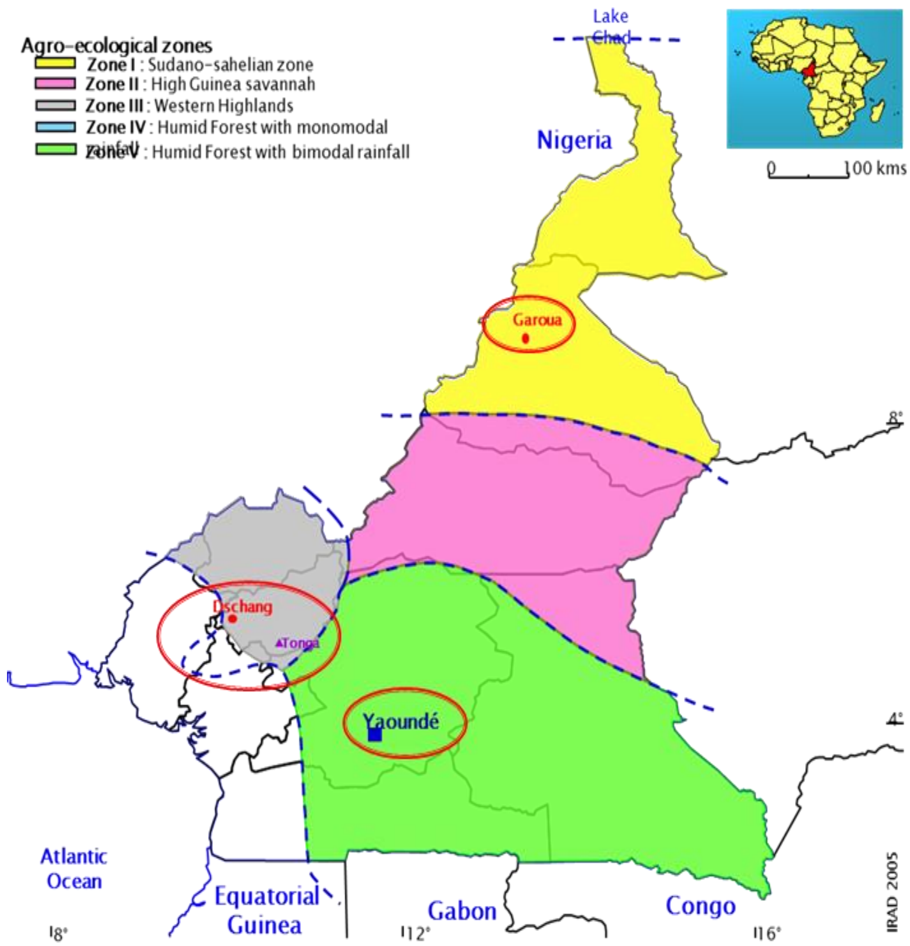

Figure 1. Cameroon agro-ecological zones.

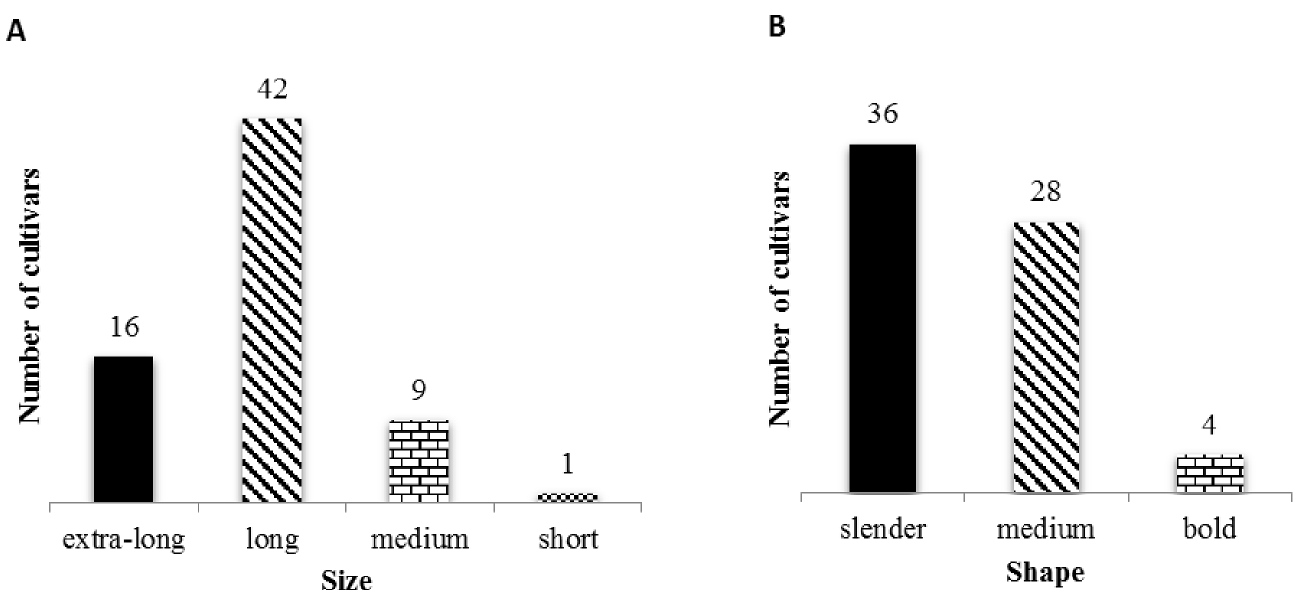

Figure 2. Kernel size (A) and shape (B) of cultivars collected.

Table 2. Principal Components for ten quantitative characters in 68 rice cultivars.

	Eigen value	Variance (%)	Cumulative (%)
PC1	4.6922	46.92	46.92
PC2	1.4182	14.18	61.10
PC3	1.3303	13.30	74.41

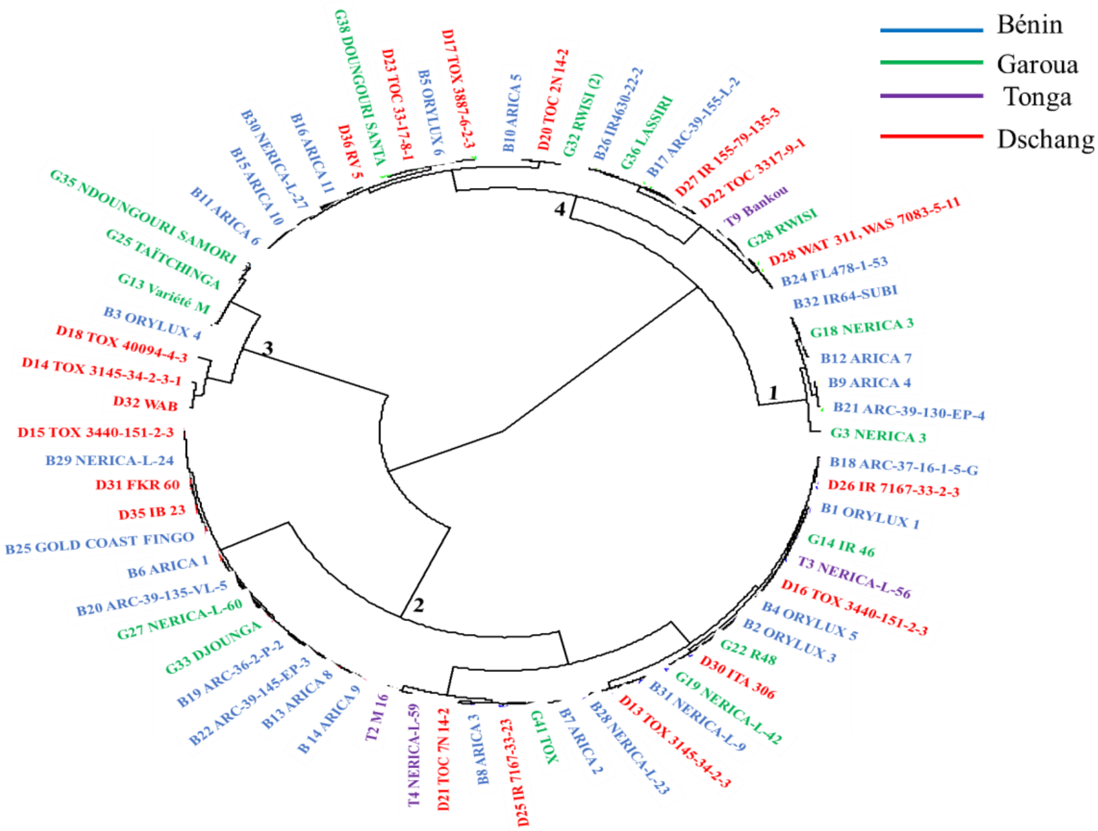

Figure 3. Dendrogram presenting the association between sixty-eight local rice varieties based on agro-morphological parameters.

Table 3. Origin and ecology of the rice varieties selected.

N°	Code	Variety	Origin	Ecology
Traditional varieties				
1	CMRGNd	G35 NDOUNGOURI SAMORI	Garoua	Rain fed upland
2	CMRGDn	G38 DOUNGOURI SANTA	Garoua	Rain fed upland
3	CMRTBa	T6 BANKOU	Tonga	Rain fed upland
4	CMRGTï	G25 TAÏTCHINGA	Garoua	Irrigated lowland
Improved varieties				
5	CMRDTx5	D17 TOX 3887-6-2-3	Dschang	Irrigate lowland
6	CMRDTc3	D22 TOC 3317-9-1	Dschang	Irrigated lowland
7	CMRDWb	D32 WAB	Dschang	Irrigated lowland
8	CMRDTx6	D18 TOX 40094-4-3	Dschang	Irrigated lowland

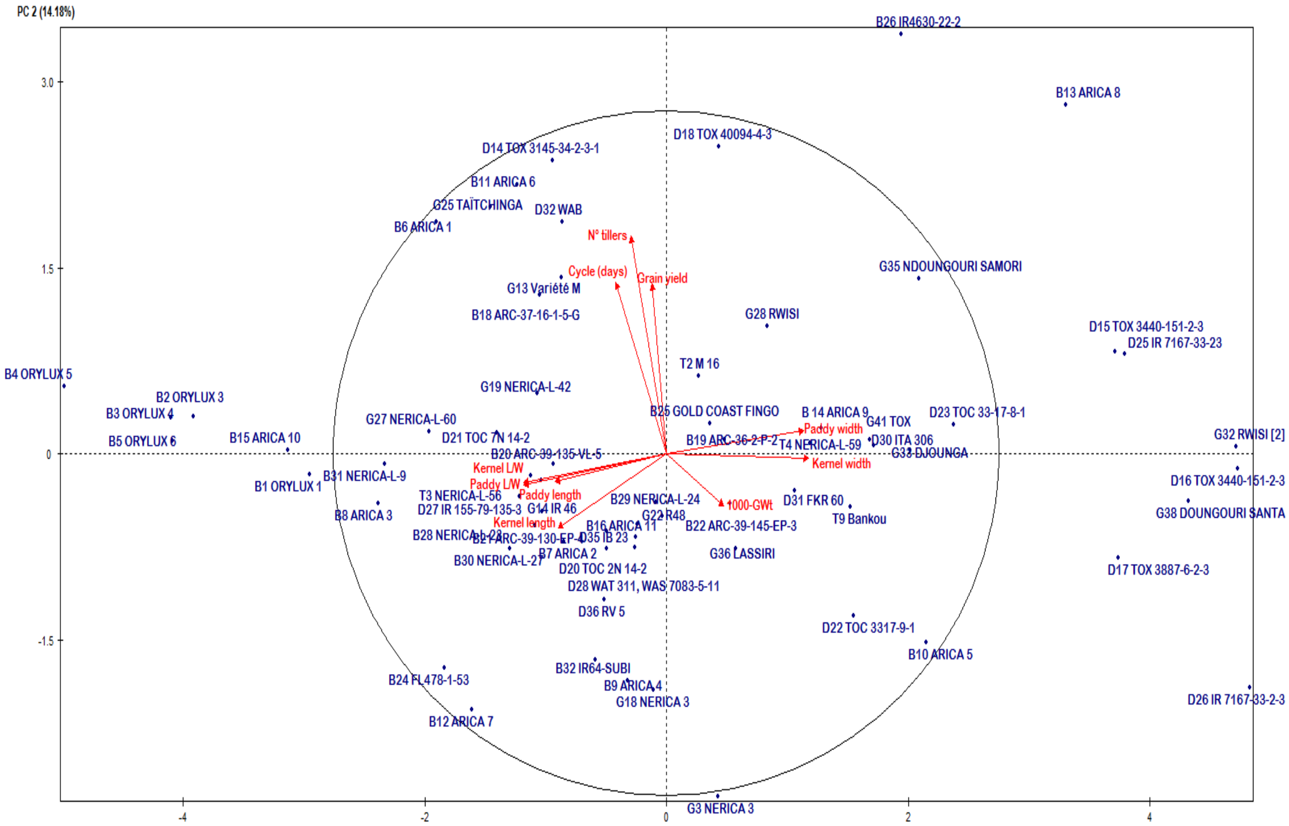

Figure 4. Principal component illustration of 68 local rice cultivars.

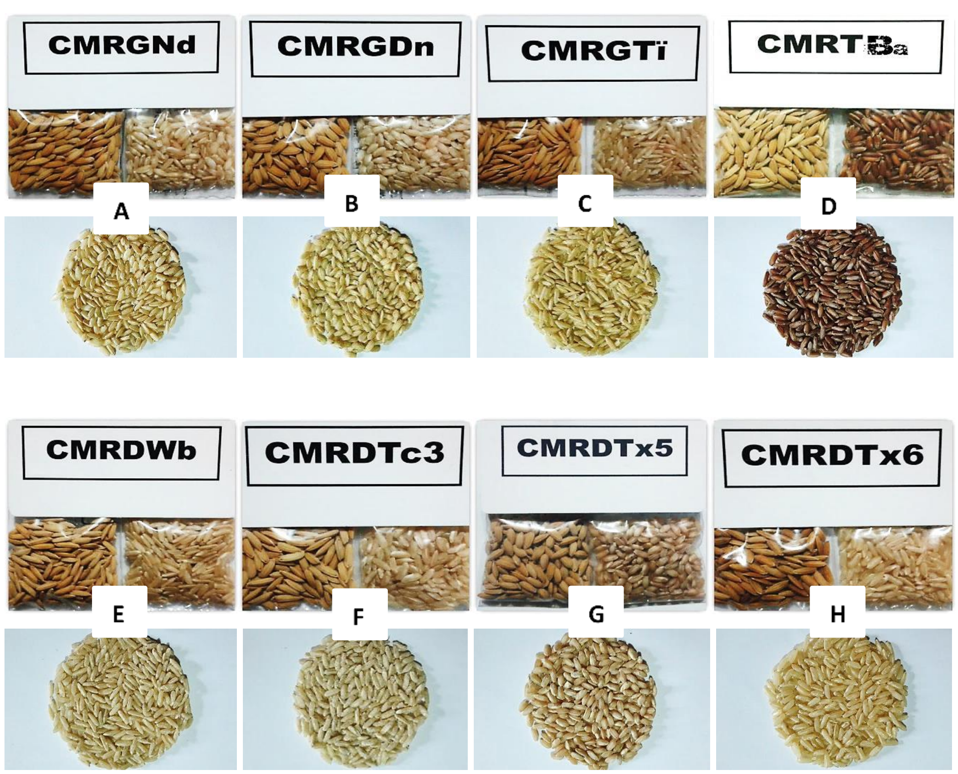

Figure 5. Samples selected for protein analysis.

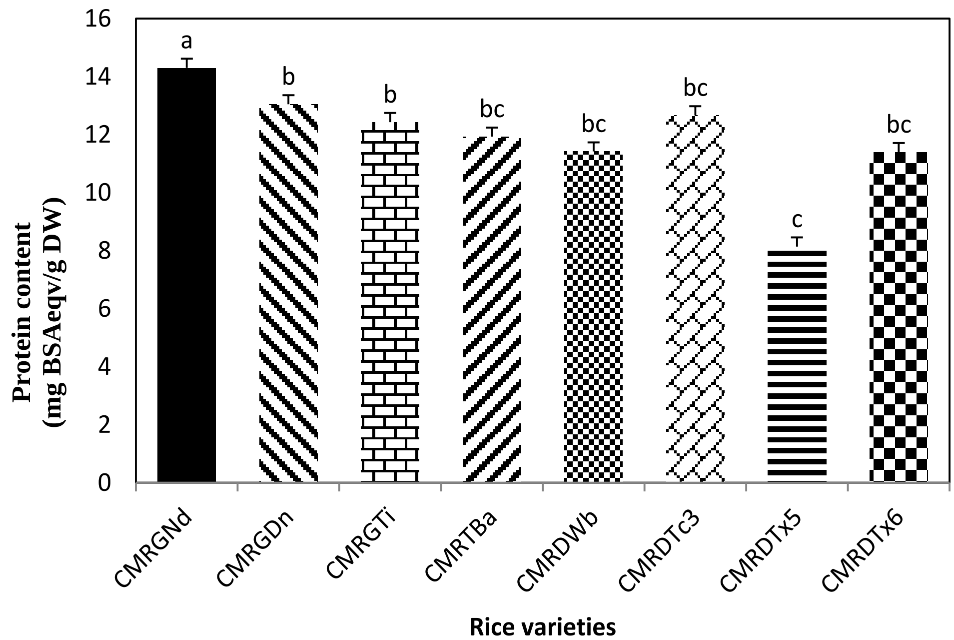

Figure 6. Total protein content of eight local rice cultivars.

Table 4. Glutelin contents (GC) and percentages (G %) of eight local rice varieties.

Variety	CMRGNd	CMRGDn	CMRGTï	CMRTBa	CMRDWb	CMRDTc3	CMRDTx5	CMRDTx6
GC (mg BSAeqv/g DW)	4.43	10.1	7.7	6.4	8.8	8.1	6.5	2.5
G %	30.9	77.7	62.1	53.8	73.3	63.8	80.2	29.1

content was obtained in CMRGNd (14.3%), followed by CMRGDn (13%) which was not significantly different from CMRGTï (12.2%). There was no significant difference between the protein contents of CMRTBa, CMRDWb, CMRDTc3 and CMRDTx6. The lowest protein content was exhibited by CMRDTx5 (8.%) which was the only variety with a protein content that fell within the reported range of 7-10%.

Glutelin extraction and quantification

Glutelin content ranged from 2.5 to 10.1 mgEqvBSA/g DW (Figure 6) and from 29.1 to 80.2% of total proteins (Table 4). Highest glutelin content was exhibited by CMRGDn (10.1 mgEqvBSA/g DW) followed by CMRDWb (8.8 mgEqvBSA/g DW), CMRDTc3 (8.1 mgEqvBSA/g DW) and CMRGTï (7.7 mgEqvBSA/g DW) which were not significantly different from each other. CMRDTx5 and CMRTBa had glutelin contents of 6.5 and 6.4 mgEqvBSA/g DW respectively, followed by CMRGNd (4.43 mgEqvBSA/g DW). Lowest glutelin content was observed in CMRDTx6 (0.25 mgEqvBSA/g DW).

Cluster analysis of the eight varieties using total protein and glutelin contents (Figure 7) presented two main clusters: A and B. cluster A branches into several sub clusters while cluster B branches to two varieties: CMRGNd and CMRDTx6. Cluster A branches at the highest level into cluster C which is linked to the variety CMRDTx5. Cluster C branches into cluster D and CMRGDn and cluster D gives cluster E and CMRTBa. Finally, cluster E gives CMRDWb and cluster F which contains the varieties CMRGTï and CMRDTc3.

Correlation results

A highly significant negative correlation (-0.863**) was observed between cycle duration and grain width, while cycle duration and grain length/width ratio showed a significant highly positive correlation (0.812*). Number of tillers presented a highly significant positive correlation with yield (0.921**), a significant negative correlation with grain width (-0.742*) and a significant positive correlation with grain length/width ratio (0.731*). Yield demonstrated a significant positive correlation with grain length (0.740*)

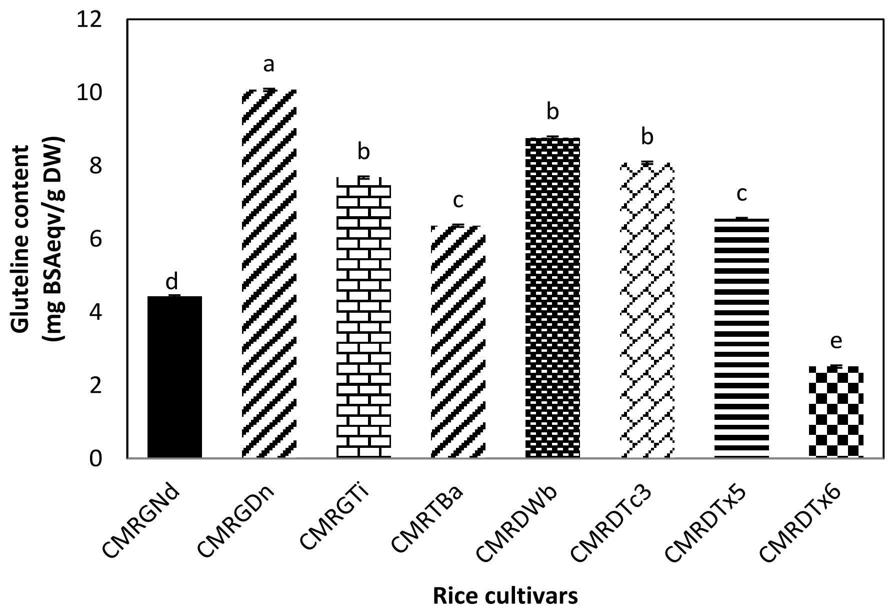

Figure 7. Glutelin content of eight local rice cultivars.

Table 5. Pearson correlation of variables involved in the study**.**

	Cycle	N° of tillers	Yield	Grain length (L)	Grain width (W)	Grain L/W ratio	Protein content	Glutelin content
Cycle	-							
N° of tillers	0.631	-						
Yield	0.552	0.921**	-					
Grain length (L)	0.602	0.593	0.740*	-				
Grain width (W)	-0.863**	-0.742*	-0.744*	-0.872**	-			
Grain L/W ratio	0.812*	0.731*	0.759*	0.927**	-0.989**	-		
Protein content	0.256	0.136	0.219	0.335	-0.117	0.185	-	
Glutelin content	-0.226	-0.344	-0.511	-0.182	0.239	-0.177	0.091	-

**Correlation is significant at the 0.01 level (2-tailed). *correlation is significant at the 0.05 level (2-tailed).

and length/width ratio (0.759*), and a significant negative correlation with grain width (-0.744*). Grain length demonstrated a highly significant negative correlation with grain width and a highly significant positive correlation with grain length/width ratio (-0.872** and 0.927** respectively) (Table 5). Grain width showed a highly significant negative correlation (-0.989**) with grain length/width ration but protein and glutelin content neither had significant correlation with either of the other variables nor with each other. These two put aside, grain width particularly demonstrated negative correlation with all other variables (Figure 8).

DISCUSSION

The size and shape categories distinguished in this study are in accordance with IRRI's Standard Evaluation System (SES) for rice (IRRI, 1996). Except the aroma, fine rice is generally appreciated for its long and slender grains. The predominance of long and slender rice grains (42 and 36 respectively out of 68) within the varieties studied is therefore indicative of the potential of these varieties for breeding programs and processing into fine rice for commercial purposes and represents a great exploitable potential for Cameroonian rice.

Cluster analysis with agro-morphological parameters yielded four clusters, with cultivars from different localities distributed within the same clusters, and cultivars from the same localities distributed in different clusters. This translates the variability that exists between varieties from the same environment and the similarity between varieties from different environments, which indicates that the decisive factors controlling these agro-morphological

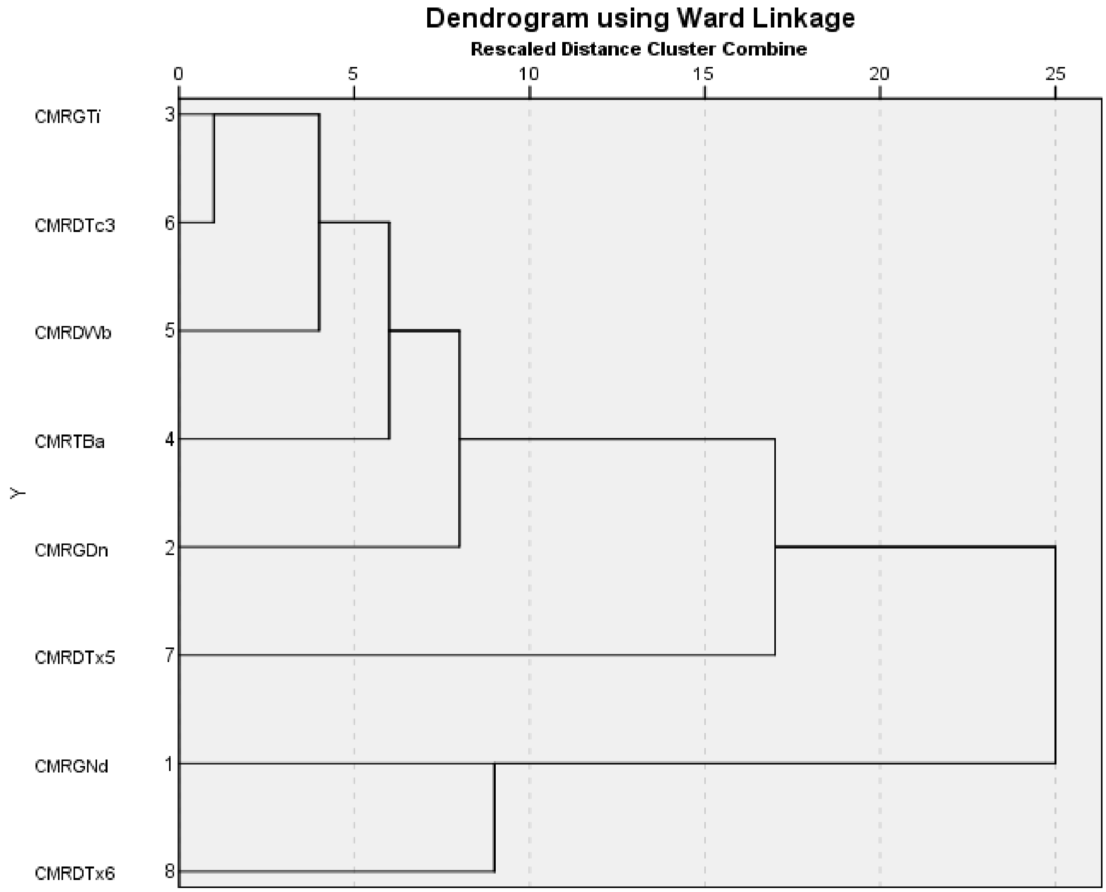

Figure 8. Dendrogram presenting the relationship between eight local rice varieties based on protein and glutelin content.

traits lie in the cropping systems and/or the rice genome. The high yielding cultivars were grouped together in cluster 3 and were mainly cultivars with slender shape, long size grains and high tiller number. Malaa et al. (2017) demonstrated farmers' preference for long grains (generally appreciated on market) and high tiller number of plants, which are associated to high yields. The high yields observed may thus be explained by the long size of the grains and the higher number of plant tillers. These high yields may as well be attributed to the genetic make-up of the cultivars, their environment and cropping conditions. In the same sense, low yields observed in cultivars grouped in cluster 1 may be explained by their low tiller number, genetic constitution and unfavourable environmental and cropping conditions. PCA analysis presented grain dimensions as the major contributors of the total variation, thereby presenting paddy length, width and length/width ratio as well as kernel length, width and length/width ratio as the major determinants of phenotypic diversity. This is in accordance with Rai et al. (2013) results on landraces of aromatic indica rice, which showed that grain length and width among other morphological characters are the major determinants of phenotypic diversity.

The protein contents of the varieties studied (8.1-14.3%) were higher than those reported by Juliano and Villareal (1993); Khush (1997) (7-10%). This might be because of adequate environmental conditions and cropping system. Buresova et al. (2010) reported that water supply, handling, application of fertilizer (soil nitrogen availability), environmental stress (such as salinity and alkalinity, temperatures and diseases), location of growing areas, growing conditions and time tend to increase grain protein content. Hence, these factors might have been adequate for the different cultivars studied. The higher protein content of the traditional varieties as compared to the improved varieties demonstrates their nutritional quality. Guo et al. (2007) reported that rice populations cropped in upland conditions had higher protein contents than those grown in lowland conditions. This explains the higher protein contents observed in CMRGNd and CMRGDn, which are upland varieties. CMRGNd, which showed highest protein content (14.3%), can be exploited to develop rice of better nutritional and technological quality. Rice protein is a major factor in determining texture (e.g. stickiness), pasting capacity, and sensory characteristics of rice. High protein content makes eating texture harder. This implies that CMRGNd, CMRGDn and CMRGTï, which demonstrated high protein content, would be adequate for cooking hard texture rice. In addition, these varieties shall be beneficial to farmers as they would provide increased returns due to less wastage during milling, which is associated to high storage proteins, as was reported by Leesawatwong et al. (2005). CMRTBa had a red pericarp, which is indicative of the presence of phenolic compounds which account for antioxidant property. Bhat and Riar (2017) obtained total protein contents of 7.24-8.85% with pigmented traditional cultivars from India. CMRTBa, which is equally a pigmented and traditional cultivar demonstrated a higher protein content (11.9%) and hence constitutes good genetic resource for breeding medicinal rice or developing a nutraceutical.

Glutelin is the most abundant and most nutritious of the four storage protein types present in rice appeared to be of significant differential content in the varieties selected. The quantities obtained were in accordance with the values reported by Kawakatsu et al. (2008), that is 60-80% of total proteins except for CMRTBa (53.8 %) and CMRDTx6 (29.1 %). CMRGDn, which showed the highest glutelin content (10.1 mgBSAEqv/g DW) is of particular interest for the development of a rice variety with better nutritional quality.

Classification of the eight selected varieties by cluster analysis based on total protein and glutelin content grouped together CMRGNd (traditional) and CMRDTx6 (improved) which are both characterized by high yields, with that of CMRGTx6 slightly higher. However, CMRGNd had higher protein and glutelin contents than CMRGTx6. This classification aligns with the one based on agro-morphology in which both varieties were grouped in the same cluster. CMRGTï (traditional) and CMRDTc3 (improved) were equally grouped in the same cluster; which is explained by their similar high protein and glutelin contents, the protein content of CMRGTï being slightly higher than that of CMRGTc3. Besides, CMRGTï presented a far greater yield than CMRTc3. With these two traditional varieties showing better traits than their improved counterparts, the quality of Cameroon's local and native varieties is clearly unveiled.

Positive correlation observed between cycle duration and grain length/width ratio might imply that slender grains are associated to a long cycle. Likewise, bold grains would be associated to a short cycle. In the same way, positive correlation observed between number of tillers, yield and grain length/width ratio would imply that we should expect high yield and slender grains from a variety that presents numerous tillers and low yield with bold grains from one that presents fewer tillers. Positive correlation observed between yield, grain length and grain length/width ratio may indicate that a variety characterised by long grains should normally be a high yielding one as opposed to one with short grains. A variety with slender grains should equally yield more than a variety with bold grains. Also, positive correlation between grain length and grain length/width ratio may indicate that long grains will generally be slender while short grains would be bold. Negative correlations observed between grain width and cycle; grain width and number of tillers may imply that a short cycle plant will yield short grains and long cycle plants will yield long grains. A variety with numerous tillers will provide long grains and a variety with few tillers will provide short grains.

Conclusion

The major findings suggest that Cameroon possesses high diversity in rice germplasms, most of which are improved varieties though a few native varieties still exist. The predominance of long, slender kernels and the high protein and glutelin contents of the varieties studied indicate the good quality of Cameroon's locally cultivated rice, especially those thought to be traditional. However, there is need to investigate the genetic make-up of these four thought traditional cultivars and confirm or not their nativity as well as provide relevant information for breeding programs.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Application technology of imidacloprid in wheat: Effects on *Schizaphis graminum* management and natural enemies

William A. Machado[1†], Stephan M. Carvalho[2], João Paulo A. R. da Cunha[1], Sérgio M. Silva[3] and Ernane M. Lemes[1]*

[1]Agrarian Science Institute (ICIAG), Federal University of Uberlândia (UFU), Gloria Farm, BR050, Uberlândia, Brazil.
[2]Entomology Department (DEN), Federal University of Lavras (UFLA), zip code: 3037, Lavras, Brazil.
[3]Agrarian Science Institute (ICA), Federal University of Jequitinhonha and Mucuri Valleys (UFVJM), St. Vereador João Narciso, n. 1380, Unaí, Brazil.

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Wheat production is dependent on costly insecticides that generate social and environmental issues. The growing demand for a rate reduction of spray applications and increased spray efficiency make essential the research for more adequate crop management. Therefore, this study aimed to evaluate technologies of imidacloprid application on wheat aphid, *Schizaphis graminum* and its natural enemies, *Chrysoperla externa* and *Orius insidiosus*. A set of experiments were performed to evaluate the effectiveness of *S. graminum* control and the toxicity to natural enemies using imidacloprid plus adjuvants sprayed with different nozzles and application rates. Improved *S. graminum* control was observed in the association between the adjuvant lauryl ether sodium sulfate and the hollow cone nozzle for both application rates (75 and 150 L ha^{-1}), and the adjuvant copolymer of polyester + silicone + d-limonene with the asymmetric twin flat-fan nozzle at a low rate (75 L ha^{-1}), since these treatments eliminate the wheat aphid after the fifth day of treatment application. The reduction of spray volume to wheat aphid control is possible with no loss of insecticide effectiveness. Imidacloprid associated with any one of the adjuvants tested is harmful to *C. externa* and *O. insidiosus*, even at the fifth day after application the insecticide continues being harmful to the natural enemies, regardless of the technology used.

Key words: *Triticum aestivum*, *Schizaphis graminum*, ecological disturbance, insecticide impacts, natural control, neonicotinoid.

INTRODUCTION

The productivity of wheat, *Triticum aestivum* L. is strongly influenced by the attack of insect pests. Among them, the aphids (Hemiptera: Aphididae) are an important pest problem to the cultivation of winter cereals (Salvadori and

*Corresponding author. E-mail: ernanefito@gmail.com.

†In memoriam

Salles, 2002). The ability of these insects to cause damage is high due to the elevated biotic potential and toxicity of the substances injected into the plant tissue during the feeding process. In intense aphid attacks during the emergency of the wheat tillering, in general, causes plant death (Gassen, 1984; Salvadori and Tonet, 2001).

The chemical control is predominantly used in the management of aphids as the green aphid of cereals, or wheat aphid (*Schizaphis graminum* Rondani, 1852), which can cause up to 50% of direct (sap suction) and indirect (fungal growth on honeydew) yield losses (Kindler et al., 2002; Van-Emden and Harrington, 2007; Royer et al., 2015). This aphid is widely distributed in the wheat production regions of the world (Blackman and Eastop, 2000). Thus, wheat production is very dependent on the use of insecticides, which increases production costs and generates several social and environmental problems (Salvadori, 1999). Chemical control of this insect pest is recommended when 10% of plants are infested during the vegetative phase or when it exceeds ten aphids per spike, until the mass phase of the wheat grain (Silva et al., 1996).

According to Mohammed et al. (2018), the neonicotinoid insecticide imidacloprid [1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine] is still effective in suppressing cereal aphid populations in wheat fields throughout the growing season; however, the negative impact of this insecticide on the biological control services and pollinators needs to be considered with caution (Whitehorn et al., 2012; Dicks, 2013; Gibbons et al., 2015; Kang et al., 2018; Lämsä et al., 2018). Although imidacloprid has been banned in several regions (Valavanidis, 2018; Fijian Government, 2019), it has still been largely used in many countries, highlighting the importance of the studies for more appropriate managements and technologies of this insecticide.

For many years of the modern age, little attention was devoted to the sustainability of agriculture, but concerning is rising (Singh and Singh, 2017; Zhang et al., 2017). The growing demands by eco-friendly farming press to decrease the application rates, to reduce the costs and to increase the efficiency of the activities. These demands turn essential the knowledge of the application of crop inputs and parameters that ensure the products will reach the target efficiently, minimizing losses and leading to more sustainable agriculture (Matuo et al., 1989; Ali et al., 2018).

The determination of the most appropriated parameters, such as the application rate and the size of the droplets, is directly related to the quality of the spray application (Matthews et al., 2014). The definition of the spray application rate depends mainly on the type of the target to be reached, the required coverage, the way the pesticide acts and the application technique. The application rate also influences the operational efficiency of the application process because the time used to sprayer reload activities significantly changes the operational capacity of the sprayers (Antuniassi, 2014).

However, the reduction of the application rate requires an enhancement of the application technology regularly used in the field. Thus, there is an increasing need for studies that allow the use of efficient nozzles and appropriate rates (Silva, 1999a). Additionally, beyond the operational gains, it is important to study the possible impacts generated by new technology and procedures on the population of beneficial insects as the natural enemies (predators) of wheat pests. Therefore, the objectives of this study were to evaluate different technologies for applying the imidacloprid insecticide on the wheat crop concerning the chemical control of *S. graminum* and the impacts of these application technologies on natural enemies present in the area.

MATERIALS AND METHODS

Site study

The experiment was conducted in duplicate, Field-1 and Field-2, installed simultaneously; both had irrigation systems via a central pivot and were installed in areas of wheat seed production (wheat cultivar BRS 404). The fields were distant 500 m from each other and located in Maringá Farm, Araguari County, Minas Gerais State, Brazil (18°34'00.23''S; 48°13'03.9''W).

Sowing of wheat crop and cultural practices

The wheat sowing was done in the no-tillage system in April 2016 in Field-1 and Field-2, respectively. The spacing used was 17 cm between rows, with a density of 350 plants m^{-2}. The cropping management followed the indications of the 'Recommendations of the South-Brazilian Commission of Wheat Research' (Recomendações da Comissão Sul-Brasileira de Pesquisa de Trigo, 2000) regarding fertilization, weed control and disease management.

Use of spray nozzles

The spray nozzles (MagnoJet, Brazil) for insecticide treatments application were: (1) MGA 015, hollow cone jet, with 90° angulation and 0.56 L min^{-1} nominal flow; (2) MGA 03, hollow cone jet, with 90° angulation and 1.13 L min^{-1} nominal flow; and (3) AS 7030, asymmetric twin flat fan, with 110° angulation and 0.75 L min^{-1} nominal flow.

Use of adjuvants

The adjuvants used were: lauryl ether sodium sulfate (LESS) - Mirus 400Si® (Superagro, Brazil), concentration of 0.6 mL L^{-1}, characteristics: anti-evaporation, acidifier, anti-drift and adjuvant anti-foaming (adjuvant 01); and copolymer of polyester, silicone and d-limonene (CPSDL) - Orlist 900Li® (Superagro, Brazil), concentration of 1.33 mL L^{-1}, characteristics: spreader, moisturizing, compatibilizer and penetrating adjuvant (adjuvant 02). The spray solutions were prepared in the sprayer tank, adding first the adjuvants in the concentration recommended by the manufacturer, together with the imidacloprid insecticide - 200 g ha^{-1} of the active ingredient.

Treatments and conditions of application

Both experiments were carried out in the strip-plot scheme, with 8 treatments: (1) control (no product application); (2) nozzle AS 7030, 75 L ha^{-1}, no adjuvant (T.1); (3) nozzle MGA 015, 75 L ha^{-1}, no adjuvant (T.2); (4) nozzle MGA 015, 75 L ha^{-1} + adjuvant 01 (T.3); (5) nozzle AS 7030, 75 L ha^{-1} + adjuvant 01 (T.4); (6) nozzle AS 7030, 75 L ha^{-1} + adjuvant 02 (T.5); (7) nozzle MGA 030, 150 L ha^{-1}, no adjuvant (T.6); and, (8) nozzle MGA 03, 150 L ha^{-1} + adjuvant 01 (T.7), each were repeated thrice. The plots had 740 m long and 27 m wide. The usable area assessed corresponded to 1,800 m^2, discarding 10 m at the beginning and the end of the plot and 1 m on each side.

The spray applications occurred when *S. graminum* reached 10% of plant infestation, according to the 'Recommendations of the South-Brazilian Commission of Wheat Research' (Recomendações da Comissão Sul-Brasileira de Pesquisa de Trigo, 2000) and to Cunha et al. (2016). A self-propelled sprayer (Case® Patriot 250 model) was used with 27 m length and nozzles were spaced by 0.5 m. The working speed established was 16 km h^{-1} (4.44 m s^{-1}) for all treatments.

The environmental conditions during the applications were monitored using a thermo-higro-digital anemometer (SKTHAL-01, Skill-Tec, Brazil). During the applications in Field-1, the weather conditions presented a minimum temperature of 25.4°C and a maximum of 29.5°C, relative humidity between 48 and 51%, and wind speeds between 5.8 and 7.9 km h^{-1}. In Field-2, the weather conditions presented a minimum temperature of 30.7°C and a maximum of 32.4°C, relative humidity between 43 and 45%, and wind speeds between 0.8 and 5.2 km h^{-1}.

Insect evaluations

The evaluation of the effectiveness of the spray treatments in the control of *S. graminum* was performed by counting (surveys) of the alive adult insects, before and after each application, using a clashing cloth with 1 m wide by 1 m length. Similarly, the population of *Chrysoperla externa* (Hagen, 1861) and *Orius insidiosus* (Say, 1832) (two important natural enemies of aphids that belong to the orders Neuroptera and Hemiptera, respectively) was evaluated (Parra et al., 2002).

A prior evaluation was performed on the same day of the application of the treatments and, subsequently, other surveys were carried out at 3, 5, 7 and 10 days after the application of the treatments. The percentage of effectiveness of the treatments was calculated by the formula of Henderson and Tilton (1955):

$$E = 1 - \left[\left(\frac{T_b}{T_a} \times \frac{t_a}{t_b}\right)\right] \times 100$$

where E: Efficacy (%); Tb: number of live insects in the control treatment before application; Ta: number of live insects in the control treatment after application; ta: number of live insects in the treatment after application; tb: number of live insects in the treatment before application. The values obtained during the evaluations, after conversion to the percentage of effectiveness, were classified as low efficacy (lower than 80%), good efficacy (from 80 to 90%), and high efficacy (greater than 90%).

The evaluations of the effects of different application technologies on the population density of natural enemies were carried out with the treatments classified in classes of toxicity. According to the International Organization for Biological and Integrated Control (IOBC) (Hassan and Degrande, 1996; Veire et al., 2002), the classes are: class 1- innocuous (<30%), class 2- slightly harmful (30≤T≤80%), class 3- moderately harmful (80≤T≤99%), and class 4- harmful (>99% of mortality); these classes are a function of the average number of adults found in the areas after 3, 5, 7 and 10 days of the spray treatment application. The percentage of toxicity (T) was calculated by the formula proposed by Henderson and Tilton (1955).

Statistical analysis

The results were submitted for the analyses of normality of residue distribution by the test of Kolmogorov-Smirnov and to the homogeneity of variances by the test of Levene (both at 0.01 significance level) before the analysis of variance (0.05 significance level). Student's t-test differentiated the averages of the treatments for each day of the evaluation for independent samples (0.05 significance level).

RESULTS AND DISCUSSION

Evaluation of the control of wheat aphid

The association between the adjuvant 01 and the hollow cone nozzle, for both application rates, and the adjuvant 02 with the asymmetric twin flat fan nozzle (75 L ha^{-1}) improved efficiency in the control of the wheat aphid (Table 1).

The improvement of the control of the *S. graminum* in these treatments can be explained, mainly by the use of adjuvants, which can modify the spray properties and improve the quality of the droplets increasing the spray spread and adhesion on the leaf surface (Kissmann, 1998; Hilz and Vermeer, 2013; Preftakes et al., 2019). Such characteristics may accelerate the absorption of the phytosanitary product, reducing its exposure to degradation/loss factors, such as solar radiation, temperature, and rain. Those characteristics help achieved good efficiency of the insecticide even after five days of spraying, where good control performance on the *S. graminum* was still observed (Cunha and Alves, 2009; Preftakes et al., 2019).

Table 2 shows the effectiveness of control of *S. graminum* at 3, 5, 7 and 10 days after the application of the insecticide. At 3 days after spray application, it could be noted that there was a difference in the *S. graminum* population among the nozzle, adjuvants and application rates. According to the classification proposed by Henderson and Tilton (1955), the treatments that showed high efficiency (> 90%) during the evaluation period were those that contained adjuvants, with emphasis to the associations between the adjuvant 01 and the hollow cone nozzle, both application rates (75 and 150 L ha^{-1}) and the adjuvant 02 with the asymmetric twin flat fan nozzle, at the rate of 75 L ha^{-1}.

Therefore, better control of the *S. graminum* was observed in the associations between the adjuvant LESS and the hollow cone nozzle for both application rates (75 and 150 L ha^{-1}), and the adjuvant CPSDL with the asymmetric twin flat fan nozzle for 75 L ha^{-1} indicating that the reduction of spray volume in the imidacloprid application to *S. graminum* control is possible with no

Table 1. Effect of application technology on the population density of *Schizaphis graminum* at 3, 5, 7 and 10 days after application (DAA) of imidacloprid, associated or not with adjuvants and types of the nozzle in two wheat fields, in the 2015/2016 harvest season.

Treatment	Average number of adults[1]									
	Before application		3 DAA		5 DAA		7 DAA		10 DAA	
	Fd-1	Fd-2	Fd-1	Fd-2	Fd-1	Fd-2	Fd-1	Fd-2	Fd-1	Fd-2
T.1	5.2 ± 1.9^{a}	6.2 ± 4.1^{a}	2.4 ± 0.9^{a}	2.6 ± 1.1^{b}	1.4 ± 0.5^{b}	1.2 ± 1.3^{b}	0.6 ± 0.9^{b}	0.4 ± 0.9^{b}	1.8 ± 0.8^{b}	2.2 ± 0.8^{b}
T.2	5.0 ± 3.5^{a}	6.0 ± 4.1^{a}	2.2 ± 1.9^{a}	2.0 ± 2.1^{b}	1.2 ± 1.1^{b}	1.2 ± 1.3^{b}	0.4 ± 0.9^{b}	0^{c}	1.8 ± 0.8^{b}	2.0 ± 0.7^{b}
T.3	5.8 ± 4.3^{a}	6.8 ± 2.2^{a}	1.6 ± 1.5^{b}	0.6 ± 0.9^{c}	0.6 ± 0.9^{b}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}
T.4	5.4 ± 4.4^{a}	6.4 ± 3.9^{a}	2.0 ± 2.1^{a}	2.0 ± 1.0^{b}	1.0 ± 1.0^{b}	0.6 ± 0.9^{b}	0.4 ± 2.2^{b}	0^{c}	1.4 ± 1.3^{b}	1.2 ± 0.8^{b}
T.5	6.0 ± 2.8^{a}	7.0 ± 3.6^{a}	1.8 ± 0.8^{b}	0.6 ± 0.9^{c}	0.8 ± 0.4^{b}	0.2 ± 0.4^{b}	0^{c}	0^{c}	0^{c}	0^{c}
T.6	5.6 ± 2.1^{a}	6.6 ± 6.0^{a}	1.8 ± 1.5^{b}	1.4 ± 1.9^{b}	0.8 ± 0.4^{b}	0.6 ± 0.5^{b}	0.2 ± 0.4^{b}	0.2 ± 0.4^{b}	1.6 ± 1.1^{b}	1.4 ± 0.5^{b}
T.7	6.2 ± 4.5^{a}	7.2 ± 5.4^{a}	1.8 ± 1.3^{b}	0.6 ± 0.9^{c}	0.6 ± 0.9^{b}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}
Control	4.8 ± 2.2^{1a2}	5.8 ± 5.0^{a}	5.0 ± 2.2^{a}	7.4 ± 2.3^{a}	6.0 ± 1.9^{a}	8.2 ± 3.0^{a}	8.2 ± 2.5^{a}	7.2 ± 1.6^{a}	9.2 ± 1.5^{a}	10.2 ± 1.6^{a}

[1]Averages followed by standard deviation. [2]Averages followed by different letters, in column, differ among treatments by Student's t test ($p < 0.05$). T.1 = nozzle AS 7030, 75 L ha^{-1}, no adjuvant; T.2 = nozzle MGA 015, 75 L ha^{-1}, no adjuvant; T.3 = nozzle MGA 015, 75 L ha^{-1}, adjuvant 01; T.4 = nozzle AS 7030, 75 L ha^{-1}, adjuvant 01; T.5 = nozzle AS 7030, 75 L ha^{-1}, adjuvant 02; T.6 = nozzle MGA 030, 150 L ha^{-1}, no adjuvant; T.7 = nozzle MGA 03, 150 L ha^{-1}, adjuvant 01. Adjuvant 01: lauryl ether sodium sulfate (LESS). Adjuvant 02: copolymer of polyester, silicone and d-limonene (CPSDL). Fd-1 = Field-1; Fd-2 = Field-2.

Table 2. Efficacy of control of *Schizaphis graminum* at 3, 5, 7 and 10 days after application (DAA) of imidacloprid, associated or not with adjuvants and types of the nozzle in two wheat fields, in the 2015/2016 harvest season.

Treatment	Percentage of control[1]							
	3 DAA		5 DAA		7 DAA		10 DAA	
	Fd-1	Fd-2	Fd-1	Fd-2	Fd-1	Fd-2	Fd-1	Fd-2
T.1	55.69	67.13	78.46	86.31	93.25	94.80	81.94	79.82
T.2	57.76	73.87	80.80	85.85	95.32	100	81.22	81.05
T.3	73.52	93.08	91.72	100	100	100	100	100
T.4	64.44	75.51	85.18	93.36	97.83	100	86.47	89.34
T.5	71.20	93.28	89.33	97.97	100	100	100	100
T.6	69.14	83.37	88.57	93.57	95.82	97.56	85.09	87.94
T.7	72.13	93.47	92.25	100	100	100	100	100

[1]Low efficacy = lower than 80%; good efficacy = from 80% to 90%; high efficacy = higher than 90%. T.1 = nozzle AS 7030, 75 L ha^{-1}, no adjuvant; T.2 = nozzle MGA 015, 75 L ha^{-1}, no adjuvant; T.3 = nozzle MGA 015, 75 L ha^{-1}, adjuvant 01; T.4 = nozzle AS 7030, 75 L ha^{-1}, adjuvant 01; T.5 = nozzle AS 7030, 75 L ha^{-1}, adjuvant 02; T.6 = nozzle MGA 030, 150 L ha^{-1}, no adjuvant; T.7 = nozzle MGA 03, 150 L ha^{-1}, adjuvant 01. Adjuvant 01: lauryl ether sodium sulfate (LESS). Adjuvant 02: copolymer of polyester, silicone and d-limonene (CPSDL). Fd-1 = Field-1; Fd-2 = Field-2.

loss of effectiveness, but the presence of the adjuvant is important to assist this control.

Silva (1999b) studied the efficiency of insecticides without adjuvants on *S. graminum* in corn after applying with spray volumes of 150 or 300 L ha^{-1}, concluded that the largest volume was the most indicated for the efficient control of this pest. The author explains that, when using a hollow cone nozzle, the insecticides are less efficient than the applications with a flat fan nozzle. It is possible that these differences, in the case of the maize crop, are related to the great distance between the point of jet projection and the target, and that fine droplets (hollow cone) are more sensitive to change the trajectory.

Similarly, Cunha et al. (2006) attest that fine droplets are ideal, because, for the same spray volume, droplets of smaller diameter are better distributed on the leaf surface, and provide better coverage of the target and greater penetration into the plant canopy improving the control efficiency. However, as mentioned, very fine droplets are more prone to drift or evaporate and very coarse droplets have problems due to their tendency of outflow and less canopy coverage.

In most cases, there was no difference between the spray volumes tested, analyzing each nozzle and adjuvant in particular. This lack of differences indicates that it is possible to reduce the spray volume of the application without loss of efficiency of the imidacloprid insecticide. It is also important to highlight that the use of

Table 3. Toxicity of spray treatments on *Chrysoperla externa* at 3, 5, 7 and 10 days after application (DAA) of imidacloprid, associated or not with adjuvants and types of the nozzle in two wheat fields, in the 2015/2016 harvest season.

Treatment	Percentage of toxicity[1]								Class[2]
	3 DAA		5 DAA		7 DAA		10 DAA		
	Fd-1	Fd-2	Fd-1	Fd-2	Fd-1	Fd-2	Fd-1	Fd-2	
T.1	62.5	76.2	100	100	100	100	100	100	4
T.2	25.0	64.3	100	100	100	100	100	100	4
T.3	100	100	100	100	100	100	100	100	4
T.4	100	100	100	100	100	100	100	100	4
T.5	100	100	100	100	100	100	100	100	4
T.6	62.5	64.3	100	100	100	100	100	100	4
T.7	100	100	100	100	100	100	100	100	4

[1]Low efficacy = lower than 80%; good efficacy = from 80% to 90%; high efficacy = higher than 90%. [2]Class of toxicity from the fifth day after application of the spray treatments: class 1 = innocuous (T<30%), class 2 = slightly harmful (30%≤T≤80%), class 3 = moderately harmful (80%≤T≤99%) and class 4 = harmful (T>99%) (VEIRE et al., 2002). T.1 = nozzle AS 7030, 75 L ha^{-1}, no adjuvant; T.2 = nozzle MGA 015, 75 L ha^{-1}, no adjuvant; T.3 = nozzle MGA 015, 75 L ha^{-1}, adjuvant 01; T.4 = nozzle AS 7030, 75 L ha^{-1}, adjuvant 01; T.5 = nozzle AS 7030, 75 L ha^{-1}, adjuvant 02; T.6 = nozzle MGA 030, 150 L ha^{-1}, no adjuvant; T.7 = nozzle MGA 03, 150 L ha^{-1}, adjuvant 01. Adjuvant 01: lauryl ether sodium sulfate (LESS). Adjuvant 02: copolymer of polyester, silicone and d-limonene (CPSDL). Fd-1 = Field-1; Fd-2 = Field-2.

adjuvants improved the quality of the application and maintain a good level of control, even with spray volume reduction (Preftakes et al., 2019).

These results corroborate with those found by Ferrari et al. (2014), who studied the insecticide to control bedbugs on soybean. They observed that the use of adjuvant resulted in the maintenance of the efficiency of control in a terrestrial application with a reduced rate (50 L ha^{-1}). To proceed with the reduction in the spray volume, it is very important to make the correct adjustment of the pressure and choice the nozzle that ensure good deposition on plant leaves, as well as consider environmental factors to reduce losses by drift (action of the wind) or drop extinction (evaporation) due to wrong height of the application boom, to low humidity and/or to high air temperature.

Toxic effects on natural enemies

The presence of two natural enemies, *C. externa* and *O. insidiosus*, was observed before the application of the treatments, and the percentage of toxicity was determined with the observation of natural enemies after the application of the treatments.

C. externa

The treatments with the presence of adjuvants, soon after 3 days from application, showed high toxicity to *C. externa*. In this way, according to the classification of the IOBC, the insecticide imidacloprid proved to be harmful to this species even 5 days after the insecticide application (Table 3), independent of the application technology employed, which compromised the ability of predation of the wheat aphid. The quantities of *C. externa* found in all treatments are presented in Table 4.

In a similar study, it was demonstrated that some neonicotinoids may have a greater capacity to penetrate the cuticle of insects, therefore being more toxic (Tomizawa and Casida, 2005). This fact can affect the development of organisms, as well as compromising the reproduction of subsequent generations, which can be considered as a sublethal effect. Carvalho et al. (2010) obtained results that corroborate those presented in this study, observing differences between the treatments in relation to the survival probability of *C. externa* where imidacloprid resulted in the survival of only 22.7%, which suggests high toxicity of this product to adults of this species. Similar results were obtained by Bueno and Freitas (2003), which found that imidacloprid caused 100% mortality of the first instar larvae of *C. externa*.

Rocha (2008) studied the selectivity of imidacloprid (0.7 g L^{-1} of a.i.) and thiamethoxam (0.5 g L^{-1} of a.i.) at different stages of the biological cycle of *C. externa*, collected in coffee plantation, reported that these compounds caused 100% mortality of larvae of second instar nymphs and adults, making it impossible to carry out assessments of the fertility and viability of eggs. The author also noted that thiamethoxam, imidacloprid, mineral oil, endosulfan, and dimethoate, when applied directly on pupae of *C. externa*, did not affect its cycle, with an average duration of this stage varying from 10.3 to 10.9 days.

The effect on the mortality of predators observed in treatments with imidacloprid may be related to the neurotoxic action, even in low doses (Ware and Whitacre, 2004; Lämsä et al., 2018). These compounds can affect various groups of insects, including natural enemies,

Table 4. Effect of application technology on the population density of *Chrysoperla externa* at 3, 5, 7 and 10 days after application (DAA) of imidacloprid, associated or not with adjuvants and types of the nozzle in two wheat fields, in the 2015/2016 harvest season.

Treatment	Average number of adults[1]									
	Before application		3 DAA		5 DAA		7 DAA		10 DAA	
	Fd-1	Fd-2	Fd-1	Fd-2	Fd-1	Fd-2	Fd-1	Fd-2	Fd-1	Fd-2
T.1	0.4±0.5[a]	0.6±0.9[a]	0.2±0.4[a]	0.2±0.4[a]	0[b]	0[b]	0[b]	0[b]	0[b]	0[b]
T.2	0.2±0.4[a]	0.4±0.5[a]	0.2±0.4[b]	0.2±0.4[a]	0[b]	0[b]	0[b]	0[b]	0[b]	0[b]
T.3	0.4±0.5[a]	0.4±0.5[a]	0[c]	0[b]	0[b]	0[b]	0[b]	0[b]	0[b]	0[b]
T.4	0.4±0.5[a]	0.2±0.4[a]	0[c]	0[b]	0[b]	0[b]	0[b]	0[b]	0[b]	0[b]
T.5	0.2±0.4[a]	0.6±0.9[a]	0[c]	0[b]	0[b]	0[b]	0[b]	0[b]	0[b]	0[b]
T.6	0.4±0.5[a]	0.4±0.5[a]	0.2±0.4[b]	0.2±0.4[a]	0[b]	0[b]	0[b]	0[b]	0[b]	0[b]
T.7	0.4±0.5[a]	0.6±0.9[a]	0[c]	0[b]	0[b]	0[b]	0[b]	0[b]	0[b]	0[b]
Control	0.6±0.5[1a2]	1.0±0.7[a]	0.8±1.1[a]	1.4±0.5[a]	1.6±1.8[a]	2.2±1.3[a]	2.0±1.9[a]	3.0±0.7[a]	2.8±1.6[a]	3.6±0.5[a]

[1]Averages followed by standard deviation. [2]Averages followed by different letters, in column, differ among treatments by Student's t test ($p < 0.05$). T.1 = nozzle AS 7030, 75 L ha^{-1}, no adjuvant; T.2 = nozzle MGA 015, 75 L ha^{-1}, no adjuvant; T.3 = nozzle MGA 015, 75 L ha^{-1}, adjuvant 01; T.4 = nozzle AS 7030, 75 L ha^{-1}, adjuvant 01; T.5 = nozzle AS 7030, 75 L ha^{-1}, adjuvant 02; T.6 = nozzle MGA 030, 150 L ha^{-1}, no adjuvant; T.7 = nozzle MGA 03, 150 L ha^{-1}, adjuvant 01. Adjuvant 01: lauryl ether sodium sulfate (LESS). Adjuvant 02: copolymer of polyester, silicone and d-limonene (CPSDL). Fd-1 = Field-1; Fd-2 = Field-2.

Table 5. Toxicity of spray treatments on *Orius insidiosus* at 3, 5, 7 and 10 days after application (DAA) of imidacloprid, associated or not with adjuvants and types of nozzle in two wheat fields, in the 2015/2016 harvest season.

Treatment	Percentage of toxicity - (T%)[1]								Class[2]
	3 DAA		5 DAA		7 DAA		10 DAA		
	Fd-1	Fd-2	Fd-1	Fd-2	Fd-1	Fd-2	Fd-1	Fd-2	
T.1	50.0	40.00	100	100	100	100	100	100	4
T.2	66.7	40.00	100	100	100	100	100	100	4
T.3	100	100	100	100	100	100	100	100	4
T.4	100	100	100	100	100	100	100	100	4
T.5	100	100	100	100	100	100	100	100	4
T.6	50.0	70.00	100	100	100	100	100	100	4
T.7	100	100	100	100	100	100	100	100	4

[1]Low efficacy = lower than 80%; good efficacy = from 80% to 90%; high efficacy = higher than 90%. [2]Class of toxicity from the fifth day after application of the spray treatments: class 1 = innocuous (T<30%), class 2 = slightly harmful (30%≤T≤80%), class 3 = moderately harmful (80%≤T≤99%) and class 4 = harmful (T>99%) (VEIRE et al., 2002). T.1 = nozzle AS 7030, 75 L ha^{-1}, no adjuvant; T.2 = nozzle MGA 015, 75 L ha^{-1}, no adjuvant; T.3 = nozzle MGA 015, 75 L ha^{-1}, adjuvant 01; T.4 = nozzle AS 7030, 75 L ha^{-1}, adjuvant 01; T.5 = nozzle AS 7030, 75 L ha^{-1}, adjuvant 02; T.6 = nozzle MGA 030, 150 L ha^{-1}, no adjuvant; T.7 = nozzle MGA 03, 150 L ha^{-1}, adjuvant 01. Adjuvant 01: lauryl ether sodium sulfate (LESS). Adjuvant 02: copolymer of polyester, silicone and d-limonene (CPSDL). Fd-1 = Field-1; Fd-2 = Field-2.

significantly reducing insect survival (Torres and Ruberson, 2004; Wood and Goulson, 2017).

O. insidiosus

The data presented in Tables 5 and 6 relate to the toxicity and survival of *O. insidiosus*, and resemble those found for *C. externa*. Again, the treatments with the presence of adjuvants, soon after 3 days from application, showed high toxicity to *O. insidiosus*. The insecticide imidacloprid demonstrated, once again, to be toxic to natural enemies studied even 5 days after the insecticide application, regardless of the technology used, impairing their ability to predation of aphids. The quantities of *O. insidiosus* found in all treatments are presented in Table 6.

These results confirm those obtained by other researchers with other species of the genus *Orius*. Delbeke et al. (1997) verified under laboratory conditions, that the 5th instar nymphs of *Orius laevigatus* (Fieber, 1860) (Hemiptera: Anthocoridae), in contact with residues of the insecticide imidacloprid (0.04 mg a.i. L^{-1}) presented 50% of mortality. The high toxicity of insecticides was also observed by Morais et al. (2003), who reported that abamectin, fenpropathrin, and imidacloprid did not allow the survival of adults of *O. insidiosus*. On the other hand, cartap and cyromazine did not affect insect survival significantly. Although such products have been classified

Table 6. Effect of application technology on the population density of *Orius insidiosus* at 3, 5, 7 and 10 days after application (DAA) of imidacloprid, associated or not with adjuvants and types of the nozzle in two wheat fields, in the 2015/16 harvest season.

Treatment	Average number of adults[1]									
	Before application		03 DAA		05 DAA		07 DAA		10 DAA	
	Fd-1	Fd-2	Fd-1	Fd-2	Fd-1	Fd-2	Fd-1	Fd-2	Fd-1	Fd-2
T.1	0.2 ± 0.4^{a}	0.2 ± 0.4^{a}	0.2 ± 0.4^{b}	0.2 ± 0.4^{a}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}
T.2	0.6 ± 1.3^{a}	0.2 ± 0.4^{a}	0.4 ± 0.9^{a}	0.2 ± 0.4^{a}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}
T.3	0.2 ± 0.4^{a}	0.2 ± 0.4^{a}	0^{c}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}
T.4	0.2 ± 0.4^{a}	0.2 ± 0.4^{a}	0.2 ± 0.4^{b}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}
T.5	0.2 ± 0.4^{a}	0.2 ± 0.4^{a}	0^{c}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}
T.6	0.2 ± 0.4^{a}	0.4 ± 0.9^{a}	0.2 ± 0.4^{b}	0.2 ± 0.4^{a}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}
T.7	0.4 ± 0.5^{a}	0.2 ± 0.4^{a}	0^{c}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}
Control	0.2 ± 0.4^{1a2}	0.6 ± 0.9^{a}	0.4 ± 0.9^{a}	1.0 ± 1.0^{a}	1.2 ± 0.8^{a}	1.2 ± 0.8^{a}	1.6 ± 0.9^{a}	1.6 ± 0.9^{a}	1.2 ± 0.8^{a}	1.4 ± 0.9^{a}

[1]Averages followed by standard deviation. [2]Averages followed by different letters, in column, differ among treatments by Student's t test ($p < 0.05$). T.1 = nozzle AS 7030, 75 L ha^{-1}, no adjuvant; T.2 = nozzle MGA 015, 75 L ha^{-1}, no adjuvant; T.3 = nozzle MGA 015, 75 L ha^{-1}, adjuvant 01; T.4 = nozzle AS 7030, 75 L ha^{-1}, adjuvant 01; T.5 = nozzle AS 7030, 75 L ha^{-1}, adjuvant 02; T.6 = nozzle MGA 030, 150 L ha^{-1}, no adjuvant; T.7 = nozzle MGA 03, 150 L ha^{-1}, adjuvant 01. Adjuvant 01: lauryl ether sodium sulfate (LESS). Adjuvant 02: copolymer of polyester, silicone and d-limonene (CPSDL). Fd-1 = Field-1; Fd-2 = Field-2.

in class 4 (harmful), it was possible to observe that abamectin caused a delayed effect, in comparison to the insecticides fenpropathrin and imidacloprid, while the first killed 36.7% of the population was evaluated until 30 days after treatment, the other two caused in the same period, 100% mortality.

Similar results were obtained by Lee et al. (1997) with fenpropathrin, which was sprayed on adults of *Orius sauteri* (Poppius, 1909) (Hemiptera: Anthocoridae) and found no survivors. The harmful effects of imidacloprid were also similar to those observed by Shipp et al. (1992), by confined adults of *O. insidiosus* in cages with leaves of cucumber previously treated and no survivors were identified.

The results found here with imidacloprid approached those found by Elzen (2001), who provided eggs of *Helicoverpa zea* (Boddie, 1850) (Lepidoptera: Noctuidae) previously treated with this insecticide to males and females of *O. insidiosus*, and observed a reduction in the survival of this predator, averaging 52.2 and 37.3% reduction, respectively. Nemoto (1995) aimed at the control of pests of eggplant in field conditions, found that throughout five sprayings of imidacloprid, there was a significant reduction in the density of *O. sauteri* and *Orius minutus* (L., 1758) (Hemiptera: Anthocoridae).

It has been observed, again, that the treatments with adjuvant caused higher toxicity to the natural enemy found in this study. According to Cunha and Alves (2009), the adjuvants, when used properly, can improve the interaction of the insecticide with water and fix some of their characteristics, with positive impacts on the efficiency of insect control targets and not targets (e.g. bees, spiders, and natural enemies).

The adjuvant 2 presents, in its composition the essential oil D-limonene, a terpene monocyclic substance that presents activity against insects, mites, and microorganisms (Hollingsworth, 2005; Hikal et al., 2017). The interest in the use of monoterpenes to control pests is based on the need for insecticides that are less harmful to the environment and which do not have negative impacts on human health, when compared with conventional chemical treatments, with low toxicity to humans, has important commercial appeal and has been appointed as an alternative to synthetic insecticides (Ibrahim et al., 2001; Regnault-Roger et al., 2012).

Therefore, imidacloprid associated with any of the adjuvants tested is harmful to the natural enemies: *C. externa* and *O. insidiosus*, even on the fifth day after application, regardless of the technology employed. It is necessary to find other combinations of insecticides and adjuvants to control the *S. graminum* without compromising the natural enemies.

Imidacloprid constitutes an example of a product from the new generation of insecticides belonging to the group of neonicotinoids, which are compounds that act as agonists of nicotinic receptors of acetylcholine (Ware and Whitacre, 2004; Regnault-Roger et al., 2012) and feature high toxicity to insects and selectivity to man. In the present study, this effect was evident in all treatments, also demonstrating its detrimental effect on natural enemies of the *S. graminum*, *C. externa* and *O. insidiosus* under field conditions. Thus, more studies are necessary to find other options of chemical control, including the adjuvants, to control the *S. graminum* without compromising the natural enemies that are very important to the integrated pest management.

Conclusion

Improved *S. graminum* control was observed in the association between the adjuvant lauryl ether sodium sulfate and the hollow cone nozzle for both application rates (75 and 150 L ha^{-1}) and between the adjuvant copolymer of polyester+silicone+d-limonene and asymmetric twin flat nozzle at low spray rate (75 L ha^{-1}).

The reduction of insecticide spray volume to *S. graminum* control is possible with no loss of effectiveness, improving the efficiency of the process of insecticide application.

The application of this insecticide associated with any one of the adjuvants tested is harmful to *C. externa* and *O. insidiosus*, even on the fifth day after imidacloprid application regardless of the technology employed.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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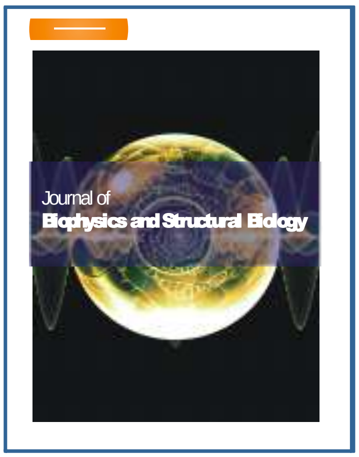

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