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Full Length Research Paper

Compatibility among insecticides, acaricides, and *Bacillus thuringiensis* used to control *Tetranychus urticae* (Acari: Tetranychidae) and *Heliothis virescens* (Lepidoptera: Noctuidae) in cotton fields

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***Tetranychus urticae* and *Heliothis virescens* are major pests of cotton and have been managed by the indiscriminate use of acaricides and insecticides. The combined use of chemical and biological products, in particular *Bacillus thuringiensis*-based products, and the rotation of active ingredients can reduce the negative effects of pesticides and can contribute to the management of resistance, control costs, and help the environment. Therefore, research on the compatibility of control tactics is essential to successful integrated pest management (IPM). The objective of this research was to determine the compatibility of chemical pesticides and a *B. thuringiensis*-based product in the control of *T. urticae* and *H. virescens* in cotton fields. Spiromesifen was compatible with *B. thuringiensis* var. *kurstaki*. However, it reduced the development of *B. thuringiensis* by 40 to 65% relative to the control. Bifenthrin was toxic or moderately toxic to *B. thuringiensis* depending on the concentration used. Compared to the control, the vegetative growth of *B. thuringiensis* was 20% lower when used with bifenthrin. Bifenthrin + carbosulfan was highly toxic to *B. thuringiensis*, decreasing its vegetative growth to less than 1% compared to the control. Spiromesifen was compatible with the recommended concentrations of *B. thuringiensis* var. *kurstaki*.**

Key words: Integrated pest management, management of resistance, pesticides, entomopathogenic bacterium, association of control tactics.

INTRODUCTION

Cotton (*Gossypium* spp.) is one of the ten most important crops for the Brazilian economy. In Brazil, the planted area with cotton has increased in the states of Mato Grosso (MT), Bahia (BA), and Goias (GO), where

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productivity is highest (Conab, 2012). In 2011 to 2012, Brazil ranked third in cotton production, with 1.043 million tons exported, 1,000,000 planted hectares, and 1.5 million tons feather production (Abrapa, 2012; Conab, 2012).

Cotton is grown with high variability of technological level employed in the field, which impacts the quantity and quality of fiber produced principally as a material to be used by textile industries. The annual harvest of cotton can be negatively affected by insect pests that cause quantitative and qualitative damage to the crop and a subsequent reduction in productivity and quality of the trade product.

The spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), is a major pest of cotton (Wilson, 1993). Its high reproductive potential, short life cycle in addition to a low environmental resistance, injection of toxins, and consumption of cellular contents cause premature senescence, defoliation, and decreases in productivity and fiber quality (Oliveira and Calcagnolo, 1975; Flechtmann, 1985).

The tobacco budworm, *Heliothis virescens* Fabricius (Lepidoptera: Noctuidae) is another major pest of cotton. It is widely distributed in Brazil due to its polyphagous behavior. It has the potential to cause qualitative and quantitative damage to the crop (Santos, 2001). The strategies to manage these pests in the field have been based solely on the use of pesticides, which has resulted in resistance of these pests to different active ingredients, population outbreaks of secondary pests, and adverse effects on natural biological control agents (Manachini, 2002). To manage resistance, farmers can use biological-based products. Among these, products based on *Bacillus thuringiensis* Berliner are the most commonly used and are effective in controlling different cotton pests but not their natural enemies (Torres et al., 2006; Brookes and Barfoot, 2008). The combined use of chemical and biological controls can be an obstacle in integrated pest management (IPM) programs due to the high risk of incompatibility, which may compromise the entire pest control system. The objective of the research was to determine the compatibility of the main synthetic insecticides and acaricides used to control *T. urticae* and *H. virescens* with a formulation of *B. thuringiensis* in cotton fields.

MATERIALS AND METHODS

The experiment was conducted at the Laboratory of Biology and Rearing Insect (LBRI), Department of Plant Protection, Faculty of Agrarian and Veterinarian Sciences, Sao Paulo State University (FCAV/UNESP), Jaboticabal, Sao Paulo, Brazil. The entomopathogenic bacterium *B. thuringiensis* var. *kurstaki* was obtained from the commercial product Dipel SC® strain HD-1 (Sumitomo Chemical Representations of Brazil Ltda.), and was used at the maximum (0.75 L/ha) and minimum (0.50 L/ha) recommended concentrations for the control of *H. virescens* in cotton (Agrofit, 2012). The synthetic pesticides used were the commercial products Oberon® (a.i. spiromesifen) (Bayer S.A.) (0.60 and 0.50 L/ha) and Talstar® 100EC (a.i. bifenthrin) (FMC Chemicals of Brazil Ltda.) (0.60 and 0.55 L/ha), which are recommended for the control of *Tetranychus urticae* on cotton, and Talisman® (a.i. bifenthrin + carbosulfan) (FMC Chemicals of Brazil Ltda.) (1.00 L/ha) (Table 1), which is recommended for the control of *T. urticae* and *H. virescens* (Agrofit, 2012).

Nutrient agar was used as the culture medium to grow the entomopathogenic bacterium *B. thuringiensis* var. *kurstaki* (Himedia Laboratories Pvt. Ltda.). Nutrient agar in distilled water (28 g/L) was autoclaved at 1 atm for 20 min. When a temperature of 45°C was reached, the chemical pesticide was added to the culture medium at the recommended concentration. The mixture was homogenized, and 25 ml was poured into individual petri dishes (9 cm diameter). The control was composed only of the culture medium (Table 2). There were 7 replicates per treatment. After solidification of the culture medium, 5 µl of a solution of the *B. thuringiensis*-based product was added at the minimum and maximum recommended concentrations for the control of *H. virescens* in cotton (Table 2). The 5 µl aliquot was placed in the center of a petri dish to better visualize the colony growth. The dishes were transferred to an incubator chamber at 27 ± 1°C, 70 ± 10% relative humidity, and 12 h photoperiod.

Colony growth was measured every 24 h for five days. The area occupied by the colonies in the petri dishes was projected onto a plain white sheet paper (A4) and traced. The colonized area was then cut out and measured with a leaf area meter (CID Bio-Science, Camas, Washington, United State of America; model CI-202). At the end of the evaluation period of vegetative growth of *B. thuringiensis*, we quantified the number of spores (CFU/ml) using an optical microscope. With a Neubauer chamber, the colonies were scraped from the agar and placed in rearing tubes with sterile water (10 ml) plus a surfactant (Tween® 0.01%). These solutions were diluted to count the spores. The experiment was conducted using a completely randomized design. The data were subjected to analyses of variance (ANOVA). The means for vegetative and reproductive growth were compared by the Tukey test ($P < 0.01$) and were performed using the statistical program SAS (SAS Institute, 2002). Data were standardized by the classification of compatibility according to Alves et al. (1998), based on the mean percentage of vegetative growth and sporulation of *B. thuringiensis*, using the following formula:

$$T = \frac{20*[CV] + 80*[ESP]}{100}$$

T = corrected value of vegetative growth and sporulation for the classification of the product; *CV* = percentage of vegetative growth relative to the control; *ESP* = percentage of sporulation or reproductive growth relative to the control.

The classification of pesticide compatibility with the entomopathogenic bacterium *B. thuringiensis* were based on the *T* values proposed by Alves et al. (1998) (Table 3).

RESULTS

The vegetative growth (cm^2) of *B. thuringiensis* var. *kurstaki* when used at the maximum recommended concentration to control *H. virescens* (0.75 L/ha) in cotton was negatively affected when in contact with the tested phytosanitary products at 24 ($F = 79.01$; df = 6; $P < 0.01$), 48 ($F = 145.50$; df = 6; $P < 0.01$), 72 ($F = 160.17$; df = 6; $P < 0.01$), 96 ($F = 152.16$; df = 6; $P < 0.01$) and 120 h ($F = 205.55$; df = 6; $P < 0.01$) (Table 4). Spiromesifen at the

Table 1. Phytosanitary commercial products used in experiments and recommended for controlling *T. urticae* in cotton fields.

Name		Chemical Group	*Max. R. C.* (L/ha)[2]	*Min. R. C.* (L/ha)[3]
Commercial	***A. I.*[1]**			
Oberon CS®	Spiromesifen	Cetoenol	0.60	0.50
Talstar 100CE®	Bifenthrin	Pyrethroid	0.60	0.55
Talisman CE®	Bifenthrin + Carbosulfan	Pyrethroid + Methylcarbamate of Benzofuran	1.00	***

[1]Active Ingredient[2]*Max. R. C.* - Maximum Recommended Concentration - Dose standardized to 100 litre H_2O/ha, [3]*Min. R. C.* - Minimum Recommended Concentration - Dose standardized to 100 L H_2O/ha, *** - Minimum recommended concentration is not available. CS = concentrated suspension; CE = concentrate emulsifiable.

Table 2. Maximum and minimum concentrations used in the different comparisons of chemical and biological products.

Treatments (n = 12)[1]	Concentration (L/ha)[2]
Control + *B. thuringiensis* (0.75 L/ha)	-----
Control + *B. thuringiensis* (0.50 L/ha)	-----
Spiromesinfen + *B. thuringiensis* (0.75 L/ha)	0.60 (*R max.*)
Spiromesinfen + *B. thuringiensis* (0.50 L/ha)	0.60 (*R max.*)
Spiromesinfen *B. thuringiensis* (0.75 L/ha)	0.50 (*R min.*)
Spiromesinfen + *B. thuringiensis* (0.50 L/ha)	0.50 (*R min.*)
Bifenthrin + *B. thuringiensis* (0.75 L/ha)	0.60 (*R max.*)
Bifenthrin + *B. thuringiensis* (0.50 L/ha)	0.60 (*R max.*)
Bifenthrin + *B. thuringiensis* (0.75 L/ha)	0.55 (*R min.*)
Bifenthrin + *B. thuringiensis* (0.50 L/ha)	0.55 (*R min.*)
(Bifenthrin + Carbosulfan) + *B. thuringiensis* (0.75 L/ha)	1.00 (*R max.*)
(Bifenthrin + Carbosulfan) + *B. thuringiensis* (0.50 L/ha)	1.00 (R max.)

[1]Number of replicates/treatment = 7.[2]Concentration of phytosanitary commercial products used: *R (max.)* = maximum concentration; *R (min.)* = minimum concentration.

Table 3. Toxicological classification (*T*) of chemicals on the entomopathogenic bacterium *Bacillus thuringiensis* var. *kurstaki* (Alves et al., 1998).

***T* value**	**Toxicological classification**
0–30	High Toxic (*HT*)
31–45	Toxic (*T*)
46–60	Moderately Toxic (*MoT*)
>60	Compatible (*C*)

maximum recommended concentration to control *T. urticae* (0.60 L/ha) significantly affected the vegetative growth of *B. thuringiensis* during the assessment period, but with lower intensity compared to the other phytosanitary products. At the minimum recommended concentration (0.50 L /ha), vegetative growth did not change significantly at the 96 and 120 h assessment periods (Table 4).

Bifenthrin at the two tested concentrations was more harmful to the vegetative growth of *B. thuringiensis* when compared to spiromesifen. The use of bifenthrin (0.55 L/ha) at the minimum recommended concentration to control *T. urticae* reduced the development of *B. thuringiensis* compared to the maximum recommended concentration (0.60 L/ha) only in the last assessment period (120 h) (Table 4).

B. thuringiensis in contact with bifenthrin + carbosulfan (1.00 L/ha) showed no vegetative growth during most assessment periods. Bifenthrin + carbosulfan was the least compatible with *B. thuringiensis* var. *kurstaki* (Table 4). At 0.60 L/ha, spiromesifen significantly reduced the development of the bacterium in all assessment periods, with growth at 40 to 60% of the control (Figure 1a). At 0.50 L/ha, spiromesifen was similar to the control to the

Table 4. Vegetative growth (cm^2) (±*SE*) of *Bacillus thuringiensis* var. *kurstaki* (0.75 L/ha) on solid culture medium containing chemicals at maximum and minimum recommended doses to control *Tetranychus urticae* in cotton fields (Temperature 27 ± 1°C; 70 ± 10% Relative Humidity, and 12 h dark: 12 h light regime).

Treatment	*C* (L/ha)[1]	Vegetative growth (cm^2) [2]				
		24 h	48 h	72 h	96 h	120 h
Control	-----	25.51 ± 2.36[a]	41.37 ± 2.98[a]	53.47 ± 2.65[a]	57.09 ± 1.83[a]	58.43 ± 1.81[a]
Spiromesifen	0.60	11.07 ± 1.54[b]	22.32 ± 1.91[b]	28.28 ± 2.62[b]	30.66 ± 3.01[b]	34.85 ± 3.64[b]
	0.50	7.95 ± 1.61[b]	18.70 ± 3.16[b]	30.78 ± 4.93[b]	42.14 ± 6.85[ab]	46.8 ± 5.51[ab]
Bifenthrin	0.60	1.53 ± 0.68[c]	3.54 ± 0.74[c]	4.97 ± 1.01[c]	5.70 ± 1.04[c]	8.69 ± 1.32[c]
	0.55	0.82 ± 0.28[c]	1.81 ± 0.27[cd]	2.74 ± 0.36[c]	3.87 ± 0.35[c]	4.61 ± 0.54[d]
Bifenthrin + Carbosulfan	1.00	0.00[c]	0.20 ± 0.13[d]	0.00[d]	0.00[d]	0.00[e]
C.V. (%)		9.28	7.80	7.95	8.26	7.04

Means followed by same letter in the column did not significantly differ by Tukey test ($P < 0.01$).[1]*C* = concentration of phytosanitary commercial products used.[2]Data were log-transformed to meet normality assumptions.

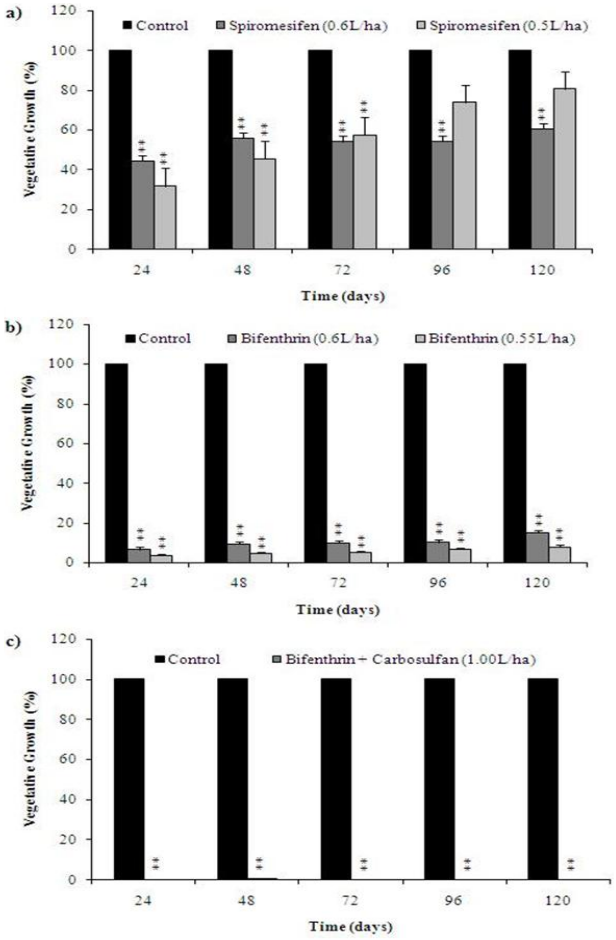

Figure 1. Percentage of vegetative and reproductive growth of *Bacillus thuringiensis* var. *kurstaki* (0.75 L/ha) in the presence of chemicals at maximum and minimum recommended concentrations to control *Tetranychus urticae* in cotton fields related to the control (Temperature 27 ± 1°C; 70 ± 10% Relative Humidity, and 12 h dark: 12 h light regime).**$P < 0.01$. The bars indicate the standard error of mean (n = 7).

Table 5. Vegetative growth (cm^2) (±*SE*) of *Bacillus thuringiensis* var. *kurstaki* (0.5 litre/ha) on solid culture medium containing chemicals at maximum and minimum recommended concentrations to control *Tetranychus urticae* in cotton fields (Temperature 27 ± 1°C; 70 ± 10% Relative Humidity, and 12 h dark: 12 h light regime).

Treatment	*C* (L/ha)[1]	Vegetative growth (cm^2)[2]				
		24 h	48 h	72 h	96 h	120 h
Control	-----	17.46 ± 2.16[a]	47.23 ± 3.62[a]	55.69 ± 1.59[a]	57.98 ± 1.52[a]	59.61 ± 1.48[a]
Spiromesifen	0.60	17.17 ± 1.84[a]	35.32 ± 5.56[a]	39.74 ± 5.30[a]	41.68 ± 5.14[a]	42.81 ± 4.91[a]
	0.50	14.74 ± 2.43[a]	29.42 ± 1.67[a]	46.66 ± 4.81[a]	49.14 ± 5.29[a]	51.28 ± 5.34[a]
Bifenthrin	0.60	0.29 ± 0.15[b]	2.33 ± 0.35[bc]	2.68 ± 0.29[b]	3.81 ± 0.50[b]	4.36 ± 0.46[b]
	0.55	1.07 ± 0.74[b]	7.95 ± 3.69[b]	9.54 ± 3.59[c]	9.62 ± 3.61[b]	9.71 ± 3.62[b]
Bifenthrin + Carbosulfan	1.00	0.00[b]	0.00[c]	0.00[d]	0.00[c]	0.00[c]
C.V. (%)		9.50	10.11	8.89	8.87	8.71

Means followed by same letter in the column did not significantly differ by Tukey test ($P < 0.01$).[1]*C* = Concentration of phytosanitary commercial products used.[2]Data were log-transformed to meet normality assumptions.

96 and 120 h assessment periods, with growth at 70% of the control (Figure 1a). The development of *B. thuringiensis* was significantly influenced by the presence of bifenthrin in the culture medium in all assessed periods, with vegetative growth less than 20% of the control (Figure 1b). Bifenthrin + carbosulfan significantly reduced the development of *B. thuringiensis*, whose vegetative growth was lower than 1% that of the control, showing a higher level of incompatibility with the formulated biological product (Figure 1c).

Vegetative growth of *B. thuringiensis* at the minimum recommended concentration to control *H. virescens* (0.50 L/ha) in cotton was negatively affected with the tested phytosanitary products at 24 ($F = 82.76$; df = 6; $P < 0.01$), 48 ($F = 97.54$; df = 6; $P < 0.01$), 72 ($F = 138.83$; df = 6; $P < 0.01$), 96 ($F = 137.65$; df = 6; $P < 0.01$) and 120 h ($F = 142.82$; df = 6; $P < 0.01$) (Table 5). However, vegetative growth of *B. thuringiensis* was not affected by spiromesifen (Table 5). A reduction in the development of *B. thuringiensis* was observed in treatments with bifenthrin. The maximum recommended concentration to control *T. urticae* (0.60 L/ha) showed lower compatibility with *B. thuringiensis* when compared to the minimum recommended concentration (0.55 L/ha), with statistical differences only in the third assessment period (72 h) (Table 5). Bifenthrin + carbosulfan inhibited the vegetative growth of *B. thuringiensis* in all assessments periods and showed higher incompatibility (Table 5).

Despite the growth of *B. thuringiensis* (0.50 L/ha) when in contact with the other analyzed pesticides, when compared to control growth, only spiromesifen did not decrease the development of the bacterium in all assessed periods, with vegetative growth higher than 60%, which suggests a high level of compatibility with the biological pesticide (Figure 2a). The development of *B. thuringiensis* was negatively influenced by the presence of bifenthrin at both concentrations. Vegetative growth was approximately 20% that of the control (Figure 2b). The absence of *B. thuringiensis* growth was verified in bifenthrin + carbosulfan, the least compatible among the tested pesticides (Figure 2c).

The mean CFU/ml of *B. thuringiensis* var. *kurstaki* (0.75 L/ha) was significantly affected when in contact with the pesticides ($F = 43.89$; df = 6; $P < 0.01$). The mean CFU/ml was lower in bifenthrin treatments compared to the control. There were no CFU in the bifenthrin + carbosulfan treatments after 120 h (Table 6). The treatments containing spiromesifen had a mean colony forming unit (CFU/ml) similar to the control after 120 h of incubation at both concentrations of *B. thuringiensis* var. *kurstaki* (Table 6).

Spiromesifen and bifenthrin treatments had mean CFU/ml values similar to those of the control at concentrations of 0.50 L/ha of *B. thuringiensis* var. *kurstaki* in the 120 h assessment period. Bifenthrin + carbosulfan significantly inhibited the formation of colonies compared to the control and the other treatments ($F = 13.34$; df = 6; $P < 0.01$) (Table 6).

The relationship between vegetative growth and CFU/ml indicated that spiromesifen was compatible with both concentrations of *B. thuringiensis* var. *kurstaki* (Table 7). Bifenthrin at maximum and minimum recommended concentrations to control *T. urticae* was classified as toxic and moderately toxic, respectively, when in contact with *B. thuringiensis* var. *kurstaki* (0.75 L/ha) according to Alves et al. (1998). However, both concentrations of bifenthrin were moderately toxic to *B. thuringiensis* var. *kurstaki* at the minimum concentration (0.50 L/ha) (Table 7). Bifenthrin + carbosulfan were classified as the most toxic to both test concentrations of *B. thuringiensis* var. *kurstaki*, according to Alves et al. (1998) (Table 7).

DISCUSSION

Compatibility between chemicals and biological pesticides can be classified as negative, neutral, or

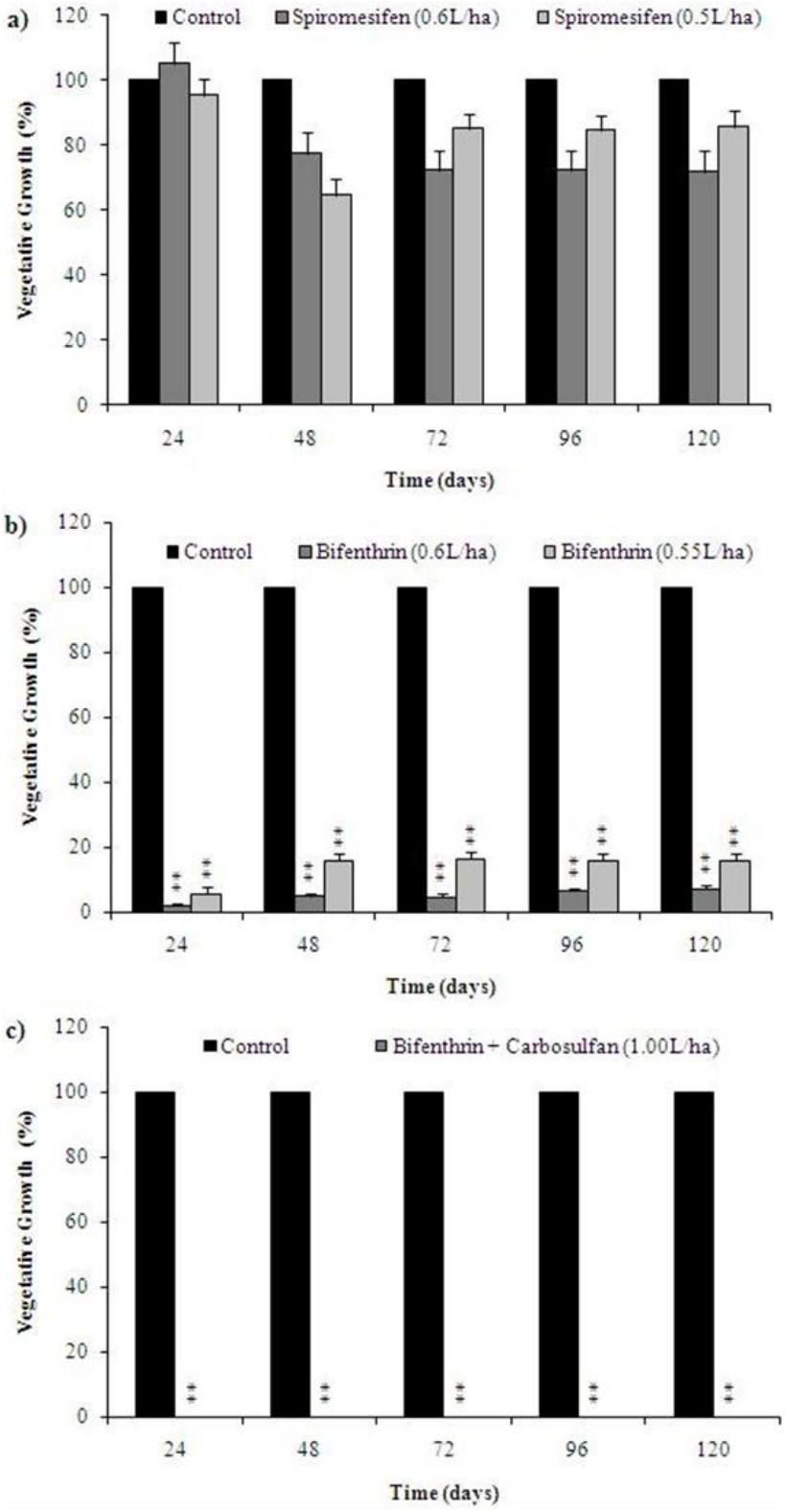

Figure 2. Percentage of vegetative and reproductive growth of *Bacillus thuringiensis* var. *kurstaki* (0.50 L/ha) in the presence of chemicals at maximum and minimum recommended concentrations to control *Tetranychus urticae* in cotton fields related to the control (Temperature 27 ± 1°C; 70 ± 10% Relative Humidity and 12 h dark: 12 h light regime).$^{**}P < 0.01$. The bars indicate the standard error of mean (n = 7).

Table 6. Number of colony forming units (CFU/mL) (±*SE*) of *Bacillus thuringiensis* var. *kurstaki* at two recommended concentrations on solid culture medium containing chemicals at maximum and minimum recommended doses to control *Tetranychus urticae* in cotton fields (Temperature 27 ± 1 °C; 70 ± 10% Relative Humidity, and 12-h dark: 12-h light regime).

Treatment	Concentration (L/ha)[1]	[1] Mean CFU/ml (×10^7)	
		***B. thuringiensis* (0.75 L/ha)**	***B. thuringiensis* (0.50 L/ha)**
Control	-----	3.89 ± 0.31^a	1.96 ± 0.20^a
Spiromesifen	0.60	2.90 ± 0.07^{ab}	1.78 ± 0.08^a
	0.50	3.59 ± 0.49^a	1.60 ± 0.21^a
Bifenthrin	0.60	1.23 ± 0.09^c	1.33 ± 0.26^a
	0.55	2.18 ± 0.38^{bc}	1.03 ± 0.58^a
Bifenthrin + Carbosulfan	1.00	0.00^d	0.00^b
C.V. (%)		4.97	5.56

Means followed by same letter in the column did not significantly differ by Tukey test ($P < 0.01$).[1]Data were log-transformed to meet normality assumptions.

Table 7. Corrected values of the vegetative and reproductive growth of *Bacillus thuringiensis* var. *kurstaki* at two recommended concentrations on solid culture medium (*T*) and toxicological classification of chemical products at maximum and minimum recommended concentrations to control *Tetranychus urticae* in cotton fields.

Treatments	Concentration (L/ha)[1]	***B. thuringiensis* (0.75 L/ha)**		***B. thuringiensis* (0.50 L/ha)**	
		T	**Classification**	***T***	**Classification**
Spiromesifen	0.60	71.77	*C*	87.00	*C*
	0.50	89.97	*C*	82.42	*C*
Bifenthrin	0.60	28.32	*T*	55.75	*MoT*
	0.55	46.44	*MoT*	45.23	*MoT*
Bifenthrin + Carbosulfan	1.00	0.00	*HT*	0.00	*HT*

C = compatible; *MoT* = moderately toxic; *T* = toxic; *HT* = high toxic.[1]Concentration of phytosanitary commercial products used.

positive (Morris, 1975; Habib and Garcia, 1981; Kusmanova, 1981; Seleena et al., 1999). The results observed for *B. thuringiensis* var. *kurstaki* in the presence of the synthetic chemical products bifenthrin and bifenthrin + carbosulfan showed a significant reduction in the activity, inhibition of sporulation and growth of *B. thuringiensis* (Alves et al. 1998; Manachini, 2002). The sensitivity of *B. thuringiensis* to bifenthrin and bifenthrin + carbosulfan, besides being related to the chemical molecule of each active ingredient, can also be negatively influenced by the presence of additional compounds in the commercial products, such as emulsifiers and other additives (Dougherty et al., 1970; Morris and Armstrong, 1975; Morris, 1977). Among the active ingredients of the endosulfan insecticide class, profenofos + lufenuron and malathion also lowered the development of *B. thuringiensis in vitro* (Almeida et al., 2003; Batista-filho et al., 2003; Pinto et al., 2012), indicating a high sensitivity of this microrganism to a range of toxic synthetic chemical compounds. Alves et al. (1998) emphasizes that the high toxicity of a tested commercial pesticide under laboratory conditions does not always have the same behavior in field, but infer an occurrence of future problems due to incompatibility.

The recommended dose of the tested phytosanitary products was also an important factor of compatibility between the chemical and biological products. This variable can be used to promote compatibility (Batista-filho et al., 2001; Manachini, 2002; Fadare and Amusa, 2003; Pinto et al., 2012). However, we observed in some cases that the highest concentration of a chemical product allowed better development of the bacterium (Figures 1 and 2).

This performance can be related to the capacity of some *Bacillus* species to degrade compounds in a chemical insecticide, aiding their own development (Rache and Coats, 1988; Das et al., 1995; Das et al., 2003). In this way, in some cases, a combination of a

chemical and a biological insecticide can control pests more efficiently when compared to a single method (Hardman and Gaul, 1990; Valentine et al., 1996). This statement is based on the principle that a conventional insecticide can act as a stressing agent to an insect and, in turn, the insect becomes vulnerable and more susceptible to infectious diseases, such as *B. thuringiensis* (Polanczyk and Alves, 2005). *B. thuringiensis* var. *kurstaki* in the presence of the commercial product based on spiromesifen showed positive results in controlling *H. virescens* and *T. urticae* under laboratory conditions. This result corroborates the synergic effect between these chemical compounds (Manachini, 2002) that can be observed 24 h after contact with the entomopathogen and spiromesifen, and which is represented by the higher vegetative growth when compared to the control (Figure 2).

Batista-filho et al. (2001) also reported a synergic effect between the insecticide fipronil and *B. thuringiensis* var. *kurstaki*. This was also reported by Hardman and Gaul (1990) who obtained high efficiency in controlling *Operophtera brumata* L. (Lepidoptera: Geometridae) and mites in apple fields with the associated use of *B. thuringiensis* var. *kurstaki* and pyrethroids, principally cypermethrin. Organophosphate and carbamate insecticides were also synergic with the sporulation and development of *B. thuringiensis* (Chen et al., 1974; Morris, 1977; DAS et al. 2003). The positive results related to the compatibility of *B. thuringiensis* were also observed in insecticides classified as growth regulators (Valentine et al., 1996), which are very important to physiological selectivity in cotton (Soares and Busolli, 2000; Carvalho et al., 2003, Czepak et al., 2005) and which have many active ingredients compatible with Bt-based products.

Conclusions

Spiromesifen was compatible with the recommended concentrations of *B. thuringiensis* var. *kurstaki*. Bifenthrin was toxic or moderately toxic to *B. thuringiensis* var. *kurstaki* at the recommended doses. Bifenthrin + carbosulfan were highly toxic to *B. thuringiensis* var. *kurstaki*.

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Performance of a local open pollinated maize variety and a common hybrid variety under intensive small-scale farming practices

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Given that the majority of maize farmers in Kenya are small-scale, improvement in maize production must focus on increased production per unit area. While hybrid maize varieties outperform local open pollinated varieties under conventional farming practices, their relative performance has not been tested under small-scale intensive production practices. A study was conducted in 2013 in Kitale, western Kenya, to evaluate performance of 'Namba Nane'; a local open pollinated maize variety, alongside a high yielding hybrid, 'Hybrid 614D' under a small-scale, intensive farming practice that utilizes deep tillage and compost/manure. Each variety was subjected to conventional and diagonal offset close spacing. The grain yield of the hybrid (12.8 tons ha^{-1}) was not statistically different from that of 'Namba Nane' (10.2 tons ha^{-1}), even though the number of rows per cob and number of ears per plant of the former were significantly greater than those of latter. However, yields of both varieties were about twice the published potential yield of improved hybrid maize (6 tons ha^{-1}) grown with conventional practices. Seed kernels of 'Namba Nane' weighed 1.6 times more than those of 'Hybrid 614D'. Diagonal off-set close spacing under this technology increased the maize grain yield of both varieties 1.3 times. The cost of producing 'Namba Nane' under the technology was significantly less than producing the hybrid and twice more profitable (gross margin). Growing 'Namba Nane' using small-scale, intensive farming practices may be a viable option for most small-scale, resource-challenged farmers to increase economic yields.

Key words: Biointensive, double digging, hybrid, open pollinated, 'Namba Nane', small-scale intensive.

INTRODUCTION

While maize is the most important cereal crop in Kenya, where it serves as both a staple food and cash crop for millions of people (Ojiem et al., 1996; Vanlauwe et al., 2008), increases in its productivity have not kept pace with increasing demand. High population pressure and repeated subdivision of land, coupled with limited

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resources available to the large proportion of the population living below the poverty index, severely constrain maize production. Kenya's population continues to increase at an average of 2.6% annually since 2002 and stood at 41.6 million in 2011 (World Bank, 2012). Average farm sizes are shrinking largely due to the traditional land inheritance practice of subdividing land among male offspring (Kilson, 1955; Karanja, 1991; Yamano, 2007; Shreffler and Nii-Amoo, 2009). Decreasing farm sizes coupled with increasing population drives a deficit spiral in which yields decrease because farmers can afford fewer and fewer inputs (Ojiem et al., 1996; Macharia et al., 2010). Part of the solution to this challenge lies in very small scale, high yielding, low-external-input farming practices (Ojiem et al., 1996; Omondi, 1996; Jeavons, 2012).

Although most farmers in Kenya recognize that modern maize hybrids generally yield more than local open pollinated varieties [OPVs (Ojiem et al., 1996; Duvick et al., 2004; Kutka, 2005; Macharia et al., 2010)], they believe that hybrid maize can perform well only under high input management practices (Ojiem et al., 1996; Macharia et al., 2010). The high cost of certified seed and fertilizer forces the majority of farmers in Kenya to either use low-yielding farm saved seeds, derived from local varieties and hybrids, and/or apply fertilizers below the recommended rates (Ojiem et al., 1996; Macharia et al., 2010). Alternative methods of farming are needed to intensify maize productivity.

For decades, maize production recommendations have tended to focus more on yield than profit, in a bid to encourage more farmers to adopt high-yielding hybrids and high-input management (Shull, 1911). This emphasis has, however, not always been successful in helping farmers to meet their economic, environmental or lifestyle needs (Brummer, 2004; Kirschenmann, 2004). Studies have shown that with little or no input of synthetic fertilizers, grain yield of OPV maize cultivars can be comparable or better than that of hybrids (Ojiem et al., 1996; Coulter et al., 2010). However, performance of local OPVs using high-yielding, small-scale, intensive farming practices has not been documented. Such practices are promoted by development organizations and often include high rates of compost, dense crop spacing, deep subsoil loosening, and other labor-intensive practices intended to produce very high yields on very small plots. Deep soil preparation (often called double digging) can increase porosity and aeration of the soil, which, combined with high rates of compost, stimulates microbial activity, root penetration, and enhanced water and nutrient supply potential (Chaudhary et al., 1985; Varsa et al., 1997; Jeavons, 2012). This allows high-density planting that can lead to very high yields on small areas.

The objective of this study was to compare the agronomic and economic performance of an indigenous OPV called 'Namba Nane' with that of a popular high yielding hybrid (Hybrid 614D) under intensive small-scale farming practices. Results could aid farmers- and agricultural development workers in helping farmers choose the most economically beneficial maize varieties for use with small-scale, intensive farming practices.

MATERIALS AND METHODS

A field study was established in 2013 at Manor House Agricultural Centre (MHAC) near Kitale town in Trans Nzoia County, western Kenya (01°00′N, 34°55′W; elevation, 1,860 m above sea level) to evaluate the performance of 'Namba Nane', under small-scale intensive farming techniques, compared to that of 'Hybrid 614D'. With mean annual rainfall of 1200 mm, Trans Nzoia experiences a bimodal rainfall pattern, with the long rains starting from mid-March to June and short rains from August to November (Jaetzold and Schmidt, 1983). Temperature ranges from an average minimum of 10°C to a maximum of 27°C (Jaetzold and Schmidt, 1983). The soils in the study site were deep, well-drained, Ferralsols (Jaetzold and Schmidt, 1983; Food and Agriculture Organization, 1988). For this study, components of a farming system known as Bio-intensive agriculture (BIA) that combines deep soil preparation, high rates of compost, and high-density planting were used. Developed by John Jeavons of Ecology Action, Willits, California, U.S.A., BIA is a combination of *French Intensive Techniques* and *Biodynamic Techniques* practiced in Europe in 1800s and early 1900s. It utilizes deep soil preparation (double digging) to 60 cm depth, composting at rates exceeding 60 Mg ha^{-1}, diagonal offset close spacing, companion planting, and biological pest control (Jeavons, 2012).

The study was a two-factor factorial randomized complete block design replicated four times. The first factor consisted of maize varieties and included two levels: 'Hybrid 614D' obtained from Kenya Seed Company Ltd., and a traditional OPV called 'Namba Nane' obtained from previous season's crop grown at the MHAC's Central garden. 'Namba Nane' is a Kiswahili phrase meaning "number eight", referring to the number of rows of kernels commonly produced on a cob. The second factor consisted of planting densities and included two levels: conventional spacing recommended by the Kenya Ministry of Agriculture of 75 cm between rows and 30 cm within the row (6 plants m^{-2}); and BIA-recommended diagonal offset spacing of 38 cm between plants (9 plants m^{-2}; Jeavons, 2012). The two factors were subjected to BIA double digging and composting practices, as described by Jeavons (2012) across the treatments.

All 12 plots (measuring 1.5 × 6 m) were double dug on 25th March, 2013, after which 60 kg of composted farm yard manure from the Ol'ngantongo Agricultural Development Corporation farm were incorporated into each plot to a depth of 10 cm (66.7 t ha^{-1}, dry weight). Plots were planted by hand on 8th April, 2013. Two seeds were planted per hole and then plants were thinned to one per hole two weeks after seed emergence, resulting in a plant population of 60 and 78 plants per plot in conventional and diagonal offset spacing respectively. Plots were weeded on 9th May and 13th June, 2013.

Yield data was determined by harvesting maize plants from an area of 2 m^2 from the middle row of each plot on 26th September, 2013. Determination of grain yield was done after maize grains were sundried to a moisture content of 13%. Dry weight of stover and cobs from the harvested samples was also recorded to determine biomass yield. Plant growth was measured as height, leaf length and width as well as leaf population $plant^{-1}$ done at 8 leaf stage and tasseling. Data were analysed using the MIXED procedure in SAS version 9.2 (SAS Institute, 2008). Differences in treatment means were determined using Fisher's protected LSD (α= 0.05).

Table 1. Cost of various inputs in Kenya shillings (KES) per deep tilled plot (9 m^2) for maize variety 'Namba Nane' (No. 8) and 'Hybrid 614D' at MHAC near Kitale, Kenya, 2013.

Breed	Spacing	Seed	Tilling	Fertilizing	Planting	Harvesting	Drying and threshing
	cm	Labor					
		KES/9m^2 plot					
Namba Nane	75 by 30	1.7	90	79	5.4	5	20
Namba Nane	38 by 38	1.8	90	79	10	5	22
Hybrid 614D	75 by 30	4.0	90	79	5.4	5	18
Hybrid 614D	38 by 38	4.3	90	79	10	5	20

Economic analysis

Economic analysis of maize from different treatments was determined using gross margin and cost-benefit analyses. Cost and benefit estimates were based on revenues and costs incurred in production of maize using the different treatments. Total revenue represented the value of maize harvested from each plot based on prevailing prices at the time of the study. The price of 2.5 kg of 'Namba Nane' at the Kitale Municipal Market and 'Hybrid 614D' from Kenya Seed Company Ltd. were KES 100 (about U.S. $1.20) and KES 425 (about U.S. $5.20), respectively, in the month of October 2013. Variable costs accrued from purchase of seeds and labor involved in land preparation (double digging), planting, gapping, thinning, weeding, harvesting, drying and shelling were determined based on the prevailing market prices (Table 1). Benefit/cost ratio was determined by dividing the revenue by variable costs from each treatment, while gross margin was obtained from revenue less variable cost accrued in each treatment. Values of costs, revenue, gross margin, and benefit/cost ratio were subjected to analysis of variance using the MIXED procedure in SAS version 9.2 (SAS Institute, 2008) and treatment differences determined using Fisher's protected LSD (α= 0.05).

RESULTS

Agronomic analysis

There was no maize variety by plant spacing interaction for kernel rows per cob, grain yield, the weight of 100 kernels, and number of cobs per plant (Table 2), thus data for maize variety and plant spacing for those agronomic yield parameters were combined for analysis. 'Hybrid 614D' had significantly more ($P < 0.0001$) kernel rows per cob than 'Namba Nane' (Table 3). Mean number of rows per cob of hybrid was 13 compared to 8.7 for 'Namba Nane.' Plant spacing had no effect on the number of rows per cob. 'Hybrid 614D' had significantly more ($P = 0.050$) number of cobs per plant than 'Namba Nane' (Table 3).

There were no differences between treatments for maize biomass yield. There were also no significant differences between the two varieties for maize grain yield (Table 3). However, maize grown at the greater density (BIA off-set spacing) had significantly greater ($P = 0.035$) grain yield than maize grown at the lower plant density [conventional spacing (Table 3). While plant density had no significant effect on the weight of maize kernels, 'Namba Nane' had highly significant ($P < 0.0001$) greater 100 – kernel weight of maize than the hybrid (Table 3). Plant height, leaf length, leaf width, and leaf population were not affected by maize variety or plant spacing.

Economic analysis

There was a significant ($P < 0.0001$) variety by spacing interaction for cost of growing maize, thus data for this parameter were analyzed separately by variety and plant density (Table 4). Results revealed that the variable cost of growing 'Hybrid 614D' was significantly ($P < 0.0001$) greater than growing 'Namba Nane' at each plant spacing whereby growing the hybrid under close spacing cost the most and growing the OPV under conventional spacing cost the least (Table 5).

There was no significant interaction between variety and plant spacing for revenue, gross margin, and benefit/cost ratio (Table 4) thus data for variety and spacing for those economic parameters were combined for analysis. While the numerical value of revenue, gross margin, and benefit/cost ratio for 'Namba Nane' were greater than the hybrid, these differences were only marginally significant [$P = 0.10$, 0.095, and 0.089 respectively (Table 6)]. However, all three economic parameters were significantly greater at the closer BIA spacing (greater plant density) than the conventional spacing (lower plant density) [$P = 0.041$, 0.054, and 0.54 respectively (Table 6)].

DISCUSSION

While results from this study revealing that the grain yield of maize variety 'Hybrid 614D' was 2.6 tons ha^{-1} greater than that of 'Namba Nane' were expected (Duvick et al., 2004; Kutka, 2005; Macharia et al., 2010), these differences were not statistically significant (Table 3). More instructive, however, were findings that the grain yield of 'Hybrid 614D' and 'Namba Nane' were doubleand 1.7 times greater, respectively, than the documented potential of 6 tons ha^{-1} of improved hybrid maize planted with adequate inorganic fertilizers under conventional

Table 2. Partial analysis of variance (*P > F)* values for maize kernel rows per cob, grain yield, and 100 kernel weight of maize at MHAC near Kitale, Kenya, 2013.

Source of variation	df	Rows per cob	Yield	Weight of 100 kernels	No. of cobs per plant
Variety	1	**	NS	**	*
Spacing	1	NS	*	NS	NS
Variety*Spacing	1	NS	NS	NS	NS

*Statistical significance at 0.05 probability level, **Statistical significance at 0.001 probability level, NS Denotes not significant.

Table 3. Mean kernel rows per cob, yield, 100 kernel weight, and number of ears per plant of 'Hybrid 614D' and 'Namba Nane' at MHAC near Kitale, Kenya, 2013.

Maize variety	Rows per cob	Yield	Weight of 100 kernels	Cobs per plant
	Value	Tons/ha	Grams	Value
Namba Nane	8.71[b]	12.80[a]	57.61[a]	1.01[b]
Hybrid 614D	13.19[a]	10.21[a]	36.97[b]	1.15[a]
P Value	*<0.0001*	*NS*	*<0.0001*	*0.050*
Spacing (cm)				
38 by 38	10.83[A]	13.18[A]	47.63[A]	1.05[A]
75 by 30	11.08[A]	9.83[B]	46.95[A]	1.11[A]
P Value	*NS*	*0.035*	*NS*	*NS*

Means within a column followed by the same letter are not significantly different (LSD, α = 0.05).

Table 4. Partial analysis of variance (*P > F*) values for economic parameters of growing maize at MHAC near Kitale, Kenya, 2013.

Source of variation	df	Cost	Revenue	Gross margin	Benefit/Cost
Variety	1	***	*	*	*
Spacing	1	***	**	**	**
Variety*Spacing	1	***	NS	NS	NS

*Statistical significance at 0.10 probability level, **Statistical significance at 0.05 probability level, ***Statistical significance at 0.0001 probability level, NS denotes not significant.

Table 5. Mean cost of growing 'Hybrid 614D' and 'Number 8' on a 9 m^2 plot using intensive farming methods at MHAC near Kitale, Kenya, 2013.

Maize variety	Spacing	Cost
		KES/9m^2 plot
Hybrid 614D	38 by 38	229[a]
Namba Nane	38 by 38	224[b]
Hybrid 614D	75 by 30	221[c]
Namba Nane	75 by 30	217[d]
P Value		*<0.0001*

Means within a column followed by the same letter are not significantly different (LSD, α = 0.05).

agricultural methods in the high potential areas of Kenya (Kipsat et al., 2004). These findings, in addition to those revealing that close spacing for both varieties yielded significantly more maize grain yield than conventionally spaced plants (Table 3), conform with observations by Jeavons (2012) that techniques that combine deep tillage, compost application, and high-density planting can increase the yield of crops per unit of land 2 to 6 times compared with the conventional average. The soil tilth obtained by double digging can allow plant roots to penetrate downwards rather than spread outwards, enabling high-density planting (Jeavons, 2012). These results are also in agreement with Chaudhary et al. (1985) who found that sub-soiling and deep digging to a depth of 45 cm increased maize plant heights by 30 to 35 cm and maize grain yield by 70 to 350% compared with maize grown under conventional tillage practices. Similar results were obtained by Varsa et al. (1997) who found that deep tillage to a depth of 60 to 90 cm resulted in increased maize grain yield by up to 47% compared to

Table 6. A comparison of revenue, gross margin, and benefit/cost ratio of growing 'Hybrid 614D' and 'Number 8' using low cost BIA techniques at two different plant spacing at MHAC near Kitale, Kenya, 2013.

Maize variety	Revenue	Gross margin	Benefit/Cost ratio
	KES/9m^2 plot		
Namba Nane	367.7^a	147.2^a	1.67^a
Hybrid 614D	294.5^b	69.68^b	1.31^b
P value	*0.11*	*0.095*	*0.089*
Spacing (cm)			
38 by 38	380.2^A	154.15^A	1.69^A
75 by 30	282.0^B	62.73^B	1.28^B
P Value	*0.041*	*0.054*	*0.054*

Means within a column followed by the same letter are not significantly different (LSD, α = 0.05).

tillage to a depth of 40 cm. The greatest increase in maize grain yield in deeply tilled soil was achieved in the year that received the least rainfall, suggesting that maize roots extracted moisture from greater depths as the depth of soil tillage increased (Varsa et al., 1997). Studies by Chaudhary et al. (1985) showed that sub-soiling and deep digging to a depth of 45 cm decreased the soil penetration resistance in the 20 to 40 cm layer to one-tenth that of conventionally plowed soil and resulted in deeper and greater rooting of maize plants. Similar results were obtained by Varsa et al. (1997) who found that deep tillage to a depth of 60 to 90 cm reduced soil bulk density, increased root proliferation, and rooting depth. Deep tillage can also break the compacted hard pan that often occurs below the plow layer where mouldboard plows are used (Vepraskas et al., 1995; Joubert and Labuschagne, 1998). Absence of significant differences in grain yield between 'Hybrid 614D' and 'Namba Nane', in spite of the hybrid having significantly greater number of kernel rows per cob and greater number of cobs per plant may partly be explained by our findings that 'Namba Nane' had significantly greater kernel weight compared to the hybrid (Table 3). These findings suggest that 'Namba Nane' may be more resource efficient than the hybrid. It is important to note that most commercial maize hybrids are developed under high nitrogen levels and fertile soils found in research stations (Muza et al., 2004), and are therefore expected to utilize nutrients more luxuriously. Most traditional OPVs were developed under conditions of low and more dispersed nutrient concentrations prevalent in more marginal regions with less fertile soils in many developing countries (Ojiem et al., 1996; Duvick et al., 2004; Macharia et al., 2010; Gudu et al., 2005; Denning et al., 2009). 'Namba Nane' plants may have invested resources to seed formation more efficiently compared to the hybrid. This is an important attribute, especially for the more resource challenged farmers in many rural areas of Africa.

While 'Namba Nane' is generally known to produce yields that are comparable with the newer improved hybrids under reduced fertilizer input (Ojiem et al., 1996), many farmers have gradually stopped growing it and/or saving seed from it. Agricultural modernization and corporate consolidation of agriculture have generally disincentivized farmers to save their local seeds such that farmers increasingly prefer purchased seed (Lewis and Mulvany, 1997; Foti et al., 2008; Connolly, 2011). As purchased seed replaces older heirloom varieties, availability of these varieties in many farming communities declines (Lewis and Mulvany, 1997). As a result, many of the available heirloom maize seed varieties no longer maintain their original purity – hence the difficulty of obtaining 'Namba Nane' seeds that produce uniform eight kernel rows per cob. This may partly explain why results from this study revealed that average kernel rows per cob of 'Namba Nane' were 8.7.

Our results revealed that it cost significantly more to grow 'Hybrid 614D' than 'Namba Nane' using this small-scale intensive production technology (Table 5), while the revenue, gross margin, and benefit/cost ratio tended to be greater for 'Namba Nane'. Furthermore, the revenue, gross margin, and benefit/cost ratio were significantly greater for growing both varieties at diagonal offset close spacing (greater BIA plant density) than conventional plant spacing (lower plant density) (Table 6). These results indicate that growing 'Namba Nane' with small-scale, intensive production practices may be a viable option for many smallholder farmers in sub-Sahara Africa to improve yields and profitability. Resource challenged small-scale farmers who cannot afford the high cost of hybrid maize seed and accompanying recommended fertilizer rates, but have parcels of land so small that they would not be daunted by the prospect of double digging or applying compost/manure, may benefit by planting 'Namba Nane' OPV and saving seed for subsequent plantings.

Further work should evaluate the performance of

'Nambe Nane' on different soil conditions and using a range of farming practices. This should include an assessment of the effects and interactions of sub-soiling (double digging), high compost (or farm yard manure) rates, high inorganic fertilizer rates and high density on the grain and biomass yield of 'Namba Nane'.

Conflict of Interest

The author(s) have not declared any conflict of interests.

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Full Length Research Paper

Effect of silicate on nutrition and yield of wheat

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An improvement in soil properties and crop development with silicate application has been confirmed in several plant species. This study investigated the effect of application of calcium silicate on nutrition and yield of wheat (*Triticum aestivum* L.). Wheat plants were grown in 8-L pots filled with clayey Rhodic Hapludox in a greenhouse. The experiment was arranged in a completely randomized design, with five treatments, and four replicates. The treatments consisted of growing wheat plants with 0 (control), 1.2, 2.4, 4.8 and 9.6 Mg ha^{-1} of calcium silicate (Ca_2SiO_4). Calcium silicate increased the pH of the soil, and the silicon concentration in leaves and stems of the wheat. Nitrogen (N), phosphorus (P), magnesium (Mg), sulphur (S), copper (Cu), and iron (Fe) concentrations in the wheat flag leaves were not affected by the application of calcium silicate, whereas the K and Ca concentrations were increased and the Zn and Mn concentrations were reduced by the application of calcium silicate rates. The application of calcium silicate rates did not affect plant height, number of spikes per pot, shoot dry matter, grain yield and harvest index of wheat.

Key words: *Triticum aestivum* L., plant mineral nutrition, silicon.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is an important winter cereal crops from Brazil. In 2012/2013 season, the wheat production area was of 2.0 million ha, producing 4.2 million tons of grains (Conab, 2013). Wheat production, mostly concentrated in the three southern-most states of Paraná, Santa Catarina and Rio Grande do Sul, is shifting further south. Paraná is the largest wheat producing state.

The calcium and magnesium silicate can be used as corrective of soil acidity and as silicon (Si) source (Ribeiro et al., 2011; Crusciol et al., 2009). Silicon is not considered an essential element for plant growth. Several studies have shown that Si application is beneficial to crops such as rice (Zanão Júnior et al., 2010), sugar

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cane (Demattê et al., 2011), maize (Zargar and Agnihotri et al., 2013) and wheat (Soratto et al., 2012), which are considered Si-accumulating species. However, Si is still relatively unknown and rarely applied in agriculture. Silicon uptake can occur actively, in an energy-spending process, even when roots are exposed to high concentrations of this nutrient (Malavolta, 2006). In plants take up Si exclusively as monosilicic acid, also known as orthosilicic acid [$Si(OH)_4$] (Elawad and Green Junior, 1979). In many cases, increasing Si availability has increased crop development and yield, once this nutrient can indirectly influence some photosynthetic and biochemical aspects, especially in plants under biotic or abiotic stress conditions (Ma and Yamaji, 2006). The largest growth and biomass accumulation of plants grown with the Si application is associated to the changes in plant architectures, making them more erect, improving the angle of leaves and light interception, avoiding the excessive self-shading, delaying senescence, increasing the structural rigidity of the tissues and improving photosynthesis and reducing lodging (Gong and Chen, 2012; Ma and Yamaji, 2008). These beneficial effects are attributed to Si deposited in the cell wall of various plant organs (Ma and Yamaji, 2006) and by other mechanisms. High deposition of Si in tissues forms a physical barrier that enhances the strength and rigidity of the tissues.

There are few studies on the effect of Si on plant nutrition, with the majority of publications reporting aspects of wheat growth and the beneficial role of this element in resistance to biotic and abiotic stress (Rizwan et al., 2012). In addition to this aspect, the beneficial effects of Si are not always observed (Dann and Muir, 2002). There is evidence that Si has no effect on dry matter yield in *Brachiaria* grasses under water stress conditions (Melo et al., 2003).

Considering that the use of silicate tends to be and most common agricultural practice in Brazil, an improved understanding of the effect of Si on wheat crop is essential in order to adopt management strategies for improving crop production. In this context, the purpose of this study was to investigate the effects of Si on nutrition and yield of wheat (*T. aestivum* L.) subjected to high rates of calcium silicate in the soil under controlled conditions.

MATERIALS AND METHODS

An experiment was carried out under greenhouse conditions, localized in the Universidade Estadual do Oeste do Paraná, Marechal Cândido Rondon, Paraná, Brazil (24°31' S, 54°01' W, and 420 m a.s.l.), in 8 L plastic pot, during the months of May and July, 2012. The soil used in the experiment was collected from the plough layer of a clayey Rhodic Hapludox (Eutroferric Red Latosol in the Brazilian classification) with 550 g kg^{-1} of clay, 370 g kg^{-1} of silt, and 80 g kg^{-1} of sand. The soil had the following properties: pH (1:2.5 soil/$CaCl_2$ suspension 0.01M) 4.5, 21 g dm^{-3} organic matter, 17 mg dm^{-3} $P_{Mehlich-1}$, 5.3 $cmol_c$ dm^{-3} Ca, 1.2 $cmol_c$ dm^{-3} Mg, 0.5 $mmol_c$ dm^{-3} K, 4.8 3 $cmol_c$ dm^{-3} H + Al, 11,8 $mmol_c$ dm^{-3} CEC, 59% of base saturation, 9.9 mg dm^{-3} $Cu_{Mehlich-1}$, 15.1 mg dm^{-3} $Zn_{Mehlich-1}$, 59 mg dm^{-3} $Fe_{Mehlich-1}$, 35 mg dm^{-3} $Mn_{Mehlich-1}$, and 18.9 mg dm^{-3} Si (Acetic acid). All the soil chemical properties were analyzed according to Embrapa (2009).

The experiment was arranged in a completely randomized design, with five treatments, and four replicates. The treatments consisted of growing wheat plants with 0 (control), 1.2, 2.4, 4.8 and 9.6 Mg ha^{-1} of calcium silicate (Ca_2SiO_4). The calcium silicate source used was AgroSilício® (10.5% Si; 25% Ca and 6% Mg). The fertilized soil was kept for 15 days with water content near the field capacity. The basic fertilization was carried out with applying 30 mg kg^{-1} of N (urea), 60 mg kg^{-1} of P (simple superphosphate), 45 mg kg^{-1} of K (potassium chloride). At 30 days after plant emergence, the application of 45 mg kg^{-1} N was applied as a urea solution.

Five seeds of wheat (*T. aestivum* L., cv. BRS Pardela) were sown, and 9 days after seedling emergence, they were thinned to three plants per pot. The soil water content was monitored daily and maintained near at the field capacity.

At maturity (115 days after plant emergence), soil samples were collected for evaluation of pH $CaCl_2$ (1:2.5 suspension solo/$CaCl_2$ 0.01 Mol L^{-1}), the crop yield was evaluated in terms of shoot dry matter production (SDM, g pot^{-1}), grain yield (g pot^{-1}) and the harvest index. The shoot length was measured (cm $plant^{-1}$) using meter scale. Plants of all treatments were harvested separately, dried for 4 days at 65 ± 2°C, and then weighed. The number of spikes per pot was also measured. Wheat flag leaves were also collected for foliar diagnosis. The collected leaves were dried in a forced-air oven for 3 days at 65 ± 2°C, grounded to smaller size and analyzed. Concentrations of K, Ca, Mg, Cu, Fe, Mn, and Zn were determined by flame atomic absorption spectrophotometry, P was determined by colorimetry, N by sulfuric acid digestion and vapor distillation, and Si by hydrogen peroxide and sodium hydroxide digestion and determined by colorimetry, as previously described (Embrapa, 2009), and Si by digestion with hydrogen peroxide and sodium hydroxide and then by colorimetry (Korndorfer et al., 2004). Original data were analyzed by analysis of variance (ANOVA) and regression analysis, and significant equations with the highest coefficients of determination (F-test, $P \leq 0.05$) were adjusted. All analyses were performed using Saeg 8.0 software for Windows (Statistical Analysis Software, UFV, Viçosa, MG, BRA).

RESULTS AND DISCUSSION

The application of silicate significantly increased the concentration of Si in the shoots of wheat in both the stem and leaves (Figure 1), the concentrations of Si in the leaves increased from 26.7 g kg^{-1} in the control to 36.2 g kg^{-1} with a maximum dose of Si (9.6 Mg ha^{-1}). The Si concentration in leaves was on average higher than the stem. In the initial values stem 12.29 g kg^{-1} in control, increasing to 17.27 g kg^{-1} at a dose of 9.6 t ha^{-1} (Figure 1). The increased concentration of Si in the shoots indicated that the source of Si used is reactive and very effective in providing Si in the soil and the plant.

Corroborating the results of Korndörfer et al. (2010), this reported that the application of Si in soil caused concentration of this nutrient in the leaves, but did not alter the production of dry matter. The differences in the concentration of Si were not enough to affect the vegetative growth of forage.

Lima Filho and Tsai (2007) also observed absorption exponentially in three cultivars of wheat and two of oats with Si added to the nutrient solution until the dose of 100 mg l^{-1}, and concluded that the two grasses, wheat and

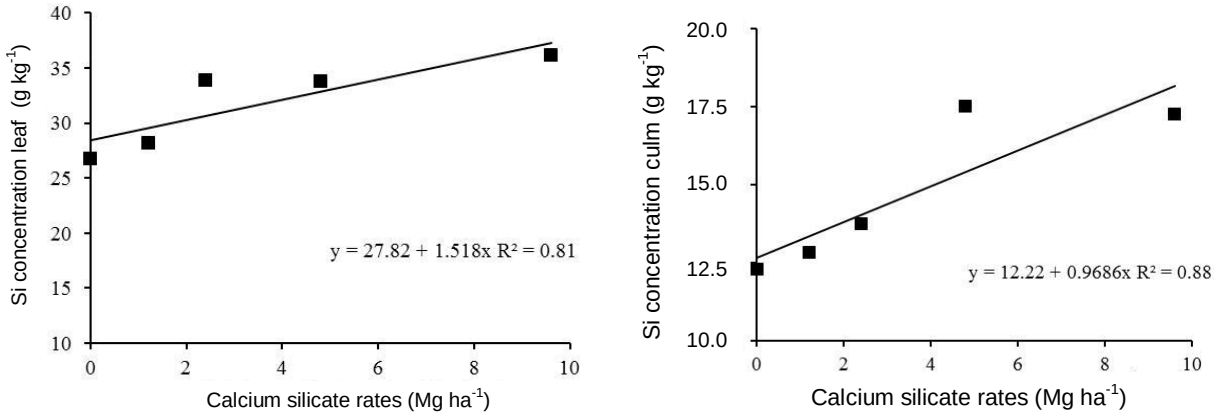

Figure 1. Effect of dose of calcium silicate on foliar concentrations of silicon on leaf and stem in wheat (*T. aestivum* L.) plats.

Table 1. Effect of calcium silicate rates on concentrations of N, P, K, Ca, Mg, S, Cu, Zn, Fe and Mn in the flag leaf of wheat (*T. aestivum* L.) plants.

Calcium silicate	N	P	K	Ca	Mg	S	Cu	Zn	Fe	Mn
Mg ha^{-1}	g kg^{-1}						mg kg^{-1}			
0	31.9	3.3	16.3	12.7	3.7	1.13	10.7	20.8	307.8	430.3
1.2	31.5	3.5	17.9	12.2	3.2	1.09	12.2	16.0	321.1	272.1
2.4	23.2	3.2	18.2	11.3	3.7	1.11	10.1	14.9	393.9	256.5
4.8	29.1	3.3	17.2	14.7	3.8	1.08	8.8	13.8	429.4	168.9
9.6	28.2	3.3	21.9	16.3	3.4	1.13	10.9	13.8	296.7	162.3
Mean	28.8	3.3	18.3	13.4	3.6	1.11	10.5	15.9	369.8	258.0
F-test	0.61^{ns}	0.19^{ns}	3.32*	15.2**	0.44^{ns}	1.73^{ns}	1.04^{ns}	4.61*	2.81^{ns}	33.5**
Regression	ns	ns	L*	L**	ns	ns	ns	L**	ns	Q**
CV (%)	31.0	12.0	12.8	30.4	20.9	4.5	52.0	17.2	26.8	14.5

^{ns}, Not significant; *** and **,** statistical significance at 5 and 1%, respectively, by F-test; **L,** linear equation; **Q,** quadratic equation; **CV,** coefficient of variation.

oats, have high absorption capacity of silicon, indicating the possibility of absorbing larger amounts if there were an increase in the availability of the substrate element.

The use of calcium silicate positively increased linearly $CaCl_2$ pH values, with threshold values of 4.6 increasing to 6.6 at the highest dose (Figure 2). Similar results with the effect of silicates in neutralizing soil acidity were also obtained by several authors (Korndörfer et al., 2010). This effect of the slag ground reaction occurred primarily by the presence of the base SiO_3^{2-} silicate generated by the reaction of compounds in the soil (Alcarde, 1992), which positively affects the pH.

Ramos et al. (2006) studied different ways of correcting the pH in a Quartzipsament and observe that the silicates were more efficient than gypsum and limestone. Prates et al. (2011) observed that the application of calcium silicate and magnesium to the soil increased the pH and did not affect the growth and development of physic nut.

Application of calcium silicate did not affect the N, P, Mg, S, Cu, and Fe concentrations in the wheat flag leaves (Table 1). These results agree to report by Soratto et al. (2012), who found that the N, P, Ca, Mg and S concentrations in the wheat flag leaves were not affected by silicon leaf application. Potassium and Ca concentrations in the wheat flag leaves were increased by the application of calcium silicate rates and the Zn and Mn concentrations were reduced (Table 1 and Figure 3). Soratto et al. (2012) also found that the silicon leaf application increased K concentration in the wheat flag leaves. Despite the effects of silicate treatments, only N, P, K, Mg and Cu concentrations were within the ranges considered appropriate by Cantarella et al. (1997), which are 20 to 34 g kg^{-1}, 2.1 to 3.5 g kg^{-1}, 15 to 30 g kg^{-1}, 1.5 to 4.0 g kg^{-1} and 5 to 25 mg kg^{-1}, respectively. Calcium, Fe, and Mn concentrations were above the optimum ranges, which are of 2.5 to 10 g kg^{-1}, 10 to 300 mg kg^{-1}, and 25 to 150 mg kg^{-1}, respectively. Sulfur and Zn concentrations were below the optimum ranges, which are of 1.5 to 3.0 g

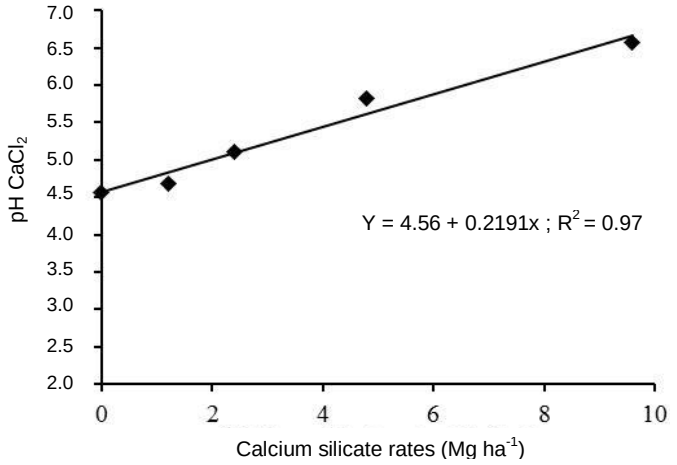

Figure 2. Mean values of pH $CaCl_2$ at the end of the experiment, after cultivation with wheat (*T. aestivum* L.), depending on the doses of calcium silicate.

kg^{-1} and 20 to 70 mg kg^{-1}, respectively.

These results confirm those obtained by Moraes et al. (2009) except for Mn, in which to assess the effect of calcium and copper sulfate on the nutritional content of bean silicate, found that doses of calcium silicate had no significant effect on the concentration of Cu, Fe and Mn in shoots, however, observed a reduction of the levels of Zn.

The application of 9.6 Mg ha^{-1} calcium silicate increased the K and Ca concentrations in the wheat flag leaves in 29 and 38%, respectively as compared to control (Figure 3a and b). The results presented here are similar to those reported by Rocha et al. (2011), who found that the residual effect of silicate tended to enhance the K concentration in sorghum leaves. This result can be a consequence of improved root growth, once Si application enhances root structures, as reported by Carvalho-Pupatto et al. (2003). In addition this increase in Ca concentration in the wheat leaves (Figure 3b) is due to supply of this nutrient with calcium silicate rates.

Zinc and Mn concentrations in the wheat flag leaves decreased progressively with increasing rates of calcium silicate. The percentage reduction of Zn and Mn concentrations was 29 and 68%, respectively, when comparing the growing wheat plants with 0 (control) and 9.6 Mg ha^{-1} (Figure 3c) and 7.0 Mg ha^{-1} of calcium silicate (Figure 3d). Lower concentrations of Zn and Mn in wheat leaves with calcium silicate rates occurred because the silicate use may rates the soil pH (Figure 2), thereby reducing the bioavailability of these micronutrients in the soil.

The Mn was higher rated for culture (Raij, 2011) range. A comprehensive range of Mn in the nutrient suitable range is between 25 to 150 mg kg^{-1}, and the plants showed mean values of 162.3 to 430.3 mg kg^{-1}. The deposition of Si on the leaves helps to improve the distribution and avoid toxicity, reducing the mean concentration of Mn in the tissues of wheat (Figure 3d).

According to Zanão Junior et al. (2010), the addition of Si to the solution increased the content of Mn in roots and decreased in leaves and sheaths, showing a lower translocation of Mn to leaves which indicates that Si reduces the toxicity caused by Mn, what may be an alternative to alleviate such adversity. In areas where excess Mn is a problem, studies to establish rates and sources in order to increase the Si availability to plants must be implemented to supplement information for fertilizer recommendations for this crop.

Lima Filho and Tsai (2007) also observed a decrease in the Mn and Zn in wheat with the supply of Si in nutrient solution; this was attributed to the fact that the accumulation of dry matter increased faster than the rate of accumulation of nutrients, resulting in a dilution effect for most nutrients studied.

Calcium silicate provided better balance in the absorption of Mn to the grain, reducing excessive amount absorbed while maintaining the appropriate concentration range (25 to 150 mg kg^{-1}) according to Raij (2011). According to Okuda and Takahashi (1964), the Si may provide better nutritional balance in the rice plant due to its ability to reduce the absorption of Mn. When rice plants are fertilized with Si, increased oxidation of Mn^{2+} is the surface of the roots, and as a result, these precipitated nutrients not being absorbed by the plant.

Rogalla and Römheld (2002) worked with cucumber plants grown with Si supply, found less than 10% of Mn in the symplast and more than 90% bound to the cell wall. Regarding plants that did not receive Si, the distribution of Mn was similar in the two compartments. These authors claim that tolerance of cucumber plants to Mn toxicity is also due to its attachment to the cell wall, which lowers its concentration in the symplast.

The application of calcium silicate rates did not affect plant height and shoot dry matter, number of spikes per pot, grain yield and harvest index of wheat (Table 2). The results presented here are similar to those reported by Melo et al. (2003) and Tokura et al. (2007); these authors found that the Si application did no effect dry matter production of *Brachiaria* grasses and rice, respectively. However, a beneficial effect of silicate application on wheat growth and yield was expected as reported by Soratto et al. (2012). Silicon positively influences plant growth and biomass production, especially monocotyledons, as a consequence of improved tissue rigidity, better angle of leaves and light interception, improving photosynthetic rate (Gong and Chen, 2012; Ma and Yamaji, 2006). According to Elawad et al. (1982), Si is involved in cell elongation and division processes as well as in hormone balance.

Studies indicated that there is an increased on number of a wheat spike per area with Si foliar application compared to the control (Soratto et al., 2012). Takahashi

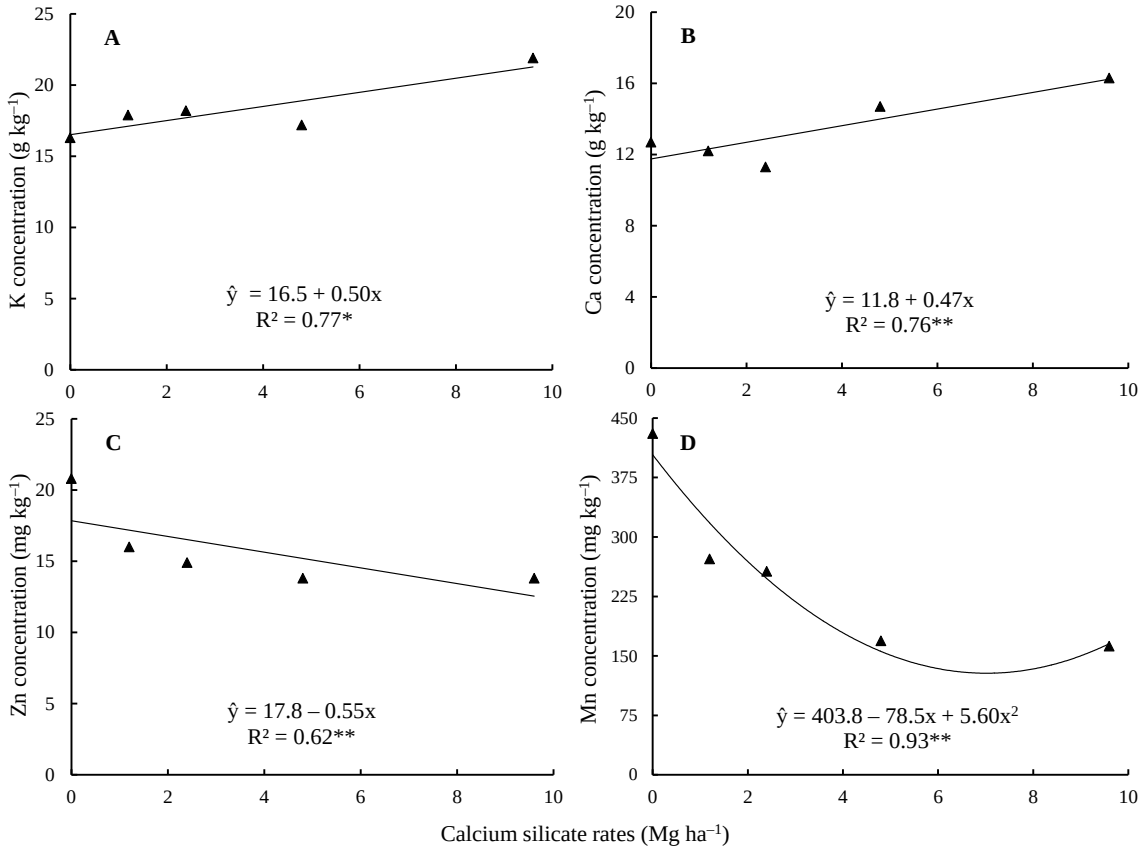

Figure 3. Effect of calcium silicate rates on potassium (A), calcium (B), zinc (C) and manganese (D) concentration in the flag leaf of wheat (*T. aestivum* L.) plants

Table 2. Effect of calcium silicate rates on plant height, shoot dry matter, number of spikes per pot, grain yield and harvest index of wheat (*T. aestivum* L.) plants.

Calcium silicate	Plant height	Shoot dry matter	Number of spikes per pot	Grain yield	Harvest index
Mg ha^{-1}	cm	g pot^{-1}	units	g pot^{-1}	
0	87.8	18.3	5.0	6.6	36.1
1.2	86.5	20.1	4.8	7.7	38.3
2.4	81.7	17.7	4.5	5.4	30.5
4.8	85.2	18.1	4.3	6.8	37.5
9.6	83.9	18.5	5.0	6.5	35.0
Mean	85.0	18.5	4.7	6.6	35.6
F test	1.26^{ns}	0.24^{ns}	0.72^{ns}	0.99^{ns}	1.23^{ns}
Regression	ns	ns	ns	ns	ns
CV (%)	4.9	15.0	16.4	24.4	19.7

^{ns}**,** not significant; **CV,** coefficient of variation.

(1995) had also confirmed the effects of Si on the number of rice panicles per area. These authors attributed higher number of spikes or panicles per area to a better Si nutrition of plants. Soratto et al. (2012) found that grain yield was significantly increased by Si leaf application compared to the control (without Si application), as a result of the higher photosynthetic area, due to the higher dry matter production and higher number of spikes per area. In this study, the numbers of spikes per pot and grain yield were not increased by silicate application (Table 2).

The lack of response to Si fertilization can be seen

when the initial Si content available soil is above the critical level, as in the work of Mauad et al. (2003). According to Korndörfer et al. (1999), the Si content available soil (extracted with $CaCl_2$ 0.05 mol L^{-1}) at least 6 to 8 mg dm^{-3}, in general, indicates a high probability of response to Si application, but the Si contents in the soil studied were high, with 18.9 mg dm^{-3} Si may be one reason for the lack of response of wheat.

Studies have demonstrated that Si is involved in a number of structural, physiological and biochemical aspects of the plant cycle, with diverse functions. As pests and diseases were controlled during the experiment and irrigation ensured the water supply, it is believed that the absence of Si effects may be correlated to the lack of biotic or abiotic stress. Beneficial effects of Si on plant metabolism has been attributed to the fact that this element activate genes involved in phenol production and enzyme activity related to defense mechanisms, especially in plants under biotic or abiotic stress conditions (Buck et al., 2008; Ma and Yamaji, 2006). The results presented here report that a larger number of studies must be conducted because the beneficial effects of silicate on wheat, Si-accumulating specie, are not always observed.

Conclusions

Calcium silicate increased the pH of the soil, and the silicon concentration in leaves and stems of the wheat. Application of calcium silicate increased K and Ca concentrations in the flag leaves of wheat and reduced Zn and Mn concentrations, whereas the treatments did not influence the concentrations of all other nutrients. Application of calcium silicate did not affect development, yield components and grain yield of wheat crop.

Conflict of Interest

The author(s) have not declared any conflict of interests.

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Soil and foliar application of Zinc to maize and wheat grown on a Zambian Alfisol

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The deficiency of zinc (Zn) in human nutrition, commonly found in cereal-based diets accounts for impaired growth (stunting) in children. Since cereals are generally low in this element, bio-fortification may represent an opportunity to increase Zn intake by humans. A study was carried out to evaluate Zn uptake by maize and wheat when they are supplied with increasing rates of foliar or soil applied Zn. Maize and wheat were grown in the field and supplied with 0, 10, 20, 30, or 40 kg Zn ha^{-1} as $ZnSO_4$ applied to the soil, or, 0,1,2,4, or 8 kg Zn ha^{-1} as foliar spray. Zinc application to soil increased maize and wheat yields beyond increments obtained with foliar application, but Zn mass concentration in maize grain was better with foliar applications. Mean maize yield was 1.78 ton ha^{-1} with soil application and 1.14 ton ha^{-1} with foliar application. This was in relation to an average of 52 mg Zn uptake by maize under each of the application methods. Wheat yield was 3.69 ton ha^{-1} under soil application and 2.74 ton ha^{-1} under foliar application. In this case, Zn uptake was higher under soil application (11.31 mg) than under foliar application (7.25 mg). Sesquioxide bound Zn was shown to be best correlated with plant Zn uptake. It was shown that Zn application is beneficial on Zambian soils, and while soil application increases crop yields, foliar application can be more useful to increase Zn mass concentration in maize.

Key words: Foliar spray, maize grain yield, wheat grain yield, zinc fractions, zinc uptake,

INTRODUCTION

Zinc (Zn) deficiency in diet is common among developing nation communities that are highly reliant on cereal-based diets (Jiang et al., 2008; Welch, 1993). This is attributed to inherently infertile soils, soil micronutrient depletion from intensification of cultivation, and general low use of fertilizers, as well as poor mobility of Zn into and within plant. Therefore health challenges such as impaired growth (stunting) in children arise (Hambridge et al., 1986). In order to reverse this trend, application of Zn fertilizer can enhance plant Zn mass concentration. However it is known that numerous factors affect Zn availability leading to reduced or enhanced availability of Zn in the soil.

Zinc deficiency symptoms tend to be slow to appear on crops in arid and semi-arid regions because deficiencies of nitrogen (N), phosphorus (P) and potassium (K) are

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Table 1. Some chemical and physical characteristics of soils used in the greenhouse study.

Soil series	Land use /designation	pH	OM	CEC	Sand	Silt	Clay	Texture
			g kg^{-1}	cmol kg^{-1}		g kg^{-1}		
Nakambala	Cropped	4.8	3.7	28.8	372	256	372	l
	Fallow	5.6	5.1	34.8	392	216	392	l
Makeni	Cropped–11North	7.0	3.9	35.5	332	286	382	l
	Fallow	6.6	8.9	44.8	472	146	382	l
Kashinka	Cropped	7.2	2.6	15.1	532	176	292	Sl
Chilimboyi	Field A	7.2	1.3	14.6	552	136	312	Sl
	Field B	7.1	1.3	13.8	512	136	352	Sl
	Field C	7.5	1.7	10.7	612	96	292	Sl
Chelstone	Field H	4.7	6.7	6.0	632	36	332	Sl
	Fallow	5.3	6.6	4.9	672	36	292	Sl
	Orchard	7.0	6.6	12.6	562	96	342	Sl
Ifisa	Cultivated	7.1	0.5	7.9	612	76	312	Sl
	Fallow	5.4	1.4	7.6	572	56	372	Sl
Chalimbana	Cultivated	6.7	2.6	15.9	652	86	262	Sl
Mushemi	Cultivated	5.7	1.3	6.1	752	36	212	Ls
	Fallow	5.7	1.4	7.1	732	36	232	Ls
Mpongwe	Cultivated	6.5	4.6	34.0	472	196	332	L
	Fallow	4.4	3.2	21.4	552	156	292	Sl
Misamfu Red	Cultivated	4.2	1.6	6.0	756	128	116	Sl
	Fallow	4.7	2.6	7.3	816	68	116	Ls
Mufulira	Cultivated	4.1	1.3	5.3	836	88	76	Ls
	Fallow	4.2	1.6	4.9	736	88	176	Sl

l = loam, ls = loamy sand, sl = sandy loam.

more likely to be expressed by affected plants much sooner than that of Zn (Mapiki and Phiri, 1995). For this reason, whereas application of Zn fertilizer should be an essential component of soil fertility management, it is still seen that compound NPK fertilizers are those normally used. In Zambia, Banda and Singh (1989) proposed a soil available Zn critical level of 0.8 mg kg^{-1} below which it is recommended to apply Zn fertilizer. Local data from various laboratories here show that Zn availability index is low in most of the soils.

Native soil Zn exists in various pools with different rates of solubility, mobility and plant availability (Adriano, 2001). This partitioning of Zn is influenced by soil pH, clay content, organic matter and sesquioxides. Arid and semi-arid region soils that are low or high in pH, low or high in organic matter content, sandy, calcareous, or water-logged are commonly deficient in Zn (Takkar and Walker, 1993). In order to supply Zn to crops grown on these soils, the method of application for effective availability and absorption by plants can be a critical concern. Therefore affordable interventions aimed at raising cereal grain Zn mass concentration could include application of Zn to soil or as foliar sprays. Traditionally, soil application is widespread, however positive response to foliar Zn application has been reported for maize (Grzebisz et al., 2008), sugarcane (Panhwar et al., 2003) and wheat (Erenoglu et al., 2002), among others. In fact, Liew (1988) suggested that foliar micronutrient application could bring about a 6 to 20 times efficiency in crop productivity. On the other hand, Rashid et al. (2000) observed that Zn fertilization to seed-bed was more effective than when broadcasted in the field. The objectives of this study were to investigate which soil Zn pool is most associated to plant Zn uptake, and to determine the response of maize and wheat crops to increasing rates of Zn applied as foliar spray or to soil.

MATERIALS AND METHODS

Greenhouse study

Three kilograms of surface soil sample obtained from cultivated and uncultivated sites at eleven locations around Zambia (Table 1) was placed in polythene pots in the greenhouse. Thereafter six grams of

Table 2. Chemical and physical properties of the UNZA Field Station soil used for the field study.

Field	pH	O.M	N	CEC	Mg	Ca	K	Na	P	Zn	Fe	Cu	Mn	Sand	Silt	Clay	Texture
		%		cmol kg^{-1}					mg kg^{-1}					%			
Maize field	7.2	1.3	0.05	14.6	2.3	5.6	0.7	0.4	7.8	0.5	4.0	0.5	22.4	55.2	13.6	31.2	sl
Wheat field	7.1	1.3	0.06	13.8	2.3	5.3	1.3	0.9	11.4	2.2	4.8	1.1	18.5	51.2	13.6	35.2	sl

sl = sandy loam.

Compound "D" fertilizer (10:20:10) was added to each pot and eight seeds of wheat (*Triticum estivum* L.) var. UNZA WV1 were planted. The pots were watered and arranged in a complete randomized design with three replications, giving sixty-six pots. Two weeks after germination, plants in each pot were thinned down to five. At the end of six weeks, above ground biomass was harvested for dry matter yield, lightly washed in distilled water and allowed to dry in a 70°C oven for 48 h before weighing. Plant dry matter was ground into fine powder and digested in hot H_2SO_4-H_2O_2 solution (Parkinson and Allen, 1975). Plant tissue Zn concentration was determined in the solution using an atomic absorption spectrophotometer. Zinc uptake was calculated as a product of dry matter yield and Zn concentration.

Laboratory analysis

The soil Zn fractionation scheme described by Johnson and Petras (1998) was used to define the various Zn fractions in the soils. However, fresh soil sample was weighed into each solution rather than use the same sample in order to reduce mixing of fractions. Briefly, the following extractions were done for the respective Zn fractions: 20 g soil in 40 ml 0.005 M DTPA for 2 h [exchangeable Zn (Exch-Zn)], one gram soil in 20 ml 1 M CH_3COONH_4/CH_3COOH mixture at pH 5 for 5 h [carbonate bound Zn (Carbo-Zn)], one gram soil in 40 ml 0.1 M $K_2P_2O_7$ for 17 h [organic bound Zn (Org-Zn)], one gram soil in 50 ml acid oxalate at pH 3 (four parts 0.2 M ammonium oxalate and three parts 0.23 M oxalic acid) for 17 h [sesquioxide Zn (Ses-Zn)], one gram soil digested in 25 ml aqua regia (one part HNO_3:three parts HCl) for twenty minutes on a hot plate [Residual Zn (Res-Zn)]. Total Zn (Tot-Zn) was calculated as a sum of all the fractions. Each soil suspension was filtered after shaking or digestion. The concentration of Zn in each extract was determined using the atomic absorption spectrophotometer. All the soils were analyzed in triplicates.

Field study

Between November, 2007 and October, 2008, a field experiment was conducted at the University of Zambia, School of Agricultural Sciences Field Station in Lusaka, located 15.25° S and 28.20° E, and 1260 m asl. The soil here is described as a sandy loam mixed isohyperthermic paleustalf (Msoni, 1985). This area receives 800 to 1000 mm rainfall per annum, primarily from November to April, with mean temperature of 24°C. For initial soil characterization soil samples were collected from 0 to 20 cm depth at ten random sites in the field and the composite soil sample was used for determination of soil physical and chemical properties using standard methods (Van Ranst et al., 1999). The study treatments included two methods of Zn application (foliar and soil application), each at four rates, applied to one crop of maize (*Zea mays* L.) and another crop of wheat (*Triticum estivum* L.) in a randomized complete block design with three replications.

Maize (*var*. MRI 724) was planted on 15th December 2007, and 200 kg ha^{-1} equivalent of Compound "D" fertilizer (10:20:10, NPK) was applied according to standard recommendation, to each of 6 × 2 m^2 plots with 75 cm spacing between the rows. On the same day, five Zn fertilization rates at 0, 10, 20, 30 or 40 kg ha^{-1} Zn were applied to the soil as $ZnSO_4.7H_2O$. At the four-leaf stage, foliar application treatment of $ZnSO_4.7H_2O$ was done uniformly on leaves to supply 0, 1, 2, 4 or 8 kg Zn in 200 L ha^{-1} using a knapsack sprayer. At the six-leaf stage an application of 70 kg N ha^{-1} was made to each of the plots using urea (46% N). A similar process was carried out for the wheat (*var*. UNZA *WV* 1) crop that was planted on 1st May, 2008, except that the 1.2 × 10 m^2 plots in this instance were each supplied with 500 kg ha^{-1} equivalent of Compound "D" fertilizer. A planter was used to drill wheat seeds into rows. At six weeks after planting and at boot stage, respectively, 45 kg N ha^{-1} was drilled in as urea.

Maize was harvested on 30th April, 2008, at the black layer stage from a 1.2 × 6 m^2 area after discarding the two border rows. The grains were air-dried for one week, weighed and corrected for moisture at 12.5%. Similarly, wheat was harvested on 10th October, 2008, from a 0.4 × 10 m^2 area after removing the border rows. Following one week of air-drying, the grains were threshed by hand and weighed. Zinc concentrations in the grains were determined after digesting milled grain sample in H_2SO_4-H_2O_2 solution (Parkinson and Allen, 1975) and measuring on an atomic absorption spectrophotometer. Zinc uptake was calculated as the product of grain weight and Zn concentration.

Statistical analysis

The data were evaluated statistically by analysis of variance using SAS statistical program (SAS 6.12). The means were compared using Duncan's Multiple Range Test. The relationship between soil Zn and plant variables were evaluated using simple linear regression analysis.

RESULTS AND DISCUSSION

Soil properties

Chemical and physical properties of the soil used in the greenhouse and field studies are presented in Tables 1 and 2, respectively. The soil was largely Alfisols, with some Oxisols and Ultisols, and their pH ($CaCl_2$) values ranged from 4.1 to 7.5. Half of them were acidic while the other half was alkaline (Table 1). Although there was no significant difference (t-test, p=0.05) in mean soil reaction between cultivated and uncultivated soil samples, cultivation generally had the tendency to reduce soil pH. The soil samples were dominated by coarse textured soils ranging between loamy sand to loam. Soil organic matter was highly variable, being very low or very high (<2.5%>) and uncultivated fields were more likely to have

Table 3. Soil Zn fractions of the 11 Zambian soils collected from different locations.

Soil series	Land use /designation	Zn fractions*					
		Exch	Carbo	Org	Ses	Res	Total
		mg kg^{-1}					
Nakambala	Cultivated	1.43	6.80	6.33	74.5	18.96	108.02
	Fallow	0.52	2.68	2.66	44.2	23.30	73.39
Makeni	Cultivated	2.28	20.13	5.71	17.5	22.00	67.67
	Fallow	9.08	16.60	Nd	57.2	13.08	96.03
Kashinka	Cultivated	2.77	4.73	4.38	7.45	14.95	34.28
Chilimboyi	Field A	0.52	28.00	1.84	4.32	9.55	44.23
	Field B	2.15	7.75	6.16	11.9	72.40	100.41
	Field C	3.16	9.40	7.61	11.5	17.9	49.62
Chelstone	Cultivated	T	3.03	1.43	11.1	6.55	22.16
	Fallow	T	2.95	Nd	30.4	5.35	38.75
	Former Orchad	T	8.95	Nd	31.3	13.25	53.54
Ifisa	Cultivated	0.09	1.95	2.38	22.6	5.85	32.92
	Fallow	T	2.28	1.97	49.6	7.60	61.50
Chalimbana	Cultivated	1.00	4.13	5.29	1.55	10.85	22.82
Mushemi	Cultivated	0.44	0.78	0.74	0.55	10.60	13.11
	Fallow	0.73	15.78	10.86	Nd	7.77	35.14
Mpongwe	Cultivated	7.45	12.18	16.06	25.7	28.27	89.71
	Fallow	T	3.00	2.14	4.80	10.59	20.53
Misamfu Red	Cultivated	0.48	8.53	0.72	34.5	7.25	51.52
	Fallow	0.42	1.95	4.73	15.5	11.60	34.20
Mufulira	Cultivated	0.86	2.13	7.72	27.3	5.30	43.37
	Fallow	0.39	1.40	2.68	9.80	6.35	20.62
Mean		1.98	7.51	4.81	22.9	14.97	52.26
SD		5.68	5.83	4.16	20.0	17.20	32.09

*Exch = exchangeable Zn; Carbo = carbonate Zn; Org = organic Zn; Ses = sesquioxide Zn; Res = residual Zn.

higher values than their cultivated analogs. The soil cation exchange capacities were between 4.9 and 44.8 cmol kg^{-1} (Table 1) with most observed to be low (< 15 cmol kg^{1}), probably due to relatively high sand and low organic matter contents of many of these soils. The mean distribution of Zn among the various soil fractions was in the order: Ses-Zn > Res-Zn > Carbo-Zn > Org-Zn > Exch-Zn (Table 3). Variable observations are reported in literature as discussed subsequently. Plant available Zn levels (Exch–Zn) were low for 14 and marginal for 4 out of the 22 soil samples (Table 3), going by the proposed 0.8 mg kg^{-1} critical level for Zn in Zambian soils (Banda and Singh, 1989). Essentially the soils that were more acidic or more alkaline in reaction were more likely to be deficient in available Zn, probably due to immobilization and reduced solubility of Zn in those soils. This observation is supported by Takkar and Walker (1993) who indicated that Zn deficiency is most common in low- and high pH soils. The other soil fertility parameters (Tables 1 and 2) were low to moderate according to the indices used by the University of Zambia Soil Analysis Laboratory, namely: Organic matter (2.5%); cation exchange capacity (12 cmol kg^{-1}); exchangeable-Ca, Mg and K (0.2 cmol kg^{-1}); extractable-Fe (2.5 mg kg^{-1}); Cu (0.2 mg kg^{-1}); and Mn (1 mg kg^{-1}).

Greenhouse dry matter yield and Zn uptake

Wheat dry matter yield in the greenhouse was not significantly different among the soils, but fallow soils generally produced more (Table 4). A similar pattern was

Table 4. Dry matter yield, Zn concentration and Zn uptake for six-week wheat crop grown in the greenhouse on 11 Zambian soils.

Soil series	Land use/designation	Total dry matter	Zn concentration	Zn uptake
		g pot^{-1}	mg kg^{-1}	mg pot^{-1}
Nakambala	Cultivated	10.00	5.0	5.00
	Fallow	16.33	3.7	6.05
Makeni	Cultivated	13.33	3.4	4.40
	Fallow	15.67	4.7	7.46
Kashinka	Cultivated	8.33	3.8	3.07
Chilimboyi	Field A	10.00	2.7	2.70
	Field B	13.33	4.0	5.41
	Field C	10.00	4.4	4.39
Chelstone	Cultivated	15.00	3.7	5.55
	Fallow	10.00	6.2	6.18
Ifisa	Cultivated	10.00	2.2	2.18
	Fallow	13.33	4.1	5.39
Chalimbana	Cultivated	8.33	4.6	3.43
Mushemi	Cultivated	13.33	3.5	4.83
	Fallow	11.67	5.8	6.79
Mpongwe	Cultivated	11.67	3.6	4.53
	Fallow	13.33	4.0	4.89
Misamfu Red	Cultivated	6.67	4.8	3.51
	Fallow	11.67	3.7	4.16
Mufulira	Cultivated	9.33	4.4	4.05
	Fallow	11.67	2.8	3.23
Mean		11.57	4.05	4.63

observed for Zn uptake and less so for Zn concentrations in plant tissue. To investigate which soil Zn pool was most likely to contribute to plant Zn uptake, the association between soil Zn and plant Zn was determined in a correlation analysis. While the correlation coefficients were generally weak, the sesquioxide bound Zn contributed significantly more to wheat plant Zn uptake than the other Zn pools (Table 5). There was no significant relationship that was observed for the exchangeable, carbonate, organic and residual Zn pools in the soil. Other authors also reported that the sesquioxide bound Zn contributed significantly to Zn uptake by wheat (Singh and Abrol, 1985) and rice (Adhikari et al., 2007; Singh and Abrol, 1985). Contrastingly, Adriano (2001) and Iyengar et al. (1981) observed that sesquoixide bound Zn was less plant available. In terms of the other soil Zn fractions, Behera et al. (2008) observed that most of the Zn from organic pool and the sorbed Zn were taken up by wheat and maize while there was a negative relationship between Zn uptake and sesquioxide bound Zn. Sinha et al. (1977) reported that the organic and clay bound Zn contributed positively and significantly to the Zn taken up by maize and wheat crops. Rico et al. (2009) analyzed 29 soils in Spain and also observed low Zn uptake from organic Zn. The variability in observations by several authors shows that all individual Zn fractions could potentially contribute to the overall Zn uptake of the plant depending on soil physico-chemical properties and the method used for fractionation.

Maize grain yield and Zn uptake

The effects of the method of Zn application and Zn rates on maize crop performance are shown in Table 6. Grain yield averaged across the different Zn rates was 56% more and significantly higher when Zn was applied to soil

Table 5. Correlation coefficients for the relationship between zinc fractions in 11 Zambian soils and zinc uptake by wheat grown for six weeks in the green house.

Soil Zn pool	Dry matter yield	Zn concentration	Zn uptake
Exchangeable Zn	0.20	0.07	0.30
Carbonate Zn	0.003	-0.02	0.05
Organic Zinc	-0.15	0.09	-0.004
Sesquioxide Zn	0.15	0.28	0.36*
Residual Zn	0.25	-0.06	0.17
Total Zn	0.23	0.18	0.38*

*Significant at 0.05 level.

Table 6. Yield and Zn uptake of maize and wheat crops supplied with increasing rates of Zn as soil and foliar applications in the field.

Method	Zn Rate	Maize		Wheat	
		Yield	Zn uptake	Yield	Zn Uptake
		t ha^{-1}	mg plot^{-1}	t ha^{-1}	mg plot^{-1}
Soil	0	1.54^{a}	31.97^{b}	3.49^{a}	8.89^{a}
	10	2.15^{a}	37.97^{b}	4.03^{a}	14.63^{a}
	20	1.69^{a}	41.48^{a}	2.98^{a}	9.83^{a}
	30	2.05^{a}	77.23^{a}	4.26^{a}	12.72^{a}
	40	1.48^{a}	71.34^{a}	3.68^{a}	10.48^{a}
	Mean	1.78	52.00	3.69	11.31
Foliar	0	1.31^{a}	22.74^{c}	2.45^{b}	7.29^{ab}
	1	1.10^{a}	35.29^{c}	2.33^{b}	5.24^{b}
	2	1.05^{a}	42.64^{b}	4.12^{a}	10.11^{a}
	4	1.12^{a}	80.52^{a}	2.59^{b}	8.49^{ab}
	8	1.10^{a}	75.77^{ab}	2.21^{b}	5.13^{b}
	Mean	1.14	51.39	2.74	7.25

^{a,b}, Means followed by the same letter do not differ significantly at 5% level by DMRT method.

compared to foliar Zn application. Foliar spraying is normally adopted to increase plant nutrient uptake when soil immobilization mechanisms reduces Zn movement in the soil. Additionally it may be a cheaper way to supply nutrients to plants. However in this case it appears that soil application and absorption through the roots was a more effective alternative to increase grain yields. Hossain et al. (2008) also observed that the soil application of Zn resulted in an increase in the grain yields of maize. Similar result though at much lower soil application rate was obtained by Abunyewa and Mercer-Quarshie (2004) in Ghana who reported a 2.18 t ha^{-1} increase in the maize grain yield from supplying 5 kg Zn ha^{-1} to the soil.

Increasing the amount of Zn applied did not affect grain yields statistically nor was there a specific trend among the rates in either the soil or foliar Zn application. However, addition of Zn fertilizer to soil resulted in 4 to 40% more grain yield than control whereas foliar Zn application reduced grain yield by 15 to 20% compared to control. Harris et al. (2007) reported a 25% increase (about 0.7 t ha^{-1}) in grain yield when they applied 2.75 kg Zn ha^{-1} to the soil of a maize field in Pakistan. They observed that increasing the rate to 5.5 kg Zn ha^{-1} produced the same results but with much lower cob weights. In the current study, maize grain yields were not significantly different among different soil Zn fertilization rates and ranged from 1.48 t ha^{-1} at 40 kg Zn ha^{-1} to 2.15 t ha^{-1} at 10 kg Zn ha^{-1}, which was the best rate.

Mean maize Zn uptake values were generally comparable between the two methods of application (Table 6). In the soil applied treatment, Zn uptake increased significantly up to 142 and 123% from the application of 30 and 40 kg Zn ha^{-1}, respectively, compared to the control. Lower application rates, on the other hand, only promoted up to 30% increase in Zn uptake. Under the foliar treatment, Zn uptake was significantly increased by 254 and 233% from the

application of 4 and 8 kg Zn ha^{-1}, respectively, compared to the control. The lower rates effected up to 86% increase in uptake. Though the average grain yields were lower with foliar spray treatment compared to soil application, the Zn uptake was similar between these two application methods (Table 6). This could be explained by higher Zn concentrations in the tissue of foliar sprayed crops. There was no visual symptom of leaf-burn observed on the crop. In this study, while foliar Zn application did not enhance maize grain yield, an application rate of 4 kg ha^{-1} was the best for increasing Zn mass concentration while 30 kg ha^{-1} applied to soil was best.

Wheat yield and Zn uptake

Soil application of Zn produced an average wheat grain yield that was 35% more and significantly different from the average grain yield produced by foliar application (Table 6). Contrary to the observation from the current study, Modaihsh (1997) reported that foliar spray of Zn at 1.8 kg ha^{-1} significantly increased grain yield of wheat grown on a calcareous sandy loam soil of Saudi Arabia. Haslett et al. (2000) concluded that foliar application of inorganic or organic Zn fertilizers were efficient in providing the Zn required by wheat for growth. Increasing rates of Zn application to soil did not affect grain yields significantly however Zn application generally resulted in 5 to 15% yield increase compared to the control. Except for a significant increase in yield when 2 kg Zn ha^{-1} was applied, increasing rates of foliar application also did not affect grain yields.

The uptake of Zn by wheat was 56% more with soil application than foliar application (Table 6). The rate of Zn applied did not significantly affect uptake when applied to soil although increases in the range of 11 to 65% over the control were obtained. Foliar Zn application treatments at 2 and 4 kg Zn ha^{-1} increased uptake over the control by 39 and 16%, respectively, while foliar Zn application at 1 and 8 kg Zn ha^{-1} decreased uptake by 30%. Sharma et al. (1988) however observed that both zinc sulphate and zinc oxide increased yield and uptake of Zn by wheat when applied within 45 days of planting.

There was no direct relationship that could be drawn between yield and uptake. This may point to the effect of metabolic mechanisms which regulate uptake, as well as positive assimilation. Nonetheless there appears to be economic benefit to be derived from the increased yields that Zn application brought about in wheat grain. Foliar spray seemed to increase Zn mass concentration however the benefit cannot be conclusive until additional work to partition Zn in the various plant parts is done. Jiang et al. (2008) have shown that there is variability in within-plant allocation and Zn accumulation in rice, which may have implications for availability to and assimilation by human. In this study, the differences in extents of response between maize and wheat crops may be attributed to their different sensitivities to Zn. Clark (1990) classified maize to be most sensitive while wheat is less sensitive to Zn deficiency, therefore their corresponding responses.

Conclusion

The current study demonstrates that application of minimum rates of Zn to soil at 10 kg ha^{-1} to maize and 30 kg ha^{-1} to wheat crops was beneficial. Soil application was more effective in raising yield levels, but foliar application between 2 and 4 kg ha^{-1} increased the Zn mass concentration in plant tissue. Zinc uptake was more from the sesquioxide bound Zn and this may be an indication that there is a positive dynamic equilibrium between this fraction and the more soluble Zn fractions.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Activation of biochemical defense mechanisms in bean plants for homeopathic preparations

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To evaluate the potential elicitor of homeopathic preparations on bean plants cv Carioca, homeopathics of *Corymbia citriodora*, *Calcarea carbonica*, *Silicea* and *Sulphur* in dynamisations 12, 24, 30 and 60CH were applied by pulverization throughout the aerial part. Samples of leaf tissue were taken at 6, 12, 24, 48 and 126 h after the treatment (HAT) to analyze the activity of peroxidase (POX), catalase (CAT), chitinase (CHI) and β-1.3-glucanase (GLU), and fifteen days after the application for the total contents of chlorophyll a and b. For the induction of phytoalexin phaseolin, seeds of the same genotype were germinated in the presence of respective treatments, and the production of phytoalexin quantified spectrophotometrically. All treatments increased the activity of POX, CAT, QUI and GLU, in at least one of the schedules evaluated in comparison to the control. The treatments *C. citriodora* and *C. carbonic* did not alter the contents of chlorophyll but induced the accumulation of phaseolin. *Silicea* and *Sulphur* caused significant reduction in the levels of chlorophyll a and b. The induction values were superior to the trading inductor (harpin), indicating that these homeopathies may come to be utilized as elicitor treatments on bean plants. The results indicate the potential of the treatments applied in the induction of biochemical mechanisms of defense in the bean plants.

Key words: Resistance induction, homeopathy, enzymes, phytoalexins.

INTRODUCTION

The plants exhibit a range of effective defense mechanisms against phytopathogens regardless to the arrival of the pathogen on the infection site. However, there are other defense mechanisms even further efficient that apparently remain inactive or dormant, only being actuated or activated after exposure of the plants to inducing agents. In this case, the resistance is said to be induced, that is, the plants realize the attacks, and in its high capacity for adaptation, active defense mechanisms allow that they survive (Pascholati et al., 2011).

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The inducible defense mechanisms during the process of resistance induction may be structural and biochemical. Among the biochemicals, it highlights the production of phytoalexins, proteins-RP, hydroxyproline rich glycoproteins, protease inhibitors, and peroxidases, among others. These can act as barriers for the production of toxic substances or repellents, creating adverse conditions for the establishment of the pathogen in the plant (Cavalcanti et al., 2005; Pascholati et al., 2011). Activation of latent defense mechanisms may be related to action agents of origin biotic or abiotic called elicitors. The presence of the elicitors renders the plant resistant to posterior infections for several weeks, with effective protection against a diversified range of pathogens (Agrios, 2005).Among the compounds that may act by inducing plant defense mechanisms can be cited in the fungal extracts, plant extracts, essential oils, and homeopathic preparations, among others. The use of homeopathy in plants can act as abiotic inducers of induced resistance, as well as being an easy technique to apply and low cost, being used in all types of living beings, thus reducing the need for agrochemicals and contributing to the conservation of the environment and human health (Rossi et al., 2007).

The classic homeopathies, or already tested in humans and animals, or prepared from different compounds have shown effects on the activation and / or inhibition of secondary metabolic compounds. Coqueiro et al. (2008) to streamline the essential oil of *Corymbia citriodora* that verified the effects of homeopathies elicitors on peroxidase activity and increased production of phytoalexins in soybean. Das Dores (2007) reports the effects of the application of homeopathies of *Sulphur* and *Phosphorus* 12CH in plants fava d'anta (*Dimosphandra mollis*) observing increased activity of phenylallanine ammonia lyase and phenolic compounds, as well as an increase of the synthesis of the flavonoid rutin. Already Fonseca et al. (2006) with a single application of *Sulphur*, *Natrium muriaticum*, *Kalium phosphoricum*, *C. carbonic*, *Silicea terra* and *Magnesium carbonicum* in dinamization 4CH, reported an increase in tannin content in cabbage-clove leaves (*Porophyllum ruderale*) when compared to the control. Thus, the present study aimed to evaluate the potential of homeopathies *Calcarea carbonic*, *Silicea*, *Sulphur* and oil *C. citriodora* (dinamized) on the induction of pathogenesis-related protein, phaseolin and chlorophyll contents in bean plants.

MATERIALS AND METHODS

Choice and preparation of treatments

The choice of treatments used in this work was primarily based on analogies with the medical references used in homeopathy for humans (Carneiro, 2011). The choice of treatments *C. carbonic*, *Silicea* and *Sulphur* was based on reports of the effects of these drugs on various plant species.

The choice of *C. citriodora* was justified by reports of the potential inducer of defense compounds in various plant species.For the preparation of homeopathic remedies the essential oil of *C. citriodora* was extracted by hydrodistillation and passed by trituration in lactose until the third dynamization (3CH) (centesimal hahnemanian scale). The fourth dynamization was prepared by diluting 1% (w/v) of 3CH, using 30% grain alcohol, followed by 100 sucussions (agitations) made in mechanical arm, obtaining the dynamization 4CH. The following dynamizations were prepared by dissolving 1% (v/v) of previous dynamization until 60CH (ABFH, 2003).

The dynamizations of *C. carbonic*, *Silicea* and *Sulphur* were prepared from the matrices 4CH. The fifth dynamization was prepared of the previous dynamization at 1% (w/v) in 30% grain alcohol, followed by 100 sucussions obtaining the dynamization 5CH. The following dynamizations were prepared on the same scale by diluting 1% (v/v), with the process of succussion done with the aid of the mechanical arm until 60CH (ABFH, 2003). The dynamizations utilized in bioassays were 12, 24, 30 and 60CH.

Plant materials

Bean seeds (*Phaseolus vulgaris*) cv Carioca, provided by the Agronomic Institute of Paraná (IAPAR) were sown in pots containing 5 L substrate prepared with soil, sand and humus (3:1:1). The soil was sieved and sequentially autoclaved three times during 1 h at 121°C with 24 h intervals. Twenty days after the end of the autoclaving process, it was sowed and maintained in two plants / pot.

The application of homeopathy 12, 24, 30 and 60CH diluted in distilled water (1% v/v) of *C. citriodora* (EC), *C. carbonica* (CC) *Silica* (SI), *Sulphur* (SU) and eucalyptus essential oil (0.5%) was performed on plants at the V4 stage (twenty days after emergence). As a negative control, we used grain alcohol (0.3%) and distilled water, and as positive control harpin (Messenger ®, 873 mg L^{-1}).

Treatments were applied with sprayer to the point of straining on adaxial and abaxial surfaces throughout the leaves. For the biochemical analyzes, samples of the first and second trifoliate leaves were taken at 6, 12, 24, 48 and 216 h after treatment. The samples were stored at -20°C for subsequent analysis of enzymatic assays.

The experimental design was completely randomized, with 20 treatments with four replications; each experimental unit consisted of two plants. The results were submitted to analysis of variance and averages compared by the test of Scott-Knott ($p \geq 0.05$).

Obtaining the protein extracts

The leaf samples were macerated in liquid nitrogen and homogenized in 4 mL of potassium phosphate buffer 50 mM (pH 7.0) containing 0.1 mM EDTA and 1% (w/w) PVP (poly vinyl pyrrolidone), in a porcelain mortar. The homogenized was centrifuged for 30 min at 7300 g at 4°C, and the supernatant obtained considered enzyme extract, and stored at -20°C. The extract was used for determination of protein content using the Bradford method (1976) and the activity of peroxidase, catalase, chitinase and β-1,3-glucanase.

Determination of the activity of guaiacol peroxidase (EC 1.11.1.7)

The guaiacol peroxidase activity was determined directly by measuring the conversion of guaiacol in tetraguaiacol by mixing 0.5 mL of the enzyme extract to 2.5 mL of prepared substrate (250 µL

and 306 µL guaiacol and hydrogen peroxide in 100 mL of 0.01 M phosphate buffer pH 6.0).

The reaction occurred at 30°C and the reading activity was performed in a spectrophotometer at 470 nm for 2 min with 10 s intervals (Lusso and Pascholati, 1999). The difference between the readings on the linear increment period was used to determine the activity. The specific activity results are expressed as absorbance $min^{-1}.mg^{-1}$ protein.

Determination of the activity of catalase (EC 1.11.1.6)

Catalase activity was determined by the stable complex formed by ammonium molybdate with hydrogen peroxide. A aliquot of 50 µL of the enzyme extract was incubated in 0.5 mL of reaction mixture containing 60 mM hydrogen peroxide in potassium phosphate buffer 60 mM pH 7.4 at 38°C for 4 min. After this time the addition of 0.5 mL of 32.4 mM ammonium molybdate stopped the consumption of hydrogen peroxide present in the extract. A blank for each sample was prepared by adding ammonium molybdate to the reaction mixture, omitting the incubation period (Góth, 1991; Tománková et al., 2006). The reaction tubes were removed and 0.1 mL material transferred plates for ELISA absorbance reading. Reading the yellow complex formed by molybdate and hydrogen peroxide which was measured at 405 nm in ELISA reader VersaMax ® Microplate Reader Molecular Divicers. The difference between the absorbance of the blank and the sample incubated indicated the amount of hydrogen peroxide used by the enzyme. H_2O_2 concentration was determined using the molar extinction coefficient $\epsilon = 0.0655\ mM^{-1}\ cm^{-1}$.

Determination of chitinase activity (EC 3.2.1.14)

The chitinase activity was assessed using the methodology described by Silva et al. (2008). To this, 600 µL of sodium acetate buffer 100 mM pH 5.2 was mixed with 200 µL protein extract and 200 µL "CM-chitin-RBV" (2 mg L^{-1}). After incubation at 40°C for 20 min, the drying was made with 200 µL 1M HCl, followed by cooling on ice and centrifugation at 10,000 rpm for 5 min. The absorbance of the supernatant was determined at 550 nm in the Elisa VersaMAx® Microplate Reader Molecular Divicers, with reference 800 µL of extraction buffer + 200 µL "CM-chitin-RBV + 200 µL HCl 1.0 M. The results are expressed in absorbance units min^{-1} mg^{-1} protein.

Determination of the activity of β-1, 3 - glucanase (EC 3.2.1.6)

The determination of the activity of β-1,3-glucanase was conducted analogously to the procedure chitinase, but using carboxymethyl-curdlan marked with Remazol Brilliant Blue - "CM-curdlan-RBB"4 mgL^{-1} as substrate. The absorbance of the supernatant was determined at 600 nm at reader VersaMAx® Microplate Reader Molecular Divicers, with reference 800 µL l of extraction buffer + 200 µL "CM-curdlan-RBB + 200 µL HCl 1.0 M. The results are expressed in absorbance units' min^{-1} mg^{-1} protein (Costa et al., 2000).

Determination of total polyphenols

Thirty days after the treatments were withdrawn from the aerial part of the plants and dried in a forced ventilation oven at 60°C to constant weight and ground. The concentration of total polyphenols was determined spectrophotometrically by the Folin-Ciocalteau accordingly Bucic-Kojic et al. (2007) from the powder of dried leaves. One gram of this powder was homogenized with 50 mL of 80% ethanol in a mixer for 2 min. After 5 min centrifugation at 5000 rpm, it was transferred to 0.2 mL of this test tube extract, adding 1.8 mL of distilled water, 10 mL of Follin 10%, and after 8 min adding 8 mL of sodium carbonate (Na_2CO_3) 7.5% and it remained in the dark for 2 h. The reading was held in the 765 nm spectrum, using white as all reagents without the sample aliquot centrifuged. The results were expressed in mg of gallic acid equivalents per gram of dry weight (mg GAE / gdw).

Determination of chlorophyll content

Fifteen days after the first treatment application, two leaf discs of 2 mm in diameter were removed from the plant for the quantification of chlorophyll. Plant tissue samples were weighed and placed in glass vials containing 10 mL of 80% acetone for 7 days in the dark at 25°C. After this period, it was held reading the spectrophotometer at 663 and 645 nm for chlorophyll *a* and *b*, respectively. The determination of chlorophyll *a* was given by the formula $(0.0127.A_{663})-(0.00269.A_{645})$ and chlorophyll *b* by the formula $(0.0229.A_{645})-(0.00468.A_{663})$ (Viecelli et al., 2009). The total chlorophyll content was obtained by summing the results of the contents of chlorophyll *a* and *b*. The values were expressed in mg g^{-1}fresh tissue.

Accumulation of phytoalexin

Bean seeds cv. Carioca after disinfection were sown in plastic boxes containing germination paper moistened with 5 mL of the same treatments described above. The gerbox with seeds and their treatments were incubated in a growth chamber at 25°C in the dark for seven days. After this period, they were highlighted with 5 cm segments of etiolated hypocotyls, washed in sterile water, wiped dry and weighed. Later, they were transferred to tubes containing 10 mL of ethanol and kept at 4°C for 48 h to extract the phytoalexin. After stirring for 1 h, the measurement was performed spectrophotometrically at 280 nm. The results were expressed in units of absorbance per fresh $weight^{-1}$ ($ABS.fw^{-1}$) (Brand et al., 2010).

The experimental design was completely randomized, with 20 treatments with four replications, each experimental unit consisting of a tube containing approximately 1 g of hypocotyls. The results were submitted to analysis of variance and the averages compared by the test of Scott-Knott ($p \geq 0.05$).

RESULTS

Activity of guaiacol peroxidase (POX) and catalase (CAT)

All treatments at least one point, resulted in changes in the activity of these enzymes, with the oscillatory behavior of induction and reduction in POX activity. Six hours after the first application of homeopathy, it was possible to observe the effects of inducing peroxidase in plants treated with EC 24CH, 60CH and SU and CC 60CH, with increases of 100, 75 and 16%, respectively (Table 1). Twelve hours after treatment (HAT) there was a trend of reduced enzyme activity in plants treated with oil 0.5%, EC 30 and 60CH. The potencies of CC, SI 12 and 60CH and SU 12CH, presented statistically lower

than the control water. This trend was maintained at the time of evaluation 24 HAT, when the inhibition of POX activity was observed in all treatments. In 48 HAT all dynamizations CC increased the POX activity, reaching 29-fold increased in dynamisations 12CH, this being the only time to differ from control alcohol; *Silicea* 30 and 60CH increased activity of POX, 4 and 5 times, respectively compared to the water control. *Sulphur* resulted in increases ranging between 3.3 and 8.3 times higher than in water control in dynamisations 60 and 24CH, respectively (Table 1).

In time 216 HAT activity of POX in plants treated with EC 12CH, CC 24CH, SI 12, 30 and 60CH, SU 12, 24 and 30CH were higher compared to the water control. The application of harpin increased enzyme activity only after 6 h of the first application treatment, increasing 2.3 times the activity of POX, with the same pattern observed for the reduction of homeopathy in 24 HAT.

The patterns of CAT activity were altered by all treatments. At 6 HAT EC 12CH inhibited CAT activity with values statistically similar to alcohol control, different from that observed in CC 12CH, when activity increased 2.6 times, as well as the dinamizations of SI 24 and 30CH which promoted increases of 2.4 to 3.6 times. The application of SU 12 and 24CH also increased the activity of CAT, exceeding water control of 2.4 and 3.2 more times, respectively (Table 1). At 24 and 48 HAT CC 24 and 30CH, SI 12CH, it increased the enzymatic activity of CAT. Already SI 60CH, 216 HAT was increased by 29% CAT activity, different from what occurred with other treatments that had significantly lower compared to the water control.

Total polyphenols

Thirty days after the treatments, the amount of phenols reduction was observed when plants were treated with dinamizations EC 30CH, 24CH CC and SU SL, compared to the water control (Figure 1).

The mean EC 30CH treated plants were 16% smaller than control plants, as well as CC 24 and 60CH reduced 42 and 21%, respectively in the amounts of phenols. SU 12CH resulted in values 18% lower compared to the water control. The other treatments did not have their values changed significantly.

Activity of chitinase (CHI) and glucanase (GLU)

The homeopathy of EC 24, 30 and 60CH reduced or were statistically equal to the water control in the first evaluations. Already at 48 and 126 HAT EC 30CH showed an increase in the activity of 30 and 56%, respectively, in the activity in relation to the water control (Table 2). Plants treated with CC only at 48 HAT showed no increase in the activity of CHI which was statistically higher than the control water, reaching 130% increase enzyme activity by CC 60CH. For SI at 48 HAT, it was observed that the dynamizations 24, 30 and 60CH showed statistically higher values than the control, with increases of 223, 282 and 312%, respectively. When plants received applications of SU, enzymatic activity increased at 6 HAT (60CH), 48 and 126 HAT with dynamizations 12, 24 and 30CH (Table 2).

All homeopathies applied, presented for purposes of elicitors' glucanase for at least one of the evaluations. The evaluation showed 6 HAT inducing effects by EC 12CH with increased activity GLU at 54% and DC 12CH increased by 63%. The application of SU in all dynamizations had inducing effects at the times 6 and 12 HAT, with increments ranging from 70% (24CH) and 318% (60CH) in 6 HAT and 47% (30CH) and 131% (12CH) at 12 HAT. At 12 HAT, SI 30 and 60CH also promoted induction in the activity of GLU (Table 2).

In the time 24 HAT, CC 12CH increased 3 times the activity of GLU, other dynamizations (24, 30 and 60CH) significantly reduced enzyme activity when compared to untreated controls, as well as all treatments SI and dynamizations 12, 30 and 60CH of SU which also reduced the activity of GLU.

After 48 h of the application of DC 12 and 30CH, and all dynamizations of SI and SU 12, 24, 30CH observed increased activity of β-1,3-glucanase. Treatments with CC 12CH increased 98% of enzymatic activity. The best promotion of SI at this time was 60CH which increased over 200%. SU 12, 24 and 30CH promoted increases of over 100%. These inducing effects were also observed in the treatment by EC 216 HAT and 30 60CH with means 4 and 3 fold higher than the untreated control values in SI 4 times greater, and SU 12, 24 and which also promote increase 30CH enzyme activity, reaching 480% in 30CH. The control treatment with harpin also induced the activity of POX, CAT and GLU, and also showed the same oscillatory pattern that homeopathy.

Chlorophyll

The amounts of chlorophyll were not affected by treatment with the dinamizations of EC (Table 3), unlike observed in plants treated with SU and SI the values of total chlorophyll *a* and *b* were reduced. Plants treated with homeopathy SI had a 33% reduction in chlorophyll content as well as the 24 and 30 CH, in 60CH in the reduction was 26%. The dynamizations of SU resulted in reductions ranging from 18% (12CH) and 26% at24 and 60CH.

Production phaseolin

All treatments altered the production of phaseolin. The hypocotyls treated with essential oil of EC increased

Table 1. Specific activity of peroxidase and catalase (absorbance min^{-1} mg^{-1}protein) in bean leaves treated with homeopathy *Corymbia citriodora* (EC), *Calcarea carbonica* (CC) *Silica* (12CH) and *Sulphur* (SU).

Tratamento	Peroxidase					Catalase				
	6 HAT**	12 HAT	24 HAT	48 HAT	216 HAT	6 HAT	12 HAT	24 HAT	48 HAT	216 HAT
Control (H_2O)	0.037[a]*	0.049[b]	0.092[b]	0.086[a]	0.085[a]	2.061[b]*	0.000[a]	0.650[a]	2.615[a]	8.972[c]
Alcohol 0.3%	0.022[a]	0.027[b]	0.106[b]	0.356[b]	0.233[b]	1.312 [a]	4.661[b]	0.501[a]	4.337[b]	7.344[c]
Harpin	0.070[c]	0.031[b]	0.034[a]	0.063[a]	0.050[a]	0.000[a]	3.860[b]	0.000[a]	4.763[b]	11.741[d]
OE 0.5%	0.024[a]	0.023[a]	0.041[a]	0.070[a]	0.068[a]	2.691[b]	16.011[c]	0.000[a]	9.003[c]	7.434[c]
EC 12CH	0.015[a]	0.039[b]	0.036[a]	0.107[a]	0.122[a]	0.057[a]	0.710[a]	0.000[a]	5.066[b]	0.000[a]
EC 24CH	0.074[c]	0.032[b]	0.050[a]	0.100[a]	0.172[a]	3.054[b]	0000[a]	0.000[a]	1.804[a]	2.510[b]
EC 30CH	0.009[a]	0.022[a]	0.015[a]	0.035[a]	0.224[b]	2.390[b]	0.000[a]	0.000[a]	3.749[b]	3.780[b]
EC 60CH	0.031[a]	0.014[a]	0.017[a]	0.045[a]	0.081[a]	2.466[b]	1.443[b]	0.603[a]	0.604[a]	5.783[c]
CC 12CH	0.032[a]	0.025[a]	0.014[a]	2.519[c]	0.145[a]	5.517[c]	0.211[a]	0.521[a]	9.334[c]	3.463[b]
CC 24CH	0.009[a]	0.014[a]	0.012[a]	0.315[b]	0.382[b]	3.166[b]	1.792[b]	4.773[c]	1.430[a]	8.287[c]
CC 30CH	0.007[a]	0.009[a]	0.010[a]	0.475[b]	0.091[a]	3.095[b]	1.892[b]	2.963[b]	12.473[c]	3.292[b]
CC 60CH	0.044[b]	0.008[a]	0.019[a]	0.544[b]	0.269[b]	2.853[b]	0.000[a]	0.000[a]	8.331[c]	4.785[b]
SI 12CH	0.012[a]	0.030[b]	0.028[a]	0.159[a]	0.278[b]	3.285[b]	0.000[a]	2.007[b]	14.501[d]	0.000[a]
SI 24CH	0.025[a]	0.008[a]	0.019[a]	0.173[a]	0.164[a]	7.434[d]	0.000[a]	0.000[a]	6.196[c]	3.885[b]
SI 30CH	0.017[a]	0.021[a]	0.013[a]	0.373[b]	0.381[b]	5.103[c]	0.000[a]	0.000[a]	18.564[e]	7.952[c]
SI 60CH	0.019[a]	0.045[b]	0.016[a]	0.483[b]	0.273[b]	1.815[b]	0.000[a]	1.101[a]	2.602[a]	11.607[d]
SU 12CH	0.024[a]	0.017[a]	0.016[a]	0.378[b]	0.276[b]	5.001[c]	0.000[a]	0.129[a]	0.000[a]	0.000[a]
SU 24CH	0.021[a]	0.040[b]	0.040[a]	0.719[b]	0.244[b]	6.581[d]	0.000[a]	0.000[a]	0.000[a]	2.973[b]
SU 30CH	0.035[a]	0.016[a]	0.010[a]	0.084[a]	0.268[b]	2.582[b]	0.000[a]	1.360[a]	0.000[a]	2.827[b]
SU 60CH	0.066[c]	0.012[a]	0.037[a]	0.284[b]	0.182[a]	2.415[b]	2.370[b]	0.756[a]	0.000[a]	0.000[a]
cv%	52.55	47.49	65.39	57.34	66.49	43.5	122.55	128.96	53.26	53.98

*Means followed by different letters in the same column differ by Scott-Knott ($p < 0.05$). **HAT = hours after treatment application.

0.5% in 23% of the production of phytoalexin when compared to the water control, and homeopathics 12 and 24CH increased an average of 10 and 23% more than the control, respectively, but with values statistically similar to the control with grain alcohol (Figure 2). The application of harpin does not alter the production of phaseolin.

The application of CC increased 27% phaseolin for the treatment 12CH, 26% for 24CH, 18% for 30CH and 21% to 60CH (Figure 2). The values observed for the test, although different potencies reported for the control of water to grains alcohol which were similar (0.3%). There were no significant differences between the results of the potencies used.

The homeopathics SI and SU caused inhibition of the production of phaseolin. The application of SI 12CH inhibited 36% the formation of phaseolin, SI 24CH 36%, 20CH 28% and 60CH 24% (Figure 2). Similarly, the potencies of SU resulted in 41%, average inhibition in 24CH values were equivalent to control.

DISCUSSION

All treatments showed potential elicitor of defense compounds in beans, showing oscillatory behavior of the activity of enzymes induced. The oscillation observed in CAT and POX activity may be related

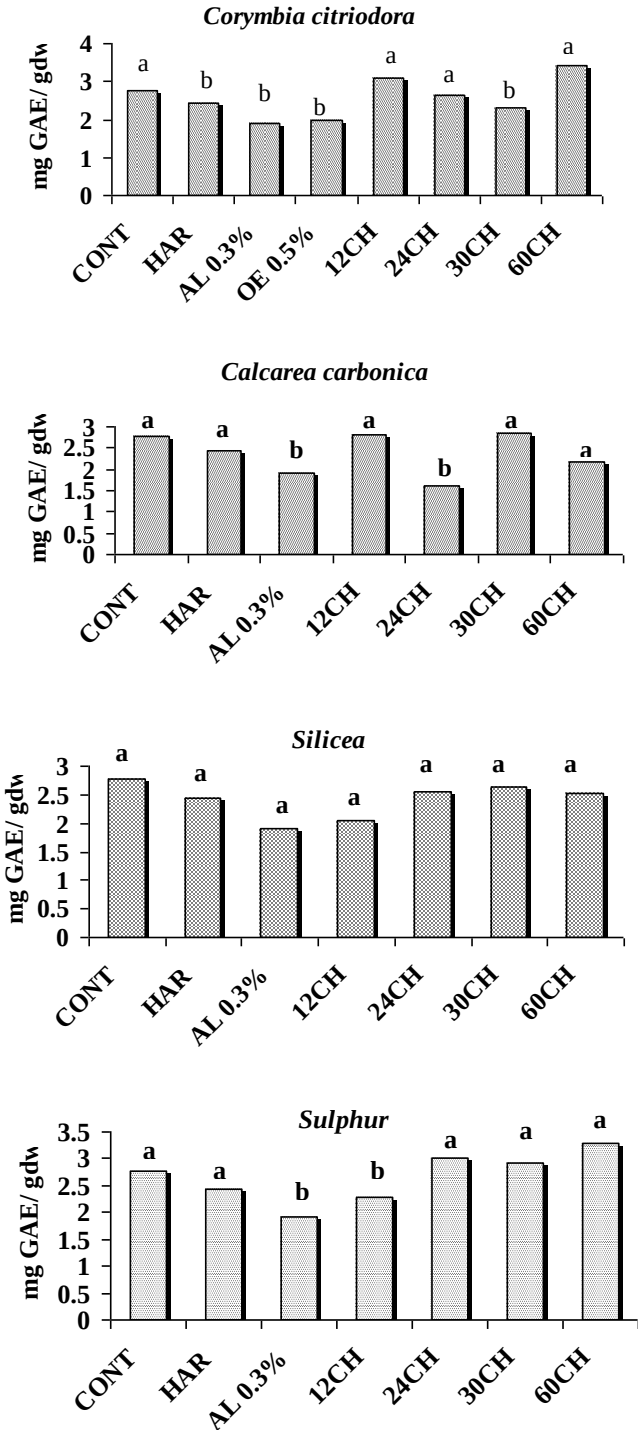

Figure 1. Total phenol content in leaves of bean cv Carioca after treatment with homeopathy *Corymbia citriodora* (EC) and eucalyptus essential oil (EO), *Calcarea carbonica* (CC) *Silica* (SI) and *Sulphur* (SU), averages of four replicates. Means followed by the same letter do not differ by Scott-Knott ($p < 0.05$).

Table 2. Specific activity of Chitinase and Glucanase (absorbance min^{-1} mg^{-1} protein) in leaves of bean cv Carioca treated with homeopathy *Corymbia citriodora* (EC). *Calcarea carbonica* (CC) *Silica* (SI) and *Sulphur* (SU).

Tratamento	Quitinase					Glucanase				
	6 HAT**	12 HAT	24 HAT	48 HAT	216 HAT	6 HAT	12 HAT	24 HAT	48 HAT	216 HAT
Control (H_2O)	0.059[b]*	0.216[a]	0.142[c]	0.039[a]	0.128[a]	0.011[c]	0.080[c]	0.055[b]	0.057[c]	0.026[d]
Alcohol 0.3%	0.058[b]	0.216[a]	0.136[c]	0.031[a]	0.226[b]	0.010[c]	0.096[c]	0.054[b]	0.045[c]	0.054[d]
Harpin	0.055[b]	0.215[a]	0.127[c]	0.038[a]	0.145[a]	0.009[c]	0.102[c]	0.053[b]	0.055[c]	0.056[d]
OE 0.5%	0.060[b]	0.243[b]	0.171[d]	0.049[b]	0.138[a]	0.006[c]	0.073[c]	0.051[b]	0.061[c]	0.035[d]
EC 12CH	0.050[b]	0.225[a]	0.106[c]	0.016[a]	0.165[a]	0.004[c]	0.077[c]	0.050[b]	0.032[c]	0.054[d]
EC 24CH	0.037[a]	0.230[a]	0.089[b]	0.051[b]	0.169[a]	0.002[c]	0.062[c]	0.049[b]	0.053[c]	0.069[d]
EC 30CH	0.032[a]	0.229[a]	0.073[b]	0.042[a]	0.200[b]	0.004[c]	0.060[c]	0.048[b]	0.057[c]	0.104[c]
EC 60CH	0.045[a]	0.226[a]	0.091[b]	0.027[a]	0.156[a]	0.017[b]	0.093[c]	0.041[b]	0.040[c]	0.089[c]
CC 12CH	0.044[a]	0.224[a]	0.079[b]	0.070[b]	0.162[a]	0.018[b]	0.059[c]	0.190[a]	0.113[b]	0.074[d]
CC 24CH	0.028[a]	0.219[a]	0.046[a]	0.057[b]	0.161[a]	0.008[c]	0.059[c]	0.020[c]	0.082[c]	0.052[d]
CC 30CH	0.036[a]	0.211[a]	0.057[a]	0.090[b]	0.169[a]	0.006[c]	0.050[c]	0.031[c]	0.117[b]	0.053[d]
CC 60CH	0.030[a]	0.222[a]	0.056[a]	0.054[b]	0.139[a]	0.008[c]	0.062[c]	0.021[c]	0.079[c]	0.062[d]
SI 12CH	0.038[a]	0.216[a]	0.033[a]	0.031[a]	0.165[a]	0.011[c]	0.072[c]	0.013[c]	0.052[c]	0.071[d]
SI 24CH	0.031[a]	0.224[a]	0.041[a]	0.126[c]	0.161[a]	0.009[c]	0.072[c]	0.005[c]	0.149[a]	0.076[d]
SI 30CH	0.031[a]	0.211[a]	0.032[a]	0.149[c]	0.246[b]	0.009[c]	0.132[b]	0.012[c]	0.173[a]	0.118[b]
SI 60CH	0.050[b]	0.230[a]	0.046[a]	0.161[c]	0.154[a]	0.011[c]	0.200[a]	0.009[c]	0.176[a]	0.082[b]
SU 12CH	0.067[b]	0.247[b]	0.062[a]	0.146[c]	0.204[b]	0.019[b]	0.185[a]	0.022[c]	0.174[a]	0.089[b]
SU 24CH	0.075[b]	0.255[b]	0.064[a]	0.081[b]	0.218[b]	0.018[b]	0.130[b]	0.041[b]	0.115[b]	0.151[a]
SU 30CH	0.065[b]	0.245[b]	0.057[a]	0.056[b]	0.208[b]	0.041[a]	0.118[b]	0.031[c]	0.117[b]	0.064[d]
SU 60CH	0.119[c]	0.299[c]	0.061[a]	0.003[a]	0.157[a]	0.046[a]	0.142[b]	0.030[c]	0.050[c]	0.091[b]
cv%	23.26	6.35	29.51	35.01	19.52	44.07	33.39	41.64	32.66	26.91

*Means followed by different letters in the same column differ by test of Scott-Knott test ($p < 0.05$). ** HAT = hours after treatment application.

to characteristics of these enzymes. A point to be analyzed was the times that there was a reduction in activity because they coincide largely with opposite effects on the activity of another enzyme. Possibly, this is due to the fact that these enzymes are involved in the production of reactive oxygen species (ROS). During this process, the superoxide radical can undergo redox reactions transformed into H_2O_2 is converted to O_2 and H_2O by the action of catalase, or it may be converted to H_2O by the action of peroxidase (Soares and Machado, 2007).

The reduction of POX activity may be related to the activation of CAT, which might explain the alternation observed in the activities, and higher activities of catalase and peroxidase inactivation and vice versa, as both work as the same substrate (Marafon et al., 2009). Cavalcanti et al. (2005) showed that the peroxidase enzyme is related to events involving induction of resistance, there is a definite pattern to their behavior, which depends on the type of inducer, its concentration, time after application and plant pathosystem studied. The possible increase in the quantity of

Table 3. Concentration of chlorophyll *a*, *b* and total in bean plants cv Carioca treated with homeopathic remedies *Corymbia citriodora*, *Calcarea carbonica*, *Silicea* and *Sulphur*. Values expressed in mg g^{-1} fresh tissue.

Treatment	Chlorophyll *a*	Chlorophyll *b*	Chlorophyll total
Test H_2O	0.121[a]*	0.044[a]	0.165[a]
Álcool 0.3%	0.133[a]	0.051[a]	0.184[a]
Harpina	0.121[a]	0.042[a]	0.164[a]
OE 0.5%	0.115[a]	0.043[a]	0.158[a]
OE 1%	0.111[a]	0.045[a]	0.156[a]
EC 12CH	0.111[a]	0.043[a]	0.155[a]
EC 24CH	0.117[a]	0.045[a]	0.162[a]
EC 30CH	0.117[a]	0.044[a]	0.162[a]
EC 60CH	0.100[b]	0.039[a]	0.139[a]
CC 12CH	0.106[a]	0.041[a]	0.147[a]
CC 24CH	0.109[a]	0.042[a]	0.152[a]
CC 30CH	0.108[a]	0.042[a]	0.151[a]
CC 60CH	0.121[a]	0.046[a]	0.168[a]
SI 12CH	0.109[a]	0.041[a]	0.150[a]
SI 24CH	0.080[b]	0.021[b]	0.102[b]
SI 30CH	0.080[b]	0.024[b]	0.105[b]
SI 60CH	0.089[b]	0.018[b]	0.108[b]
SU 12CH	0.099[b]	0.021[b]	0.121[b]
SU 24CH	0.090[b]	0.019[b]	0.109[b]
SU 30CH	0.096[b]	0.021[b]	0.118[b]
SU 60CH	0.090[b]	0.020[b]	0.111[b]
cv%	16.58	17.09	15.54

*Means followed by different letters vertically differ by Scott-Knott test ($p < 0.05$).

phenolic compounds may contribute to reducing levels of POX (Brum, 2010).

This effect on the amount of phenolic compounds has been verified by Das Dores (2010) in bean plants treated with homeopathic *Sulphur* 12CH in which it was found that there was an increase in the production of phenolic compounds evaluated, different that observed in this study. The reduction in the accumulation of phenolic in treated plants may be due to an imbalance between the production and consumption of phenolic intermediates, contributing to reduce the amount of phenolic compounds. More than that, higher activities of enzymes downstream in the phenylpropanoid pathway, such as chalcone synthase (CHS), and cinnamyl alcohol dehydrogenases, that could have an effect promoting drain on soluble phenolic substrates. There were reports of lower levels of phenolic compounds in tissues which were observed by POX activity in the similarity observed in this study (Cavalcanti et al., 2006). As observed for the CAT and POX homeopathies tested, it increased the activity of CHI and GLU. These enzymes have received attention as important components of the arsenal of plant defense proteins, that hydrolyse the major carbohydrate cell wall of fungi (chitin and β-1.3-glucan), with direct action on fungi inhibiting their growth and releasing oligosaccharide elicitors that induce the production of phytoalexins (Di Piero and Garda, 2008).

In this study we observed a similar pattern of increase for both enzymes in the same time of evaluation. Typically, chitinases and β-1.3-glucanases exhibit patterns of regulation, that are co-regulated which would explain the decrease in activity at certain moments as the period of gene transcription. Costa et al. (2000) verified that the deletion of chitinase activity, demonstrated in his work with transgenic plants was due to the duration of the process of gene transcription.

The control treatment with harpin also induced the activity of POX, CAT and GLU, and also showed the same oscillatory pattern of homeopathy. The increases caused by harpin were significant in the initial assessments between 6 and 24 HAT. This response is in agreement to that described by Barbosa-Mendes (2007) which the application of harpins within minutes after application, changed the membrane potential, causing oxidative burst and changes in ion flux. The harpins are also related to increases in the activity of phenylalanine ammonia-lyase, responsible for the synthesis of phytoalexins (Danner et al., 2008).

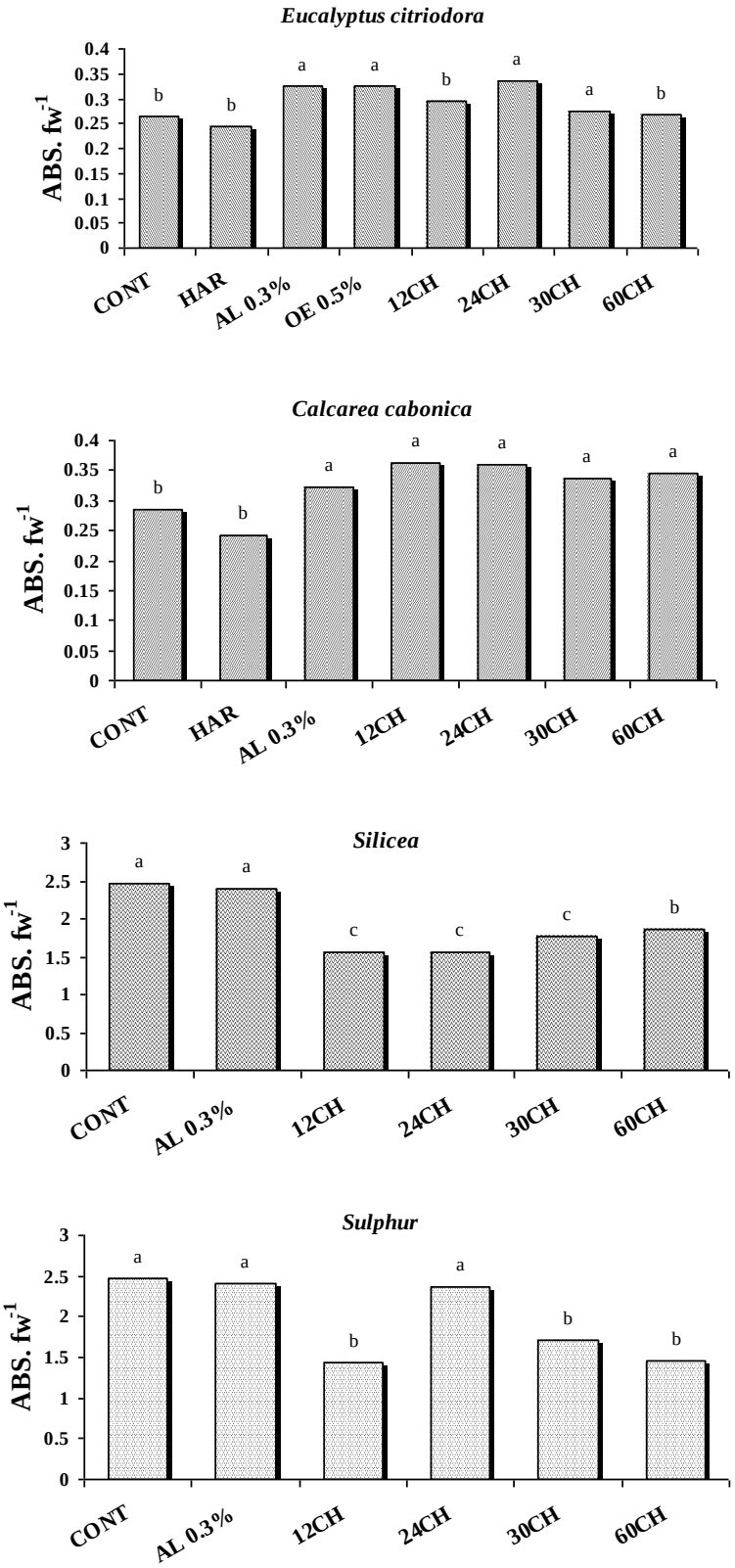

Figure 2. Production of Phaseolin in hypocotyls etiolated bean cv Cariocaafter treatment with homeopathy *Corymbia citriodora* (EC) and eucalyptus essential oil (EO). *Calcarea carbonica* (CC) *Silica* (SI) and *Sulphur* (SU).averages of four replicates, means followed by the same letter do not differ by the Scott-Knott test ($p < 0.05$).

However, this research was not induced with the production of chitinase by phaseolin and application of harpin. The comparison between the mean values, the result of the application of homeopathy and those resulting from the application of harpin show that the homeopathy when provoked induction were more efficient compared to the commercial product, in quantities as in the period of sustained elicitation. As the molecules of chlorophyll *a* and *b* are the two pigments responsible for the absorption and transfer of radiant energy (Viecelli et al., 2009), these results suggest a shift in the production of primary energy for synthesis of plant defense compounds.

It can be seen that the treatments applied in this work which reduced the amount of chlorophyll (SI, SU) were the same with the highest results in induction of catalase and peroxidase. These results suggest a deviation of carbon skeletons that directed the prime route to the sideline plant metabolism.

Regarding the production of phaseolin, the inhibition observed in plants treated with SI and the potencies 12, 30 and 60CH of SU may be related to resumption of homeostatic balance, which would require less production of defensive compounds and consequently lower metabolic cost due the balance of the plants. The metabolic cost required to produce phytoalexins, can result in disfavoring the primary pathways for the production of defense compounds, resulting from the activation of latent defense mechanisms (Barros et al., 2010).

However, increased production of these compounds as found in treatments with CE, CC and 24CH of SU, is important in the process of plant defense, justifying the expense required for its production. Considering that the antimicrobial phytoalexin substances are widely associated with vertical resistance or immunity (Matiello et al., 1997).

As observed in this study, there is a variation in the response to homeopathic treatments, which can act both in induction or reduction in the synthesis of secondary compounds. Fonseca et al. (2006) found effects of inducers and reducers of homeopathy on the tannin content in *P. ruderale* (cauliflower cloves), which varied according to homeopathy, promotion and treatment period. CC and SU single application increased tannin content assessed as SU and SI under multiple acted in the opposite direction.

The absence of universal responses described for homeopathic treatments was also observed in this study. According to the results observed, the effects are of variations between the different potencies of the same drug, but with no dose effect dependence.

It was found that for peroxidase activity, catalase, and production of phaseolin, the best treatments were potencies CC (*C. carbonica*); in the induction of chitinase and β-1.3-glucanase, applications of SU (*Sulphur*) were higher values relative to the water control and consistency between the hours.

Conclusion

In this study, the *C. citriodora* homeopathy, *C. carbonica*, *Silicea* and *Sulphur* showed potential in the elicitation of peroxidase, catalase, quitinase, β-1.3-glucanase and phytoalexin. These results show the potential of these preparations in the process of plant protection that can be a tool in the search for mechanisms for controlling plant pathogens, which are less harmful to the environment.

Conflicts of interest

The author(s) have not declared any conflict of interests.

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Growth and yield in chickpea (*Cicer arietinum* L.) genotypes in response to water stress

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Twenty chickpea genotypes were grown under rainout shelter to investigate the influence of water stress treatments imposed at varied growth stages; T_1; Control, T_2; One pre-sowing irrigation, T_3; withholding irrigation at flower-initiation, T_4; withholding irrigation at pod-initiation stage. The plant height, branches, dry weight of stem, leaves and root plant^{-1}, leaf area, leaf area index were recorded at 120 days after sowing (DAS) which showed significant variation with water stress at varied growth stages. The maximum reduction in height and branches was observed when irrigation was restricted at T_2 stage. Restricted irrigation decreased the biomass of stem, leaves and roots leading to reduced leaf area and leaf area index as well. The yield traits viz. 100 seed weight, total number of pods, percentage filled pods were reduced significantly under stress. The grain yield under restricted conditions was reduced by 40.50 to 55.91% over irrigated control in T_4 to T_2, respectively. Among the tested genotypes, GL28151, RSG963, PDG3 maintained higher growth, yield and yield traits showing their tolerance to water stress, while GL22044, RSG1861 and RVSSG4 were adversely affected most in growth traits and yield as well.

Key words: Water stress, chickpea, growth traits, yield.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the fourth largest grain legume crop in the world, with a total production of 10.9 million tons from an area of 12.0 million ha and a productivity of 0.91 t ha^{-1}. Major producing countries include India, Pakistan and Iran (FAO, 2010b). About 90% of chickpea in the world is grown under rainfed conditions where drought is one the major constraints, limiting its production. Drought affects various morphological and physiological processes, resulting in reduced growth, development and economic yield of crop. Water stress has prominent effect on leaf number, total leaf area and secondary branches causing invariable reduction under rainfed conditions (Basu et al., 2007). The major characters affecting crop grain yield are number of pods and seeds per plant which reduce under drought stress (Davies et al., 2000). Several studies have shown that optimum yield can be obtained by irrigation at branching, flowering and pod

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formation stages (Prihar and Sandhu, 1968).

The reactions of plants to water stress vary depending upon intensity and duration of stress as well as plant species and its stage of growth. Stress during vegetative phase reduce grain yield through reducing plant size, restricting leaf area, dry matter accumulation and limiting number of pods (Sadasivan et al., 1988). However, water deficits at the flowering and the post flowering stages have been found to have greater adverse impact than at the vegetative stage (Cortes and Suidaria, 1986). Present study was conducted to investigate genotypic response towards deteriorative effects of water stress by determination of growth and yield traits in twenty diverse chickpea genotypes and to find most sensitive growth stage in chickpea to water stress by imposition of stress at varied growth stages.

MATERIALS AND METHODS

Twenty chickpea genotypes for studies on moisture stress tolerance (GL21107, GL22044, GL26054, GL26074, GL281137, GL28151, GL28186, GNG1594, GNG1861, DCP 92-3, GG1362, RSG811, RVSSG4, RSG963, RSG957, BGM547, PDG3, PDG4, PBG1, GPF2) were procured from Pulses section, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, Punjab, India and grown in the field area of Department. Ludhiana represents the Indo-Gangetic plains and is situated at 36°-54'N latitude, 25°-48'E longitude and at a mean height of 247 m above sea level. The field was ploughed and leveled properly and divided into 120 plots each measuring 1.8 m^2. Trial was sown in three replications in split plot design. Sowing of seeds was undertaken in the field on 22nd November, 2011 during *Rabi* season 2011 to 2012.

Experimental design included following irrigation treatments. T_1- Without stress (control) given irrigation as and when required; T_2- One pre-sowing irrigation; T_3- Stressed by withholding irrigation at flower initiation; T_4- Stressed by withholding irrigation at pod initiation. The experiment was conducted under rainout shelter to meet the stress levels and data on growth parameters of randomly selected plants were recorded at 120 days after sowing. Plant height, branches, leaf number of randomly selected plants per plot was recorded. Stem dry weight, root dry weight, leaf dry weight of selected plants were derived by chopping the parts of plants and drying in oven at 72°C till a constant weight was derived. Leaf area was measured by leaf area meter CID Inc-213 and expressed as cm^2. Leaf area index was measured by Sun scan canopy analyzer. Yield characteristics viz. 100 seed weight, number of pods, percentage filled pods and yield were recorded at final harvest.

RESULTS

The results showed that the effect of water stress treatments, genotypes and the interactions of genotypes × treatment were significant in all growth traits viz. plant height, branches, leaf number, dry weight of stem, leaves and root, leaf area and leaf area index. Exception was dry weight of root where interaction of genotype × treatment was found to be non significant. Imposed water deficit reduced various growth traits in stressed treatments, in comparison to control. Treatment sown with one presowing irrigation (T_2) was affected most, followed by T_3, where irrigation was with held at flower initiation. Among the genotypes, GL28151 showed least reduction in plant height, number of branches (Table 1) and leaf number (Table 3) under stress treatments, except T_3, where RSG963 performed better with respect to number of branches showing percentage reduction of 2.11%. Effect of water stress was most deteriorative in RVSSG4, reducing plant height under treatments T_2, T_3 and T_4 by 30.32, 17.56 and 8.00%, respectively. GL22044 showed marked reduction in branches and leaf number under all stress treatments, however highest decline was observed under treatment T_2 where branches and leaf number reduced in GL22044 by 50.90 and 52.49%, respectively over control.

Dry weight of stem, leaves and roots decreased under stress treatments in comparison to control in all genotypes. Among genotypes, stem dry weights of GL28151 and PDG3 were recorded with minimum reductions (Table 2) whereas PDG3 recorded least percentage reduction of 13.14% under treatment T_4. GNG1861 and RVSSG4 showed tremendous decline over control under stress treatments, though highest decrease of 42.42% was noticed in GNG1861 under treatment T_2. RSG963 was able to resist drop in leaf dry weight (Table 2) under all stress treatments, though marked reduction in GNG1861 were observed, which showed highest alterations of 39.29% occurred under treatment T_2. Among, genotypes, RSG963 and PDG3 were observed with minimal percentage reductions in root dry weight (Table 3), where least reduction of 8.60% was recorded in RSG963 under treatment T_4. Decrease in root dry weight was high in GL22044 and RVSSG4, with tremendous decline of 38.67% in RVSSG4 under treatment T_2.

Water stress reduced leaf area and leaf area index significantly in all genotypes under water stress treatments in comparison to control (Table 4). Water deficit posed least affect on leaf area of RSG963 showing change of 10.88% under T_4 treatment. Dramatic reduction was noticed in GNG1861 under stress treatments; highest was 49.52% under treatment T_2. GL28151 depicted least alterations in leaf area index among genotypes. Percentage reduction varied between 31.21 to 16.14% under treatment T_2 and T_4, respectively. GL22044 and GNG1861 were observed to show highly deteriorating effects of water stress on LAI, however, maximum change over control was 60.96% observed in GL22044 under treatment T_2.

Drought stress imposed resulted in lower yield and yield traits in all genotypes under stress treatments in comparison to control. Highest reductions were observed under treatment T_2, followed by T_4. Least change in 100 seed weight (Table 5) was noticed in RSG963 under

Table 1. Effect of water stress imposed at pod initiation stage (120 DAS) on plant height (cm) and number of branches of chickpea (*Cicer arietinum* L.) genotypes.

Genotype	Plant height (cm)				Number of branches			
	T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4
GL21107	58.33±0.95	45.66±1.61	54.66±1.86	57.00±1.01	23.67±1.25	7.67±0.73	13.33±0.58	15.33±1.47
GL22044	59.09±0.81	45.66±1.20	54.33±1.58	55.09±0.87	37.33±0.70	9.00±0.06	18.33±0.07	19.22±0.59
GL26054	60.00±0.80	51.88±0.91	55.66±0.68	58.33±1.38	20.67±1.14	9.00±0.06	11.33±0.17	19.22±0.23
GL26074	55.00±0.89	50.33±0.93	52.00±1.47	53.66±1.11	22.33±1.61	13.33±0.64	13.89±0.53	15.33±1.24
GL28137	57.33±1.50	53.33±0.95	54.66±2.46	55.00±0.87	32.67±0.70	19.00±0.57	20.33±2.00	23.33±0.99
GL28151	58.00±0.87	56.33±0.62	56.88±0.85	57.00±1.41	25.33±0.90	20.56±0.99	22.67±0.43	24.33±1.05
GL28186	54.80±0.26	47.66±0.85	50.33±0.90	51.66±0.93	19.00±0.13	13.67±0.81	14.44±1.03	17.33±0.73
GNG1594	51.00±0.68	48.00±1.12	48.66±0.97	49.00±0.60	15.67±0.73	9.00±0.04	12.89±1.38	14.99±1.01
GNG1861	55.00±1.33	44.33±0.59	46.33±1.41	51.00±2.97	25.00±0.44	15.67±0.70	17.56±0.54	18.67±1.49
DCP 92-3	56.66±0.76	43.66±1.94	48.66±0.67	52.90±1.46	14.33±0.55	11.00±0.61	12.55±0.83	13.33±1.20
GG1362	62.00±0.94	50.00±0.61	57.661.03	58.66±0.70	26.00±0.61	17.80±0.51	19.00±0.42	20.00±0.59
RSG 811	62.00±1.56	45.00±0.88	52.00±0.87	58.00±1.66	30.00±0.68	12.00±0.72	23.67±0.54	28.00±0.75
RVSSG 4	62.66±0.76	43.66±0.74	51.66±0.83	57.65±0.78	30.67±1.51	15.67±0.35	20.22±0.21	20.22±0.64
RSG 963	60.66±0.86	56.00±0.82	56.33±0.91	57.00±1.76	24.67±1.97	17.89±0.39	20.89±0.73	24.00±1.09
RSG 957	61.66±1.39	47.66±0.57	55.66±0.69	57.33±0.57	30.67±0.93	18.67±0.95	24.44±0.52	27.67±0.84
BGM 547	62.33±0.56	53.44±1.32	58.33±00.42	60.00±0.74	33.00±0.64	18.99±0.13	20.00±0.55	21.33±0.67
PDG3	55.00±0.64	45.34±0.61	46.77±1.93	47.00±0.97	27.66±1.20	20.33±1.80	23.38±0.69	24.00±0.84
PDG4	57.66±1.69	47.00±1.88	48.66±0.99	52.66±1.93	24.00±0.82	18.00±0.65	20.00±1.34	20.67±0.62
PBG1	60.66±1.74	49.33±2.55	55.33±0.78	57.44±0.40	21.67±1.66	13.33±1.05	17.00±0.23	18.22±0.64
GPF2	56.66±0.59	48.00±0.76	49.66±3.27	52.33±1.66	18.96±0.68	12.34±0.58	14.44±0.57	15.32±1.49
	LSD (0.05)G = 0.790, LSD(0.05) T = 1.768, LSD (0.05) G×T = 3.536				LSD (0.05)G = 0.548, LSD(0.05) T = 1.226, LSD (0.05) G×T = 2.452			

*Different values in each column represent mean ±S.E.

stress treatments T_3 and T_4, while GL28151 performed well under stress treatment T_2, with lowest change of 4.17% over control. Highest decrease in 100 seed weight was noticed in GNG1861 under treatment T_3 and T_4, where maximum reduction of 13.07% was shown in GL22044 under treatment T_2. However, effect of stress treatments on total number of pods was recorded least in GL28151 (Table 5), with minimum reduction of 3.53% under stress treatment T_4. Percentage decline in pod number was maximum in RVSSG4 and GNG1861 with highest drop of 52.62% in RVSSG4 under treatment T_2.

Percentage filled pods were least affected in RSG963, PDG3 and GL28151 under varied treatments, with least reduction of 2.13% in PDG3 under treatment T_4 (Table 6). However, stress treatments remarkably altered percentage filled pods in RVSSG4, where maximum decrease of 17.08% noticed under treatment T_2. Yield was less affected in GL28151 under T_2 (13.04%) and T_4 (11.11%) treatments showing its tolerance. Under T_3 treatment, RSG963 performed better showing least reduced yield (8.54%) over control. Yield under stress treatments was determined highly affected in GL22044 under all stress

Table 2. Effect of water stress imposed at pod initiation stage (120 DAS) on dry weight of stem plant^{-1} and dry weight of leaves plant^{-1} of chickpea (*Cicer arietinum* L.) genotypes.

Genotype	Dry weight of stem plant^{-1} (g)				Dry weight of leaves plant^{-1} (g)			
	T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4
GL21107	6.01±0.17	4.20±0.07	4.98±0.11	5.11±0.07	4.85±5.70	3.42±0.05	3.90±0.02	4.11±0.09
GL22044	6.33±0.05	3.75±0.12	5.01±0.03	5.08±0.06	6.10±0.267	3.82±0.07	4.10±0.07	4.28±0.02
GL26054	6.26±0.03	4.35±0.07	5.19±0.03	5.25±0.03	5.36±0.07	4.30±0.04	4.54±0.04	4.76±0.06
GL26074	6.44±0.15	4.11±0.01	5.20±0.04	5.23±0.04	5.55±0.07	4.76±0.13	4.85±0.13	4.92±0.12
GL28137	6.27±0.09	4.06±0.02	5.10±0.00	5.20±0.05	5.40±0.11	4.44±0.11	4.67±0.11	4.85±0.04
GL28151	6.20±0.01	5.11±0.05	5.25±0.03	5.38±0.08	6.16±0.04	5.45±0.06	5.50±0.06	5.58±0.24
GL28186	6.19±0.03	4.44±0.11	5.05±0.03	5.14±0.04	5.88±0.11	4.65±0.24	4.90±0.24	5.20±0.06
GNG1594	6.19±0.02	4.06±0.03	5.00±0.06	5.19±0.03	5.07±0.11	4.31±0.13	4.48±0.11	4.52±0.50
GNG1861	6.35±0.11	3.65±0.14	4.76±0.06	5.09±0.02	5.14±0.05	3.12±0.03	3.34±0.11	3.54±0.04
DCP 92-3	6.30±0.09	4.12±0.07	5.02±0.02	5.10±0.05	5.30±0.04	3.76±0.36	4.11±0.18	4.32±0.06
GG1362	6.33±0.12	4.89±0.06	5.06±0.15	5.22±0.06	6.38±0.04	4.88±0.12	5.00±0.11	5.45±0.04
RSG 811	6.38±0.08	4.90±0.01	5.05±0.03	5.17±0.08	6.09±0.04	4.45±0.11	4.66±0.06	4.90±0.02
RVSSG 4	6.48±0.13	3.91±0.11	5.04±0.02	5.11±0.12	5.93±0.01	3.65±0.23	4.00±0.06	4.22±0.07
RSG 963	6.31±0.02	5.18±0.05	5.22±0.06	5.38±0.08	5.98±0.13	5.32±0.10	5.38±0.04	5.44±0.07
RSG 957	6.40±0.10	4.54±0.06	5.26±0.03	5.36±0.13	6.04±0.16	4.75±0.07	5.11±0.09	5.35±0.07
BGM 547	6.45±0.09	4.35±0.06	5.25±0.03	5.31±0.18	4.90±0.21	4.10±0.15	4.23±0.07	4.40±0.07
PDG3	6.02±0.06	4.76±0.04	5.02±0.05	5.22±0.11	6.30±0.07	5.45±0.08	5.60±0.18	5.69±0.05
PDG4	6.11±0.10	4.39±0.08	5.15±0.13	5.18±0.03	4.86±0.09	3.27±0.08	3.88±0.41	4.18±0.05
PBG1	6.47±0.06	4.23±0.07	5.24±0.04	5.32±0.10	5.18±0.52	3.90±0.08	4.25±0.19	4.66±0.07
GPF2	6.45±0.07	4.08±0.02	5.22±0.06	5.29±0.08	5.40±0.04	4.45±0.11	4.68±0.22	4.86±0.03
	LSD (0.05)G = 0.048, LSD(0.05) T = 0.107 , LSD (0.05) G×T = 0.213				LSD (0.05)G = 0.083, LSD(0.05) T = 0.186, LSD (0.05) G×T = 0.372			

*Different values in each column represent mean ±S.E.

treatments (Table 6), though highest reduction of 55.91% under treatment T_2, depicting its lesser adaptive behavior towards water deficit.

Under control conditions (T_1), there were positively non-significant correlations between yield and most of the traits, except significant positive and positive correlation with 100 seed weight (r = 0.3307**) and percentage filled pods (r = 0.2183), respectively (Table 7). However, plant height (r = -0.0307) and dry weight of root (r = -0.0201) were negatively correlated with yield under control.

Under stressed treatments, yield showed positive and highly significant correlation with dry weight of stem, root and leaves, leaf number, leaf area, leaf area index and yield traits viz. 100 seed weight, percentage filled pods and total number of pods.

Number of branches showed non-significant positive correlation (r = 0.2413) with yield under stress treatments T_2 (Table 8), T_3 and T_4, Plant height was found to show non-significant positive correlation (r = 0.1550) under treatment T_3 (Table 9) whereas negative correlation (r = -0.1223) of plant height with yield was observed under treatment T_4 (Table 10).

DISCUSSION

This investigation showed the negative effect of drought on growth traits of chickpea. Treatment T_2 with one pre-sowing irrigation was affected most,

Table 3. Effect of water stress imposed at pod initiation stage (120 DAS) on dry weight of root plant^{-1} and number of leaves plant^{-1} of chickpea (*Cicer arietinum* L.) genotypes.

Genotype	Dry weight of root plant^{-1} (g)				Number of leaves plant^{-1}			
	T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4
GL21107	3.10±0.19	2.11±0.07	2.24±0.14	2.52±0.17	164.00±9.72	87.33±5.67	94.34±2.63	112.33±4.96
GL22044	3.16±0.03	2.05±0.04	2.19±0.10	2.28±0.08	258.66±13.69	122.90±6.39	146.50±4.18	160.00±3.18
GL26054	3.21±0.08	2.13±0.02	2.52±0.12	2.64±0.06	206.66±6.84	123.66±7.58	151.00±6.85	167.80±7.20
GL26074	3.27±0.07	2.15±0.20	2.43±0.14	2.68±0.51	193.00±5.31	128.00±8.51	150.66±6.34	172.30±5.21
GL28137	3.10±0.05	2.14±0.09	2.43±0.05	2.53±0.06	208.00±2.64	155.70±11.17	172.30±4.20	182.00±4.38
GL28151	3.13±0.01	2.27±0.11	2.59±0.03	2.59±0.26	255.00±6.97	220.80±5.58	223.40±10.29	36.80±10.10
GL28186	3.22±005	2.11±0.08	2.52±0.06	2.60±0.23	234.66±6.39	151.66±7.94	179.66±2.93	192.50±7.11
GNG1594	3.02±0.05	2.36±0.06	2.37±0.02	2.41±0.20	176.33±4.54	110.90±3.94	144.30±6.95	150.33±3.20
GNG1861	3.18±0.07	2.21±0.01	2.26±0.20	2.32±0.10	187.00±10.50	95.00±4.06	112.00±5.88	126.66±7.55
DCP 92-3	3.12±0.05	1.98±0.10	2.51±0.14	2.56±0.22	172.66±3.91	135.30±11.49	137.90±8.20	150.33±7.15
GG1362	3.16±0.11	2.28±0.09	2.39±0.09	2.46±0.07	269.66±9.63	172.60±4.99	199.00±11.63	211.00±10.64
RSG 811	3.21±0.06	2.46±0.18	2.49±0.21	2.54±0.11	250.66±9.41	172.66±4.31	186.54±11.57	210.80±2.83
RVSSG 4	3.31±0.05	2.03±0.00	2.37±0.09	2.45±0.08	241.00±6.15	102.30±3.58	152.34±4.35	173.00±16.36
RSG 963	3.51±0.05	2.87±0.06	3.12±0.21	3.21±0.07	240.00±6.67	175.30±4.01	192.31±12.80	212.20±5.86
RSG 957	3.45±0.05	2.68±0.18	2.67±0.05	2.75±0.03	247.66±13.39	166.00±3.31	190.00±5.93	210.90±3.73
BGM 547	3.28±0.09	2.58±0.11	2.61±0.06	2.68±0.04	231.33±5.79	145.67±9.90	168.33±6.29	184.33±5.05
PDG3	3.15±0.04	2.58±0.08	2.59±0.07	2.61±0.04	278.00±5.36	167.00±6.63	199.00±11.66	210.00±2.95
PDG4	3.14±0.05	2.44±0.11	2.48±0.10	2.49±0.06	241.00±6.17	169.80±3.26	184.33±4.20	193.33±3.79
PBG1	3.30±0.05	2.47±0.12	2.57±0.28	2.60±0.01	222.00±9.43	131.00±.7.41	161.23±3.49	189.00±7.52
GPF2	3.28±0.05	2.45±0.11	2.55±0.18	2.59±0.10	176.39±4.59	103.28±3.60	140.28±3.21	143.39±6.67
	LSD (0.05)G = 0.078, LSD(0.05) T = 0.175, LSD (0.05) G×T = NS				LSD (0.05)G = 4.572, LSD(0.05) T = 10.222 , LSD (0.05) G×T = 20.445			

*Different values in each column represent mean ±S.E.

followed by stress at flower initiation stage (T_3). Our findings are in agreement with Yaqoob et al. (2012), who suggested that moisture stress at pre flowering stage being harmful and detrimental is the most critical stage for screening chickpea germplasm under drought prone conditions. Significant reductions occurred in plant height and number of branches under varied stress treatments. Similar results were given in chickpea (Shamsi et al., 2010). Reduction in plant height could be attributed to decrease in cell division, cell enlargement under water stress (Manivannan et al., 2007a). Under water deficiency, cell elongation of higher plants can be inhibited by interruption of water flow from xylem to thesurrounding elongating cells leading to reduced number of branches.

In our study, dry weight of stem, root and leaves decreased under stress treatments in all genotypes. The reduction in biomass could be due to either impaired reduced cyclin dependent kinase activity resulted in lesser cell division and vegetative growth reducing dry weight of shoot (Schupper et al., 1998). Decrease in dry weight of root was noticed in chickpea (Millan et al., 2006). It is attributed to reduce partitioning of biomass towards root (Pimratch et al., 2008).

Table 4. Effect of water stress imposed at pod initiation stage (120 DAS) on leaf area and leaf area index of chickpea (*Cicer arietinum* L.) genotypes.

Genotype	Leaf area (cm^2)				Leaf area index			
	T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4
GL21107	754.39±22.38	483.25±4.32	574.39±8.28	624.39±5.13	4.50±0.30	1.77±0.29	2.47±0.05	2.93±0.12
GL22044	900.34±10.56	490.67±8.35	540.12±2.79	586.55±4.60	5.38±0.13	2.10±0.12	2.64±0.13	2.80±0.07
GL26054	812.67±7.64	592.12±12.71	648.79±11.97	702.34±8.03	7.50±0.21	3.45±0.17	3.93±0.21	4.11±0.03
GL26074	854.44±15.00	678.87±10.90	700.56±10.84	734.56±5.73	6.90±0.10	3.13±0.06	3.73±0.10	4.13±0.22
GL28137	865.39±46.81	644.56±10.65	700.65±10.84	745.56±4.62	8.20±0.04	4.12±0.06	4.45±0.04	4.83±0.06
GL28151	1034.67±44.49	845.67±29.22	865.77±21.42	912.35±6.96	7.56±0.17	5.20±0.09	6.12±0.17	6.34±0.11
GL28186	964.38±74.56	672.34±4.92	734.55±8.88	811.34±16.62	7.44±0.11	4.07±0.05	5.21±0.11	5.64±0.12
GNG1594	812.56±11.55	612.33±9.37	645.56±5.69	700.67±6.20	6.23±0.06	2.65±0.05	3.21±0.06	3.45±0.04
GNG1861	823.45±17.15	415.68±3.42	468.58±1.97	510.45±16.19	8.20±0.07	3.27±0.10	3.55±0.07	3.66±00.06
DCP 92-3	845.78±5.35	534.22±5.70	612.34±6.53	652.34±4.32	7.27±0.09	3.17±0.03	3.66±0.09	3.88±0.13
GG1362	976.54±12.52	678.46±19.00	710.67±9.95	782.34±13.39	6.53±0.21	2.88±0.17	3.24±0.21	3.53±0.15
RSG 811	996.84±31.31	654.33±15.16	702.19±14.09	748.38±9.91	7.13±0.05	2.88±0.06	3.55±0.05	4.12±0.03
RVSSG 4	956.47±6.71	502.45±6.49	572.34±13.56	609.88±15.22	5.43±015	2.12±0.06	2.52±0.15	2.64±0.08
RSG 963	1023.67±50.41	848.78±31.93	880.94±10.93	912.22±6.66	7.50±0.27	4.12±0.06	5.12±0.27	5.33±0.07
RSG 957	995.67±25.52	712.34±13.19	790.67±17.61	834.55±5.71	7.40±0.08	3.37±0.02	3.80±0.08	4.12±0.06
BGM 547	784.34±24.04	573.56±7.54	614.22±14.79	662.34±6.26	6.50±0.09	3.37±0.03	4.10±0.09	4.23±0.02
PDG3	1134.00±56.81	886.56±69.31	942.12±18.01	984.33±34.04	6.37±0.06	3.53±0.21	4.80±0.06	5.12±0.06
PDG4	882.34±56.31	522.34±6.19	645.55±3.50	721.34±6.775	6.23±0.06	3.23±0.12	3.67±0.06	3.81±0.06
PBG1	823.54±5.78	536.67±4.81	600.42±2.78	688.45±7.78	7.45±0.07	3.80±0.08	4.11±0.07	4.20±0.04
GPF2	876.54±10.54	623.35±5.58	682.34±3.22	745.55±11.58	7.49±0.04	3.87±0.06	4.21±0.04	4.33±0.03
	LSD (0.05)G = 13.485, LSD(0.05) T = 30.153 , LSD (0.05) G×T = 60.307				LSD (0.05)G = 0.0721, LSD(0.05) T = 0.161 , LSD (0.05) G×T = 0.322			

*Different values in each column represent mean ±S.E.

Reduced dry weight of leaves was observed in soybean (Silvente et al., 2012). Drought stress inhibits the dry matter production largely through its inhibitory effects on leaf expansion, leaf development and consequently reduced light interception (Nam et al., 2001).

Our results revealed tremendous reduction in the leaf attributes viz. number of leaves, leaf area and leaf area index. Reduced number of leaves and leaf area in response to water deficit was noticed in chickpea (Salehpour et al., 2009). Drought stress leads to lower production of leaves, higher leaf senescence, decreased leaf size which may be attributed to decrease in vegetative growth (Pagter et al., 2005). Drought induced reduction in leaf area is ascribed to suppression of leaf expansion through reduction in photosynthesis (Rucker et al., 1995). Decreased LAI in response to increased water deficit was observed in chickpea (Khamssi et al., 2010). Decrease in leaf area index may be attributed to reduced growth and expansion of leaves (Hall, 2004).

Grain yield and its attributes were reduced significantly due to water stress. Reduction in 100 seed weight, number of pods, percentage filled pods and yield was observed under various

Table 5. Effect of water stress imposed at pod initiation stage (120 DAS) on 100 seed weight and Total number of pods in chickpea (*Cicer arietinum* L.) genotypes.

Genotype	100 seed weight (g)				Total number of pods			
	T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4
GL21107	18.23±0.54	16.33±0.40	17.60±0.98	17.23±0.42	40.29±0.95	29.35±1.96	33.54±1.29	30.33±0.53
GL22044	15.12±0.42	13.14±0.35	14.00±0.32	13.78±1.32	40.33±0.47	23.00±0.75	27.45±1.37	25.67±1.39
GL26054	22.34±0.64	19.94±0.26	22.11±0.65	20.66±0.60	51.67±0.78	32.66±0.71	38.34±0.73	35.45±0.77
GL26074	16.93±0.26	15.23±0.55	16.13±0.37	16.00±0.38	55.44±1.36	36.78±0.75	40.00±0.89	37.8±0.69
GL28137	19.11±0.26	17.62±0.68	18.42±0.91	18.26±1.30	43.00±0.62	31.64±0.76	35.43±0.93	32.76±1.25
GL28151	25.85±1.04	24.77±0.35	25.00±0.44	24.90±1.13	48.33±0.69	42.76±0.22	45.34±0.50	43.23±1.25
GL28186	19.22±0.48	18.10±0.36	18.32±1.21	18.25±0.41	42.25±1.07	27.34±0.70	32.90±0.88	30.23±0.45
GNG1594	17.00±0.30	15.80±0.56	16.14±0.68	16.02±0.27	52.45±0.69	34.44±0.67	38.77±0.74	36.78±0.77
GNG1861	16.47±0.39	14.77±0.20	14.92±0.75	14.80±0.66	57.50±1.70	30.33±0.63	37.33±0.47	35.40±0.82
DCP 92-3	16.03±0.41	15.07±0.26	15.45±0.72	15.27±0.10	56.67±1.22	31.22±0.50	38.45±1.33	36.65±0.58
GG1362	22.03±0.83	19.89±0.79	21.00±0.72	20.60±1.45	49.33±0.57	32.00±0.98	37.8±1.24	35.45±1.62
RSG 811	24.82±1.03	22.41±0.40	24.29±0.53	23.24±0.48	50.57±2.25	32.75±0.35	34.00±0.42	33.32±0.95
RVSSG 4	17.61±0.63	15.53±0.61	16.23±0.94	16.10±0.39	44.33±1.08	21.00±0.46	28.99±0.96	25.48±1.27
RSG 963	27.19±0.77	25.80±1.08	26.69±1.65	26.23±1.03	54.67±0.45	46.56±0.72	48.76±1.16	47.44±1.25
RSG 957	25.44±1.16	23.45±0.97	24.34±0.98	23.80±1.06	49.00±1.83	32.23±0.65	36.00±0.40	34.56±0.72
BGM 547	26.03±1.06	23.44±0.55	24.87±0.71	24.63±0.93	34.75±0.77	21.11±0.61	26.77±0.27	25.46±1.27
PDG3	26.54±0.76	24.80±0.69	25.43±0.40	25.15±0.52	50.33±0.80	32.45±0.65	36.67±1.03	34.75±0.80
PDG4	19.04±0.60	17.34±0.55	18.13±0.38	18.00±0.99	51.90±1.25	35.00±0.89	40.00±0.66	36.75±0.86
PBG1	17.03±0.31	15.37±0.53	16.14±0.20	15.90±0.86	43.67±0.89	26.74±0.51	32.00±0.30	30.25±0.60
GPF2	18.94±0.33	17.54±0.68	18.00±0.29	17.95±0.94	32.00±0.79	21.24±1.12	23.45±0.51	22.00±0.44
	LSD (0.05)G = 0.425, LSD(0.05) T = 0.951 , LSD (0.05) G×T = NS				LSD (0.05)G = 0.524, LSD(0.05) T = 1.171, LSD (0.05) G×T = 2.342			

*Different values in each column represent mean ±S.E.

stress treatments. However, water stress affected yield and yield traits maximum under treatment T_2, which was grown with one pre-sowing irrigation followed by T_4, where stress was given at pod initiation stage. Stress at flower-initiation (T_3) had lesser influence on yield attributes in comparison to stress at pod initiation stage (T_4), depicting it as critical stage. This study is supported by investigations of other researchers. The reproductive stage is well known for its sensitivity to drought stress; thus seed yield being the most sensitive traits to water stress treatment imposed at post flowering and pod development stages as observed in mungbean (Uprety and Bhatia, 1989). Results showing reduced number of pods werereported earlier in chickpea (Khurgami et al., 2009). Fertile pods decreased as drought stress was imposed in chickpea (Mcphee and Muehlbauer 2001). Number of pods and percentage filled pods per plant reduction under drought stress may be attributed to the abscission of the reproductive structures. Reduced 100 seed weight and yield losses under drought were reported in chickpea (Shaban et al., 2012). Decrease in 100 grain weight under drought stress conditions might be due to lower photosynthetic translocation in the developing

Table 7. Phenotypic correlation coefficients between grain yield and growth, yield traits under water stress treatment: T_1.

Trait	Plant height	No. of branches	Dry wt of stem	Dry wt of leaves	Dry wt of root	Leaf number	Leaf area	Leaf area index	100 seed weight	Total number of pods	Percentage filled pods	Grain yield
Plant height	1											
No. of branches	0.4927**	1										
Dry wt of stem	0.3310*	0.6031**	1									
Dry wt of leaves	0.2874*	0.2550	0.5560**	1								
Dry wt of root	0.4319**	0.1635	0.0644	0.2530	1							
Leaf number	0.3839**	0.5382**	0.3370*	0.5091**	0.1333	1						
Leaf area	0.1460	0.2567	0.0902	0.4705**	0.2324	0.6797**	1					
Leaf Area Index	-0.0830	-0.1958	-0.9540	0.0992	0.1514	-0.0536	0.1024	1				
100 seed weight	0.3756**	0.2611*	0.1591	0.2756*	0.2885*	0.5297**	0.5242**	0.2021	1			
Total number of pods	-0.2515	-0.2938*	-0.1971	0.0481	-0.0297	-0.0144	0.1586	0.2515	0.0138	1		
Percentage filled pods	0.0375	-0.1222	-0.0553	0.0335	0.1613	0.0759	0.1832	0.2249	0.5341**	-0.0914	1	
Grain yield	-0.0307	0.1201	0.0550	0.0894	-0.0231	0.1169	0.0974	0.0274	0.3307*	-0.0199	0.2183	1

* and ** represent significant correlation at 5% and 1% level of probability respectively.

Table 8. Phenotypic correlation coefficients between yield and growth, yield traits under water stress treatment: T_2.

Trait	Plant height	No. of branches	Dry wt of stem	Dry wt of leaves	Dry wt of root	Leaf number	Leaf area	Leaf area index	100 seed weight	Total number of pods	Percentage filled pods	Grain yield
Plant height	1											
No. of branches	0.3702**	1										
Dry wt of stem	0.4499**	0.4300**	1									
Dry wt of leaves	0.5090**	0.4274**	0.6838**	1								
Dry wt of root	0.3113*	0.3707**	0.4842**	0.3600**	1							
Leaf number	0.4653**	0.6279**	0.7830**	0.6219**	0.3443**	1						
Leaf Area	0.4729**	0.4989**	0.7712**	0.9027**	0.4799**	0.7103**	1					
Leaf Area Index	0.6202**	0.5501**	0.4629**	0.5452**	0.2578*	0.5939**	0.5718**	1				
100 seed weight	0.4723**	0.5871**	0.8277**	0.6917**	0.5700**	0.6890**	0.7690**	0.4949**	1			
Total number of pods	0.4399**	0.2615*	0.6108**	0.4732**	0.2425	0.5678**	0.5868**	0.4186**	0.4512**	1		
Percentage filled pods	0.5352**	0.3602**	0.6542**	0.6734**	0.5304**	0.5523**	0.7377**	0.6034**	0.7088**	0.5910**	1	
Grain yield	0.5790**	0.2413	0.7209**	0.6209**	0.3365**	0.6138**	0.7077**	0.4935**	0.5724**	0.7895**	0.7306**	1

* and ** represent significant correlation at 5% and 1% level of probability respectively.

Table 9. Phenotypic correlation coefficient between yield and growth, yield traits under water stress treatment: T_3.

Trait	Plant height	No. of branches	Dry wt of stem	Dry wt of leaves	Dry wt of root	Leaf number	Leaf area	Leaf area index	100 seed weight	Total number of pods	Percentage filled pods	Grain yield
Plant height	1											
No. of branches	0.1936	1										
Dry wt of stem	0.4041**	0.1631	1									
Dry wt of leaves	0.2667*	0.4113**	0.3514**	1								
Dry wt of root	0.1876	0.2762*	0.3356**	0.4574**	1							
Leaf number	0.2978*	0.6285**	0.4252**	0.6997**	0.4402**	1						
Leaf Area	0.1111	0.4824**	0.3866**	0.8814**	0.4930**	0.7298**	1					
Leaf Area Index	0.1092	0.2838	0.3581**	0.6404**	0.4372**	0.5998**	0.7025**	1				
100 seed weight	0.0370	0.1530	0.0987	0.3590**	0.2619*	0.3500**	0.4791**	0.4063**	1			
Total number of pods	0.3609**	0.6228**	0.4256**	0.6329**	0.4668**	0.6600**	0.7478**	0.5429**	0.3329**	1		
Percentage filled pods	0.2140	0.1963	0.4651**	0.6902**	0.4101**	0.4863**	0.6892**	0.5919**	0.2898*	0.5987**	1	
Grain yield	0.1550	0.2541	0.3192*	0.6422**	0.4750**	0.5574**	0.7466**	0.6553**	0.7261**	0.5793**	0.6298**	1

* and ** represent significant correlation at 5% and 1% level of probability respectively.

Table 10. Phenotypic correlation coefficient between yield and growth, yield traits under water stress treatment: T_4.

Trait	Plant height	No. of branches	Dry wt of stem	Dry wt of leaves	Dry wt of root	Leaf number	Leaf area	Leaf area index	100 seed weight	Total number of pods	Percentage filled pods	Grain yield
Plant height	1											
No. of branches	0.1677	1										
Dry wt of stem	0.2394	0.2469	1									
Dry wt of leaves	0.0538	0.3803**	0.4465**	1								
Dry wt of root	0.1538	0.0331	0.2854*	0.3404**	1							
Leaf number	0.2174	0.6558**	0.3410**	0.7125**	0.2776*	1						
Leaf area	-0.1125	0.4492**	0.4317*	0.8797**	0.4219**	0.7247**	1					
Leaf Area Index	-0.1030	0.2817*	0.3956**	0.6561**	0.3264*	0.5869**	0.7573**	1				
100 seed weight	-0.0548	0.1376	0.1959	0.3649**	0.2917*	0.3731**	0.4837**	0.4571**	1			
Total number of pods	0.2791*	0.5684**	0.4603**	0.6483**	0.4435**	0.6469**	0.7329**	0.5852**	0.3863**	1		
Percentage filled pods	0.0384	0.1062	0.4366**	0.5344**	0.3391**	0.3589**	0.6416**	0.6022**	0.3864*	0.6010**	1	
Grain yield	-0.1223	0.1541	0.3073*	0.5666**	0.3698**	0.4876**	0.6901**	0.5887**	0.7901**	0.5623**	0.5992**	1

* and ** represent significant correlation at 5% and 1% level of probability respectively.

grain. The yield loss caused was mainly due to an increased rate of floral and pod abortion and detrimental effects of drought avoidance on CO_2 assimilation.

As observed in our study, under high moisture stress, high correlation coefficient values were found between seed yield and related traits. It is similar to results observed earlier in chickpea. (Rahman and Uddin, 2000). These traits should be considered in improving yield stability of chickpea under moisture stress conditions. Yield was found positively correlated with plant height, number of branches, 100 seed weight, number of pods per plant (Shamsi et al., 2010), total dry matter (Islam et al., 2008), leaflet number (Farshadfar and Farshadfar, 2008), leaf area (Ali et al., 2010), leaf area index (Khamsi et al., 2010) in chickpea. Significant correlation between fertile pod number and yield in lentil (Azizi-e-Chakherchaman et al., 2009). These traits can be used as reliable criterion in selection of water stress tolerant chickpea cultivars.

Conclusion

An appreciable variation was noticed in all recorded parameters, due to differences in genetic constitution and environmental interactions. All recorded growth traits and yield attributes showed significant reductions under water stress at varied growth stages. These traits showed positively significant correlation with yield, showing that these can be effectively used for field screening of chickpea for drought tolerance. Among twenty genotypes studied, GL28151, RSG963 and PDG3 were more efficient in tolerating the adverse effect of water stress, whereas GL22044, GNG1861 and RVSSG3 showed sensitivity to drought. Effect of stress treatment T_2, grown with one pre sowing irrigation was adverse on growth and yield traits. Treatment stressed at flower initiation (T_3) was affected more in reducing growth traits than pod initiation stage (T_4) stress. However, pod initiation stage was more critical for yield traits than flower initiation.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Productivity of fertirrigated sugarcane in subsurface drip irrigation system

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The productivity and fresh phytomass index of sugarcane cultivated at different water replacement levels using a subsurface drip system, with or without N, were analyzed. Sugarcane plants underwent five water replacement levels (100, 75, 50, 25 and 0%), with or without N application (100 kg ha^{-1}) in urea. At harvest-time, stalk productivity, water-use efficiency, gross sugar yield, gross alcohol yield, fresh phytomass of tip, dry leaf phytomass, total fresh phytomass and the ratio between the fresh phytomass of tip and stalk productivity were evaluated. A 100% water replacement increased stalk productivity by 40% compared with drought-stricken area management (water replacement 0%) and high efficiency in the exploitation of photoassimilated in stalk production. N-urea application increased by 14% the gross sugar and alcohol yield. Water deficit (water replacement 0%) caused severe decrease (26%) in total phytomass of the sugarcane plant´s aerial section.

Key words: *Saccharum officinalis*, water replacement, irrigation, nitrogen, water deficit.

INTRODUCTION

With its positive potential energetic balance, it has been brought to the attention of producers that sugarcane culture is a source of energy production (Renouf et al., 2008; Smeets et al., 2009). Sugarcane has traditionally been employed as forage for animal feed or as raw matter for the manufacture of candy, syrup, brandy, sugar and alcohol fuel. However, sugarcane productivity is limited by edaphoclimatic factors such as water and nitrogen deficiency (Gava et al., 2010, 2011).

Water deficit is a main factor in production decrease in most cultures worldwide (Bray et al., 2000), even though its effects must be minimized by irrigation systems. Irrigation of sugarcane plantations has triggered improvements in the number of harvests and the culture cycle with a productivity increase of over 100% (Dalri et al., 2008). The rational management of water in sugarcane culture by irrigation technology is basic for the maximization of production.

Drip irrigation systems have proved to be highly efficient in water-saving in agriculture. The formation of a wetted bulb in the cultivated soil, especially in areas of intense microorganism activity and high concentration of the culture´s root system is reported (Thorburn et al., 2003). Since nutrient balance is associated with the correct management of irrigation water, nitrogenated fertilizers in the soil undergo chemical and microbial

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Table 1. Chemical characterization of soil in the experimental area.

Layer (m)	pH -	OM ($g\ dm^{-3}$)	P ($mg\ dm^{-3}$)	K	Ca	Mg	Al	H+Al	S	CTC	V (%)
				---------- ($mmol\ dm^{-3}$) ----------							
0.00 - 0.20	6.2	63.42	7.06	2.04	20.40	16.80	0.0	57.75	41.80	99.55	41.99
0.20 - 0.40	6.6	44.47	2.65	4.09	14.40	13.20	0.0	44.55	31.69	76.24	41.57

pH in distilled water. P and K – extractor Mehlich^{-1}. O.M – Organic matter. V – Saturation by bases.

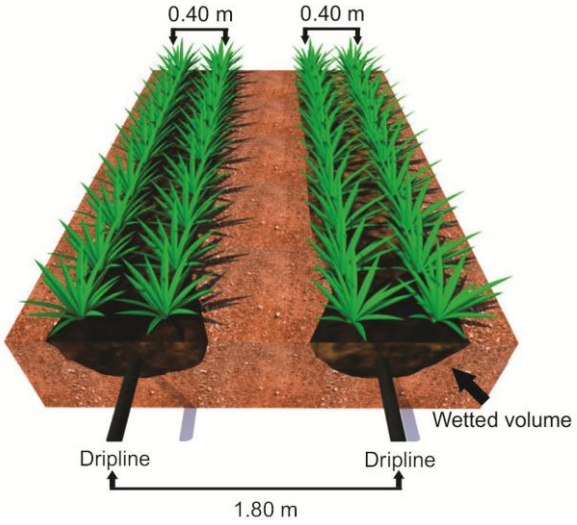

Figure 1. Outline of W-shaped planting and the laying of driplines in treatments with water replacement. Prepared by Eugênio Ângelo Ribeiro Batista and Marconi Batista Teixeira (2013).

transformations which may cause losses to vegetation. For reasons of cost, the development of adequate management of nitrogenated manure is underscored, so that N in sugarcane cultures may be better exploited (Franco et al., 2008). In fact, N deficiency in plants triggers a decrease in chlorophyll and synthesis of essential aminoacids, with a subsequent reduction of photosynthetic rates and less energy for the production of carbohydrates (Epstein and Bloom, 2006).

The productivity response of irrigated sugarcane depends on a series of factors such as the quantity of water and fertilizers (Dantas Neto et al., 2006), irrigation management, cultivar type, cutting age, and type of soil and climate (Smit and Singels, 2006). Current research characterizes the productivity index and fresh phytomass of sugarcane cultivated at different levels of water replacement using a subsurface drip system, with and without N, allotted throughout the culture cycle.

MATERIALS AND METHODS

The experiment was performed in the experimental area of the Federal Institute of Goiás, campus Rio Verde GO Brazil, 17°48'28"S and 50°53'57"W, mean altitude 720 m, slightly rolling ground relief (slope 6%), red dystrophic latissoil, (LVdf) with mean texture 458, 150 and 391 g kg^{-1} sand, silt and clay, respectively, and chemical characteristics as shown in Table 1.

The experimental design comprised randomized blocks in a 5x2 factorial scheme, with four replications. Treatments consisted of five levels of water replacement (100, 75, 50, 25 and 0%) and two nitrogen (urea) doses (0 and 100 kg N ha^{-1}).

The planting of sugarcane, cultivar RB855453, was performed in a double row (W-shaped), 8 m long, with 1.80 m spacing between the double rows. The distance between the crops in the double row was 0.40 m, with a total area of 35.2 m^2 in each paddock. For treatments with water replacement (WR) a drip tube was placed in the ground at a depth of 0.20 m among the furrows of the double row (Figure 1). The drip tube (DRIPNET PC 16150) comprised a thin wall, 1.0 bar pressure, nominal discharge 1.0 L h^{-1}, and 0.50 m spacing between drippers.

On planting, all furrows of the plots were fertilized with30 kg N ha^{-1} (urea), 120 kg P_2O_5 ha^{-1} (single superphosphate) and 80 kg K_2O ha^{-1} (potassium chloride). Nitrogen was applied by fertirrigation at a dose of 100 Kg ha^{-1}, at 30-day intervals, with 10 applications throughout the development of the sugarcane culture. Potassium fertilization was done partially, in 30% of the furrows, and the remaining part was treated with the irrigation water. Nitrogen and potassium were spread only in the treatment with 0% water replacement.

Table 2. Water volume received at each water replacement level.

WR (%)	WA (mm)	R (mm)	TVW (mm)
RH 0	0	1618	1618
RH 25	126	1618	1744
RH 50	252	1618	1870
RH 75	378	1618	1996
RH 100	504	1618	2122

WR – water replacement; WA – water applied during the experiment; R – rainfall; TVW – Total volume of water received.

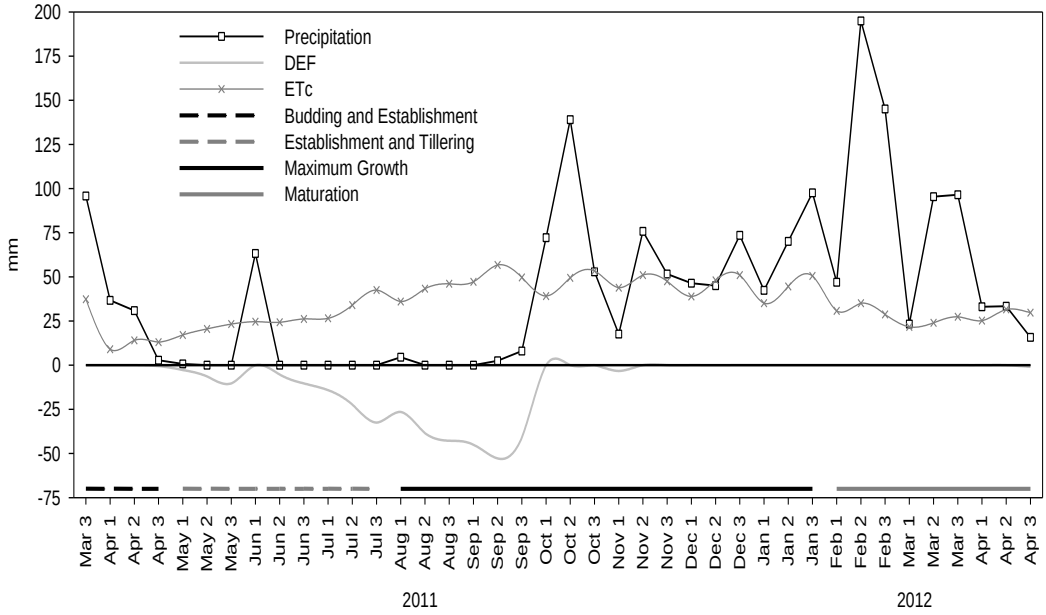

Figure 2. Water balance of sugarcane (0% water replacement) during the experiment. DEF – water deficit; ETc – Evaporation-transpiration of the culture; culture phases (adapted from Doorenbos and Kassam, 1994): Budding and Establishment (Kc = 0.6); Establishment and Tillering (Kc = from 0.9 to 1.1); Maximum growth (Kc = 1.3); Maturation (Kc = from 0.7 a 0.9). Source: INMET – Rio Verde GO Brazil.

Water demand was calculated by a 0.1 kPa puncture digital tensiometer. Tensiometric sensors were placed at a depth of 0.20, 0.40, 0.60 and 0.80 m, at a distance of 0.15, 0.30, 0.45 and 0.60 m from the drip tube, with daily readings of water tension in the soil. The soil's physical and water characteristics were determined by the water retention curve in the soil, with an available water capacity (AWC) of 100 mm. Soil was kept at field capacity in treatments with 100% WR. By the end of the experiment, the water supplemented to the soil was calculated to determine the volume of water provided (Table 2).

A water balance was estimated every ten days and water deficiency (WD) was calculated for the culture period (March 2011 to April 2012) from rainfall data, according to the method by Thornthwaite and Mather (1955), adapted by Camargo (1962). Reference evapotranspiration (Et_0) was calculated according to the equation by Penman-Monteith (Monteith, 1973), with results shown in Figure 2. Total evaporation-transpiration and precipitation reached 1549 and 1618 mm, respectively in the treatment without water replacement.

The useful area in each plot was harvested (central linear meter of the main row) after 395 days of planting. Stalk, tip and dry leaves mass were calculated. These values were used to determine the fresh phytomass of the tip (PT, Mg ha^{-1}), phytomass of the dry leaf (DP, Mg ha^{-1}), total fresh phytomass of the aerial part (TP, Mg ha^{-1}) and the ratio between tip and stalk phytomass (TP:SP, %). The TP:SP ratio was calculated by the division of TP by stalk productivity (SP) and multiplied by 100 for the percentage. The stalks of ten plants per treatment were collected and analyzed in a laboratory to determine gross sugar (GSY) and alcohol (GAY) yield. Stalk productivity (SP, Mg ha^{-1}) was calculated by the proportional ratio of the stalk weight of the sampled area per hectare. The efficiency of water usage (WUE, mm Mg^{-1} ha^{-1}) was determined by the total volume of received water (mm) divided by stalk productivity. Gross sugar (GSY, Mg ha^{-1}) and alcohol (GAY, m^3 ha^{-1}) yield were calculated following the method by Caldas(1998). Results were analyzed by ANOVA. In significant cases, regressions

Table 3. Summary of the analysis of variance for stalk productivity (SP), efficiency in water usage (EWU), gross sugar yield (GSY) and gross alcohol yield (GAY) of sugarcane at different levels of water replacement, with and without N application.

Source	GL	Mean square			
		SP	EWU	GSY	GAY
Water replacement (WR)	4	8424.45**	5.98^{ns}	245.03**	122.70**
Nitrogen (N)	1	6038.38^{ns}	9.43^{ns}	222.45*	110.50*
Interaction WR x N	4	441.10^{ns}	1.06^{ns}	12.61^{ns}	6.31^{ns}
Blocks	3	1712.05^{ns}	4.19^{ns}	36.54^{ns}	18.53^{ns}
Waste	27	1866.16	3.80	40.73	20.63
CV (%)		20.23	21.31	20.58	20.57
Nitrogen (N)			**Means**		
with N		225.82^{a}	8.67^{a}	33.36^{a}	23.74^{a}
without N		201.25^{a}	9.64^{a}	28.64^{b}	20.42^{b}
DMS		28.03	1.27	4.14	2.95

* Significant at 0.01 probability by test F; ** Significant at 0.05 probability by test F; ^{ns} Not significant at 0.05 probability by test F; Means followed by the same letter in the columns do not differ statistically at 0.05 probability by Tukey´s test.

of linear and quadratic were performed for water replacement levels. Nitrogen application means were compared using Tukey test at significance degree α = 0.05.

RESULTS

There was no significant interaction between water replacement and nitrogen doses for any of the characteristics evaluated in the sugarcane plants (Table 3). The water replacement (WR) factor caused a significant effect at 1% probability for the following variables: stalk productivity (SP), gross sugar yield (GSY) and gross alcohol yield (GAY). On the other hand, application of nitrogen (N) significantly affected the GSY and GAY results (Table 3), although the water-use efficiency (WUE) was not influenced by any factor evaluated (Table 3).

Stalk productivity (SP) responded to water replacement with a linear increase, following regression analysis (R^2 = 0.75) (Figure 3A). Consequently, a 0.4% increase in stalk productivity was obtained for each 1% water replacement provided, equivalent to 0.7 Mg ha^{-1} yield. Maximum stalk productivity was obtained in the 100% WR management with an estimated mean of 40% higher than that of drought management (WR 0%) at 178 Mg ha^{-1} (Figure 3A).

The estimated maximum gross sugar yield (GSY) amounted to 35 Mg ha^{-1} obtained by 80.2% water replacement, and therefore a 61% increase compared with treatment without any water replacement (Figure 3B). The maximum curve peak showed gross alcohol yield (GAY) of 25.34 m^3 ha^{-1} obtained by 79.7% water replacement. Mean GAY reached 22.15 m^3 ha^{-1}, whereas the lowest rate was 15.82 m^3 ha^{-1}, in the drought treatment (WR 0%), or rather, 56.25% decrease when compared with the highest yield (Figure 3C).

Mean GSY and GAY rates in sugarcane fertilized with 100 kg N ha^{-1} were respectively 33.36 Mg ha^{-1} and 23.74 m^3 ha^{-1} (Table 3). Nitrogenated manure had an increase of approximately 16% for these variables compared with plants which did not receive any nitrogen (Table 3).

Total phytomass (TF) and tip and stalk phytomass ratios (PT:SP) were significantly affected by water replacement treatments. Nitrogen affected PT significantly (Table 4) but the interaction between water replacement and nitrogen application (WR x N) did not significantly influence any of the variables analyzed (Table 4). Total phytomass in drought reached 249.5 Mg ha^{-1}, with a 35.3% decrease in yield obtained with 100% water replacement and a response of 337.7 Mg ha^{-1} (Figure 4A). The effects of nitrogenated fertilization only occurred for tip phytomass (PT), with a 14.7% increase (Table 4).

The relationship between tip and stalk phytomass (PT:SP) underscored the response of the development of the tip compared with the sugarcane stalk yield. The variable provided a quadratic response due to water replacement (R^2 = 0.74), where the highest PT:SP was reported in drought management (WR 0%), with mean PT:SP at 37% (Figure 4B). The above result revealed a tip development of 0.37 Mg ha^{-1} for each megagram of stalk produced. However, when the stalk´s low productivity with this treatment was compared with the others, the significant PT:SP response became a limiting factor attributed to the low exploitation of photosynthetic products in the production of stalks.

Further, treatment with 100% WR also had a high PT:SP ratio with a mean rate of 33.3%. Since the SP yield was high, the ratio became a positive factor. A high PT:SP ratio is very important for culture yields when one

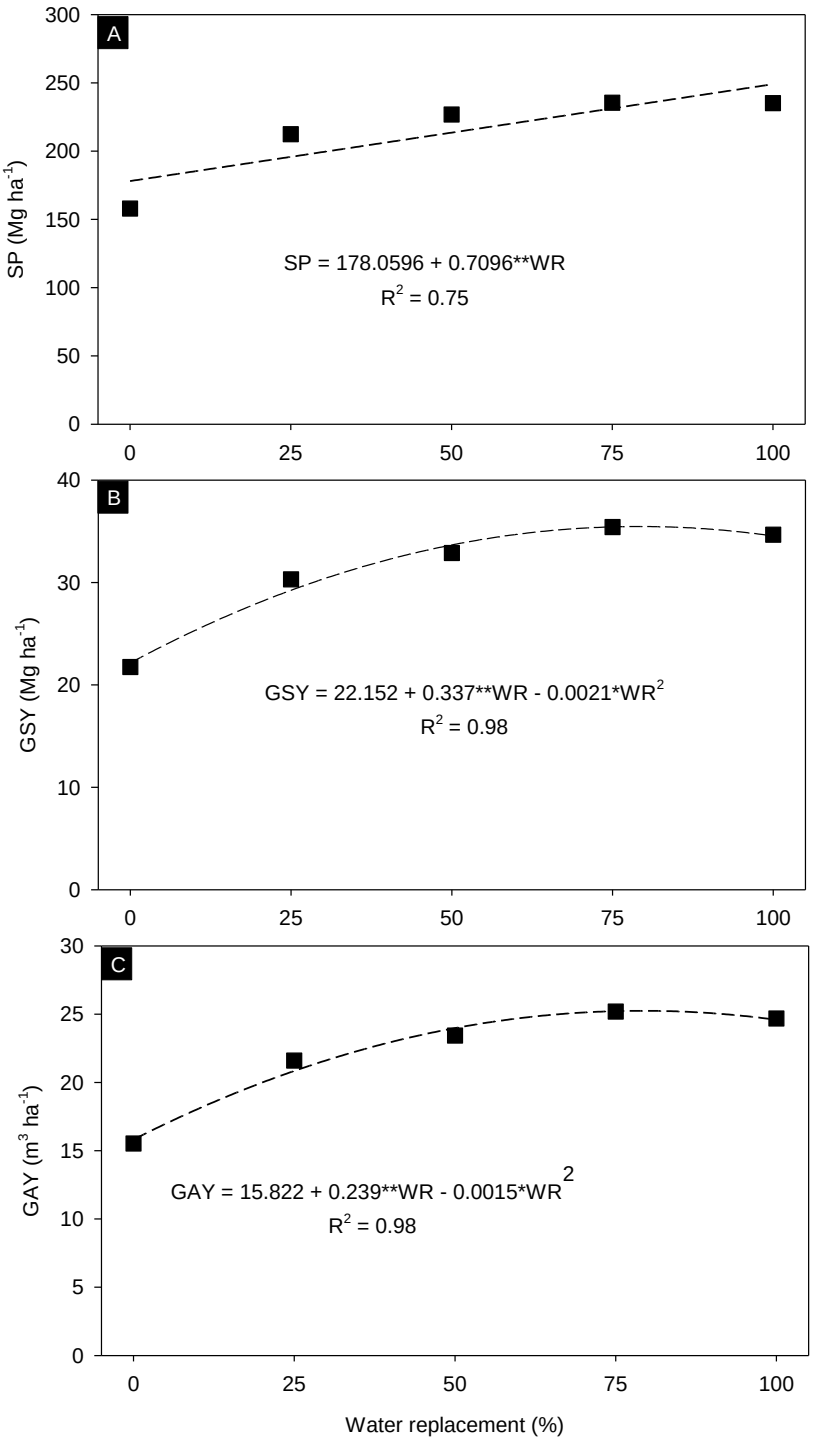

Figure 3. Effects of water replacement (WR) on the productivity of stalks (SP) (A), o gross sugarcane yield (GSY); (B) gross alcohol yield (GAY); (C) in sugarcane.

considers that the higher the production of carbohydrates by photosynthesis the higher the architecture of the leaf area. However, the assimilation of carbohydrates should be taken into account so that they can be exploited in stalk production.

High production of sugarcane tips should also be considered with regard to the hay wastes on the ground during harvest. The lowest PT:SP ratio was reported in

Table 4. Summary of analysis of variance for stalk phytomass (SP), phytomass tip (PT), dry leaf phytomass (DP), total phytomass (TF) and ratio between phytomass tip and stalk phytomass (PT:SP) of sugarcane at different levels of water replacement, with and without nitrogen.

Source	GL	Mean square			
		PT	DP	TF	PT:SP
Water replacement (WR)	4	466.81^{ns}	29.38^{ns}	12479.74*	68.70*
Nitrogen (N)	1	1060.28*	4.85^{ns}	11678.62^{ns}	16.11^{ns}
Interaction WR x N	4	394.20^{ns}	18.90^{ns}	1766.33^{ns}	43.97^{ns}
Blocks	3	39.48^{ns}	26.77^{ns}	1281.65^{ns}	56.80
Waste	27	213.36	33.69	3281.06	23.32
CV (%)		22.51	38.27	19.51	15.70
Nitrogen (N)					
with N		70.03^{a}	14.81^{a}	310.67^{a}	31.39^{a}
without N		59.73^{b}	15.51^{a}	276.50^{a}	30.12^{a}
DMS		9.47	3.76	37.16	3.13

* Significant at 0.01 probability by test F; ** Significant at 0.05 probability by test F; ^{ns} Not significant at 0.05 probability by test F; Means followed by the same letter in the columns do not differ statistically at 0.05 probability by Tukey´s test.

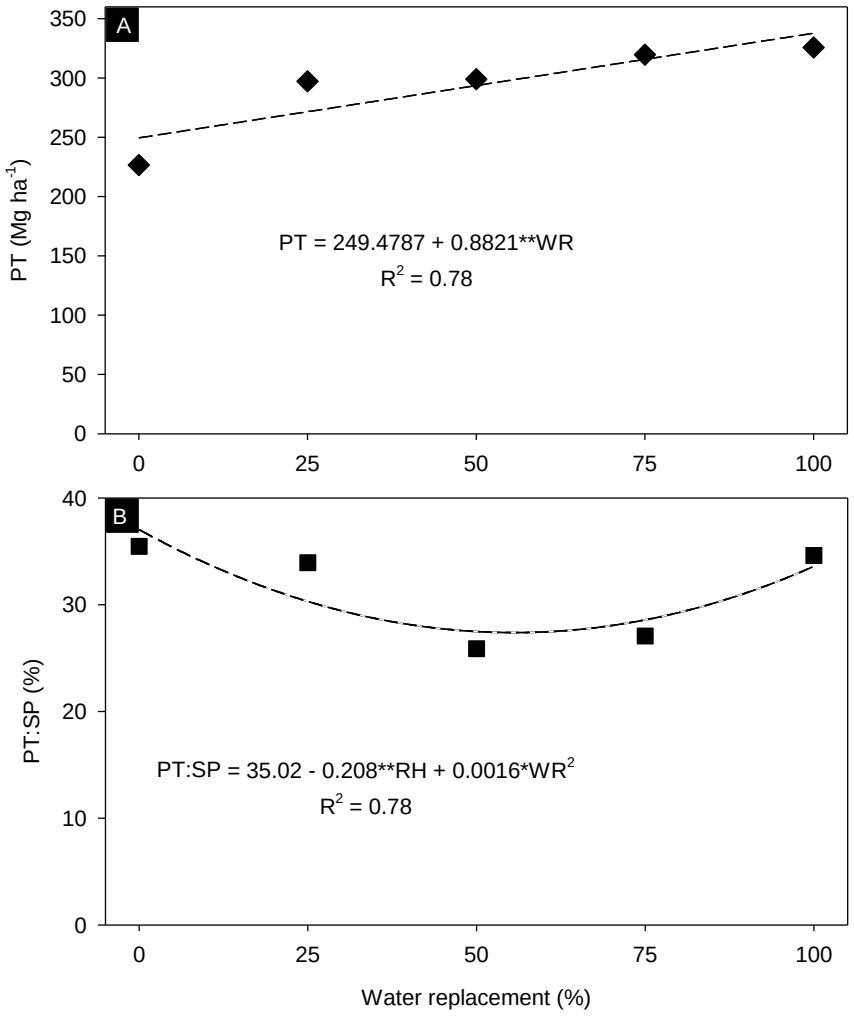

Figure 4. Effects water replacement (WR) on total phytomass (TF) (A), and ratio of phytomass tip and stalk (PT:SP) (B) in sugarcane.

water replacement at 56%, with an estimated mean of 27.4%.

This means that 0.274 Mg ha^{-1} of sugarcane tips was actually required for each megagram of stalks per hectare (Figure 4B). Given that a 218 Mg ha^{-1} stalk yield is attained with 56% water replacement, according to the trend for SP (Figure 2), the plants' high efficiency in the assimilation of carbohydrates produced in photosynthesis must be recognised.

DISCUSSION

The reported water balance showed that during phases with high water demand by the sugarcane, namely, during tillering and initial growth, a water deficit occurred. The intensity and duration of this period, especially at the start of the growth phase, decreased the productivity through a reduction in growth rates. Within the context of drought, water failed to put a turgescence pressure on the cell wall and, therefore, growth increase failed to occur (Taiz and Zeiger, 2013).

Similar to most poaceae, sugarcane requires great quantities of water for development since it has a high efficiency in using and recovering CO_2 from the air under conditions of high irradiancy and temperatures (Segato et al., 2006).

The highest rates in the productivity of stalks and the accumulation of total phytomass were reached when 100% water replacement took place, with a respective increase of 40 and 35% in productivity compared with plants during drought (WR 0%). This showed that the long dry winter caused yield decrease in spite of the occurrence of adequate rainfall during the summer. In some periods, irregular rainfall may also limit the growth of sugarcane plants (Ometto, 1980).

Mean stalk production, estimated at 213.5 Mg ha^{-1} was higher than that reported by Carvalho et al. (2009) and Oliveira et al. (2009). These results were due to the high rainfall rate during the experimental period (1618 mm) and to water availability coupled with the application frequency of the irrigation treatments. The data corroborated the importance of irrigation technology for the maximization of the culture´s genetic potential and for obtaining high productivity rates.

Water replacement and nitrogenated fertilization by fertirrigation affected the productivity and quality of the sugar. Sugarcane cultivar RB 855536 had a 57% increase, or rather, a 24.7 Mg ha^{-1} yield of sugar when it received a total water volume of 1714 mm during the cycle (Gava et al., 2011), whereas sugarcane cultivar RB 72454 had a loss of technological quality when irrigation was 130 mm higher than control treatment (Dalri et al., 2008).

Doses of 157 kg ha^{-1} of N and 148 kg ha^{-1} of K_2O for cover fertilization provided a significant increase in sugarcane technological quality, with a respective increase of 39.8 and 42.2% for GSY and GAY, featuring a yield of 12.58 Mg ha^{-1} sugar and 8.91 m^3 ha^{-1} alcohol (Dantas Neto et al., 2006).

The relevant effect of nitrogen on PT may be assigned to small doses of fertilizers throughout the culture cycle, with an absorption increase and a beneficial usage of nitrogen (Singh and Mohan, 1994; Ng Kee Kwong et al., 1999) due to a higher synchronization of availability and nutrient absorption by the plants (Gava et al., 2010). The treatment mean PT was 70 and 59.7 Mg ha^{-1}, respectively, with and without nitrogen.

Consequently, water replacement triggered an increase in stalk productivity compared with drought management. Nitrogen-urea applications in small doses throughout the culture cycle improved the sugarcane's technological indexes. Lack of water caused a heavy decrease in the total phytomass of the sugarcane's aerial parts. High stalk yield was recorded compared with tip phytomass with 100% water replacement and demonstrated the plants' high efficiency in the assimilation of carbohydrates produced by photosynthesis. When the plant phytomass was taken into account, nitrogen only increased the production of the tip phytomass.

Conflict of Interest

The author(s) have not declared any conflict of interests.

Abbreviations: AWC, Available water capacity; **DP,** Dry leaf phytomass (Mg ha-1); **ET0,** Reference evaporation-transpiration; **GAY,** Gross alcohol yield (m3 ha-1); **GSY,** Gross sugarcane yield (Mg ha-1); **Mg,** Megagram; **PT:SP,** Fresh phytomass of tip and stalk productivity ratio (Mg ha-1); **PT,** Fresh phytomass of tip (Mg ha-1); **R,** Rainfall (mm); **SP,** Stalk productivity (Mg ha-1), **TP,** Total fresh phytomass (Mg ha^{-1}); **WD,** Water deficit; **WUE,** Water-use efficiency.

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Economic impacts of climate change on agriculture and implications for food security in Zimbabwe

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This study measured the economic impacts of climate change on agriculture in Zimbabwe based on a cross-section survey of over 700 farming households. We applied the Ricardian approach to analyse the response of net revenue from crop and livestock agriculture across smallholder farming systems in the country to changes in climate normals (that is, mean rainfall and temperature). The sensitivity of net farm revenues was used to make inferences on the food security implications of climate change in the country. Results show that net farm revenues are affected negatively by increases in temperature and positively by increases in precipitation. The results from sensitivity analysis suggest that agricultural production in Zimbabwe's smallholder farming system is significantly constrained by climatic factors (high temperature and low rainfall). Farms with irrigation are more resistant to changes in climate, indicating that irrigation is an important adaptation option to help reduce the impact of further changes in climate. Dryland farming predominantly typical in Zimbabwe is the most vulnerable to warming and lower rainfall, whereas the irrigated systems are the most tolerant. These results have important policy implications especially for the need to support dryland smallholder adaptation strategies for agricultural development in the country in light of expected climate changes. For example, irrigation offered better adaptation options for farmers against further warming and drying predicted under various future climate scenarios.

Key words: Economic impacts, climate change, food security, policy implications, Zimbabwe.

INTRODUCTION

Climate change impact studies have shown that the productivity of agricultural activities is highly sensitive to climate change. The effect of changes in climate on agricultural activities both physical and economic has been shown to be significant for low input farming systems, such as subsistence farming in developing countries in sub-Saharan Africa that are located in marginal areas and have the least capacity to adapt to changing climatic conditions (Rosenzweig and Parry, 1994; Reilly and Schimmelpfennig, 1999; Kates, 2000; McGuigan et al., 2002).

The effect of climate change on agricultural systems can be seen in the interaction between changes in climate variables and the stresses that result from actions taken to increase agricultural production. Impacts on crop yields, agricultural productivity and food security vary

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depending on the types of agricultural practices and systems (Watson et al., 1997). There is growing evidence that further increases in global warming leading to changes in main climate variables - temperature, precipitation, sea level rise, atmospheric carbon dioxide content and incidence of extreme events - may significantly affect African agricultural production (Watson et al., 1997), with the result that the livelihoods of subsistence farmers and pastoral peoples, who make up a large portion of rural populations in sub-Saharan Africa, could be negatively affected. For instance, in areas where temperatures are already warm, such as Zimbabwe and most parts of sub-Saharan Africa, further increases in temperature may actually slow down rather than stimulate plant growth, culminating in a general decrease in expected yield for most of the current food crops. The indirect effect of the increased temperatures is the anticipated reduction in effective rainfall even when current amounts of rainfall are sustained, culminating in greater incidence of crop failure.

Empirical agronomic studies in Zimbabwe have revealed that climate change has a negative effect on the agricultural performance of major crops. For instance, Muchena (1994) and Magadza (1994) showed that a 2°C rise in ambient temperature and a rise of mean temperature by 4°C significantly lowered yields. In another study, Makadho (1996) assessed the potential effects of climate change on corn, using a Global Circulation Model (GCM) and the dynamic crop growth model CERES-maize, and the results indicated that maize production was expected to significantly decrease by approximately 11 to 17%, under conditions of both irrigation and non-irrigation. A reduced crop growth period because of increases in temperature, particularly during the grain filling and ripening stages, has been found to be the main factor contributing to decreased yields. There has been extensive research on the impacts of climate change, but little on the economic impacts on agriculture in Zimbabwe. To fill this empirical gap, this study carries out an economic analysis of the potential impacts of climate change on Zimbabwe's agricultural sector at the farm level. It also incorporates a brief analysis of adaptation strategies being used by farmers to cushion themselves against changing climatic conditions. The agricultural sector remains the key sector of the economy in Zimbabwe, but agricultural policy making has not yet given enough attention to the impacts of climate change and related issues.

The main objective of this study is therefore to apply empirical methods to assess the economic impacts of climate change on agriculture in Zimbabwe.

METHODOLOGY

Analytical model

Impacts of climate change have been estimated using two main approaches: (a) structural modelling of crop and farmer response, which combines crop agronomic response with economic/farmer management decisions and practices; and (b) spatial analogue models that measure observed spatial differences in agricultural production (Adams et al., 1998; Schimmelpfennig et al., 1996). Other impact assessment methods that have been used are the integrated impact assessment method and the agro-ecological zone method (Mendelsohn, 2000; Kurukulasuriya and Mendelsohn, 2006).

The main problem with using structural approaches (agronomic-economic models) is that in aggregate studies inferences made to large areas and diverse agricultural production systems are based on results from very few laboratory and experimental sites (Adams et al., 1998; Shimmelpfenning et al., 1996). The spatial analogue approach on the other hand, uses cross section evidence to make statistical (econometric) estimations of how changes in climate would affect agricultural production across different climatic zones. The main advantage of this approach is that it gives evidence of changes in farmer management practices and decisions in response to changing climatic conditions (Mendelsohn and Dinar, 2003; Mendelsohn et al., 1994).

An example of the spatial analogue approach is the Ricardian cross-sectional approach that measures the performance of farmers, households and firms across spatial scales with different climates. The technique draws heavily on the underlying observation by Ricardo that under competition, land values reflect the productivity of the land (Mendelsohn and Dinar, 1999, 2003; Mendelsohn, 2000; Mendelsohn et al., 1996, 1994).

This study adopts the cross-section Ricardian approach to measure the economic impacts of climate on net farm revenue in Africa. The study uses cross section data and econometric analyses to estimate the impacts of climate variables (temperature and precipitation), soils, hydrological and socio-economic factors on net farm revenue. Due to lack of African data on land rents, the study uses total net farm revenues defined as the sum of net revenues from three main farming activities (a) dryland crops (b) irrigated crops and (c) livestock as the measure of farm performance. Farm net revenue (R) is assumed to reflect the present value of future net productivity and costs of individual crops and livestock:

$$R = \int P_{LE} e^{\delta t} dt = \int \left[\sum P_i Q_i (X, F, Z, H, G) - \sum P_x X \right] e^{\delta t} dt \quad (1)$$

where P_{LE} is the net revenue per farm, P_i is the market price of crop *i,* Q_i is output of crop *i,* F is a vector of climate variables, Z is a set of soil variables, H, is a set of hydrological variables, G is a set of economic variables, P_x is a vector of purchased input prices, t is time, and δ is the discount rate.

The Ricardian method assumes that each farmer will seek to maximize net farm revenues by choosing inputs (X) subject to climate, soils and economic factors. The resulting net revenue function observes the loci of maximum profits subject to a set of climate, soils and economic factors and the Ricardian model is a reduced form hedonic price model of the observed loci of profits. The standard Ricardian model relies on a quadratic formulation of climatic variables:

$$R = \beta_0 + \beta_1 F + \beta_2 F^2 + \beta_3 Z + \beta_4 G + \beta_5 \log(H) + u \quad (2)$$

Where u is the error term. To capture the nonlinear relationship between net farm revenues and climate variables, the estimation includes both the linear and quadratic terms for climate variables, *F* (temperature and precipitation).

Table 1. Variability in net farm revenue across provinces and the whole sample (US$/ha).

Province	Mean net farm revenue	Range
Manicaland	281.31	2094.95
Mashonaland Central	915.23	5036.57
Mashonaland East	499.93	2769.09
Mashonaland West	240.23	1879.34
Masvingo	231.71	2744.46
Midlands	375.13	2665.44
Total sample	355.89	5859.27

Description of data

This study is based on a cross-sectional farm household survey conducted in a number of provinces and selected districts across the country as part of the Global Environment Facility/World Bank (GEF/WB)-CEEPA funded Project: Climate, Water and Agriculture*:* Impacts on and Adaptations of Agro-ecological Systems in Africa (Nhemachena, 2009; Dinar et al., 2008). The survey covered most of the country's provinces except two, which were omitted because of budgetary constraints. However, the sampled households give a fair representation of the farming systems in the country. The survey collected data for the 2002/2003 and 2003/2004 farming seasons for both crop and livestock production activities. It provided information about relevant socio-economic variables such as farm size, household size, household assets (for example, ploughs) and access to extension services, for use in the Ricardian analysis. The surveyed districts were selected on the basis of agro-climatic and hydrological zones, provincial representation and latitude. The district sample of smallholder dryland farmers was based on the proportion of district population to the total population of all selected districts. The target sample size was 1000 smallholder households, but because of budgetary constraints and the inaccessibility of some areas only 700 were finally surveyed. Only smallholder farmers were surveyed because former large-scale and now resettled areas were not readily accessible. The fact that large-scale farms were not included in the sample meant that the study also could not assess the effects of technology on net farm revenues.

As mentioned earlier, this study used net farm revenue to measure farm performance due to lack of data on land rents. Total net farm revenue is defined as the sum of net revenues from production of crop and livestock activities. The Ricardian approach is traditionally based on analysing net revenue or land value per hectare. As most farmers in Africa graze livestock on open access communal land it was very difficult to measure the amount of land farmers allocate to livestock production. Therefore, since this study combined net revenue for both crop and livestock production we could not use net revenue per hectare and instead used net revenue per farm making the unit of analysis in this study the farm.

The explanatory variables consist of seasonal climate variables, soils, water flow and socio-economic factors (Nhemachena, 2009). The study relied on long-term average climate (normals) for districts in Africa (Dinar et al., 2008; Kurukulasuriya et al., 2006). Soil data came from the Food and Agriculture Organization (FAO, 2003). Data on hydrological variables (e.g. flow and runoff for each district) were obtained from Strzepek and McCluskey (2007). The explanatory variables included in this study have been shown to affect net farm revenue in many other African Ricardian models (Dinar et al., 2008; Kurukulasuriya and Mendelsohn, 2008; Mano and Nhemachena, 2007).

A prior expectation was that farm net revenues would vary across spatial scales and in this case across provinces. Because the provinces cover more than one agro-climatic zone they generally exhibit spatial differences in climatic variables and it was therefore expected that this would cause net farm revenues to vary both within provinces and across all the sampled households. Table 1 shows variability in net farm revenue. The results show great variability in net farm revenue within provinces and across the whole sample, indicating that net revenue may be influenced by differences in climatic conditions in the various agro-climatic zones in each province. The empirical analysis therefore tried to find the climatic, soil, socio-economic and hydrological variables that would help explain this variability.

RESULTS AND DISCUSSION

The Ricardian model results are shown in Table 2. Among the socio-economic variables, more years of education and increased access to extension services are associated with improved farming information that is important for agricultural productivity. The results also show that small farms are more productive on per hectare basis compared to large farms. The possible reason for this observation is that small farms use fixed resources such as household labour and other inputs over a smaller area compared to large farms. Other important factors that have significant effects on net farm revenues include: short distances from the capital, high livestock index, access to irrigation.

The important policy message from this finding is that the government, private sector and Non-governmental organisations (NGOs) can improve net farm performances for smallholder farms by ensuring increased farmer training, and helping farmers acquire more livestock. Another important policy message is that short distances to the capital are important in improving net farm revenues. The implication of this finding is that there is crucial need to provide easy access to both input and output markets in the country to help shorten distances to markets. The results show that irrigation and livestock are important factors significantly affecting net farm performances in the country. The policy message from this result is that these two factors can provide a useful channel of farmer adaptation strategy and help in improving net farm revenues in the smallholder farming

Table 2. Response of farm net revenue to climate, soil and socio-economic variables.

Variable	All farms coefficient	Dryland coefficient	Dryland and Irrigate coefficient
Constant	809.559 (3.61***)	1050.436 (6.59***)	-356.491 (-4.22***)
summer_temp	-27.647 (-3.11***)	-39.124 (-2.05**)	-15.212 (-6.54***)
autum_temp	-94.305 (-1.24)	-108.713 (-2.53**)	
winter_temp	83.012 (4.79***)	122.861 (2.77**)	254.331 (3.35***)
spring_temp			
summer_tempsq	-1.083 (-3.17***)	-1.317 (-2.14**)	-1.591 (-6.56***)
autum_tempsq	3.232 (2.24**)	3.420 (2.57**)	1.049 (4.88***)
winter_tempsq	-1.481 (-5.02***)	-2.343 (-3.03**)	-5.608 (-3.16***)
spring_tempsq	-1.042 (-1.76*)	-1.186 (1.60*)	-1.301 (-2.15**)
summer_precip	263.981 (2.50**)	146.694 (1.77*)	135.076 (7.87***)
autum_precip	-5.197 (-3.67***)	-6.355 (-2.13**)	
winter_precip	?	?	?
spring_precip	?	?	?
summer_precipsq	-0.811 (-2.24**)	-0.417 (-4.54***)	-0.397 (-6.54***)
autum_precipsq	0.202 (3.49***)	0.160 (1.81*)	0.122 (9.11***)
winter_precipsq	1.548 (1.37)	1.631 (1.96*)	1.392 (8.22***)
spring_precipsq	-1.221 (-1.08)	-1.026 (-1.85*)	-1.109 (-8.00***)
Soil perci	-1023.718 (-2.61**)	-859.403 (-1.78*)	
Soil perclcFU	105.639 (3.97***)	429.295 (2.29**)	221.024 (6.24***)
Soil perclfCU	1264.994 (2.16**)	294.798 (1.96*)	
Soil perclgCU	1029.376 (2.69***)	338.8254 (4.64***)	
Soil percC_qc1~1a	262.422 (1.70*)	145.679 (2.16**)	
Population_density	9.726 (0.93)	-1.05 (-1.76*)	-0.093 (-1.54)
Extension_contact	2.869 (5.14***)	154.764 (3.03***)	600.641 (6.99***)
Household_size	21.805 (2.98***)	14.539 (1.37)	52.854 (6.77***)
Education_years_head	12.96162 (3.47***)	12.718 (2.38**)	30.532 (7.13***)
Total_cropped_area	-80.653 (-15.02***)	-84.623 (-11.12***)	-58.784 (-8.31***)
Distance_capital	-13.964 (-2.30**)	-22.107 (-1.81*)	-29.411 (-2.79**)
Livestock_index	4.234 (10.62***)	4.892 (6.83***)	3.271 (9.56***)
Irrigation (1/0)	110.737 (2.89***)		
Pseudo R^2	0.1871	0.2312	0.2458
Number of observations	500	377	123

*, **, ***, Significant at 10, 5 and 1% levels respectively.

sector in the face of changing climatic conditions.

The irrigation variable was also significant and positive in explaining variability of net farm revenues. This result further emphasize the importance of irrigation as an important factor in helping farmers particularly during the winter season and mid-season dry spells in summer. Farmers with access to irrigation have the capacity to cushion themselves against the harsh temperatures and limited rainfall during the dry periods. The important policy message from this finding is that promoting irrigation is very important in helping farmers cushion themselves against further changing climate. For example, the countrywide irrigation programme being implemented by the department of irrigation in the office of the president can go a long way in helping farmers in the face of further increases in climate if the implementation of the programme reaches the needy smallholder farmers in the country.

Another important point to note is that livestock is very important and can be another important source of livelihood for the smallholder farmers. Livestock, particularly cattle are an important asset in the farming system and can do well in dry climate. In this case promoting livestock production as a switch option and or complementary option to crop production in dry areas is an important safety net in the face of changing climate in the country. The policy message therefore is that livestock improvement programmes by the government's department of veterinary services and private companies is vital in sustaining farming households in the face on changing climate.

We also estimated models that included the effects of

Table 3. Marginal effects of seasonal temperature and precipitation on net revenue.

Season	All farms regression	Dryland regression	Irrigation regression
Temperature			
Summer	-86.34*** (-4.82)	-98.63*** (-7.26)	-76.74*** (-3.79)
Autumn	39.05** (2.26)	32.39*** (2.19)	43.28* (1.74)
Winter	34.08*** (1.58)	45.44** (2.47)	69.04** (2.22)
Spring	-44.13* (-2.63)	-50.36* (-3.51)	-55.24* (2.28)
Precipitation			
Summer	39.54*** (15.37)	31.29** (12.16)	25.21*** (9.81)
Autumn	30.90*** (7.76)	22.23** (5.58)	21.80* (5.47)
Winter	23.07* (0.48)	24.30** (0.51)	20.74* (0.43)
Spring	37.80 (1.64)	31.76* (1.38)	34.33* (-1.49)

*, **, ***, Significant at 10, 5 and 1% levels respectively. The numbers in brackets represent the elasticities.

including runoff as an additional source of water. The results (not shown in this paper) show positive relationships between net farm revenues and run off as an additional source of water for farms with irrigation and all farms and the relationship is negative for dry land farms. The possible explanation for this is that increases in runoff are more beneficial to farms with irrigation compared to dry land farms that do not use any runoff. These results are consistent with the expectation that additional water will increase water availability for agricultural activities and augment rainwater in times of seasonal dry spells. In this case, additional water sources in the form of runoff can be used as sources of water for irrigation during seasonal dry spells and help improve crop productivity and hence farm net revenues.

Adding interaction terms between mean runoff and climatic variables (temperature and precipitation), did not change the results much. For all farms and dryland farming, runoff and the interaction term between runoff and precipitation had both significant positive sign at 1% significant level. The results indicate that additional sources of water are very important for improving net farm performances for farmers in the country.

The results have important policy implications on the importance of providing additional water sources to rainfed smallholder agriculture particularly through irrigation. This further point to the significance of irrigation and the government can play an important role in providing additional water source to farmers through irrigation. On the other hand for farms with irrigation, both the interaction terms were also significant at 1% significant level.

Marginal effects of climate variables on net farm revenues

The study also calculated marginal impacts of climate variables (temperature and precipitation) using results from the Ricardian model (Table 2). The marginal impacts of a change in each climate variable were calculated to help interpret the climate coefficients. The marginal values depend on the regression equation that is being used and the climate that is being evaluated. The marginal effect of temperature and precipitation is evaluated at the mean for each sample, for instance the marginal effect of summer temperature on dryland is evaluated at the mean temperature of dryland.

The results on Table 3 are based on the results from using coefficients in Table 2. The results also indicate that higher summer temperatures have negative effects on net farm revenues implying that further increase in temperature would be harmful to agricultural activities in the country. A further increase in summer temperature by 1°C degree would reduce net farm revenues by about $86 per hectare for all farms and about $98 for dryland and $76 for farms with irrigation. As with summer temperatures increases in spring, temperatures results in decreases in net farm revenues. Increases in winter and autumn temperatures are beneficial to crop production and increases net farm revenues by about $34 per hectare for all farms and about $45 for dry land and $69 for farms with irrigation.

On the other hand, the increase in precipitation has positive effects on net farm revenues. The benefits are high for summer and spring precipitation increase and an increase in summer precipitation by 1 mm would result in an increase in net farm revenues of about $39; $31 and $25 per hectare, respectively for all farms, dryland farms and farms with irrigation. The increases in winter and autumn precipitation show almost similar results and both have positive effects on net farm revenues as withsummer and spring precipitation. The results points to the importance of more summer rain for successful farming in the country. More rainfall is associated with positive gains in net farm revenues, and the possible explanation for this observation is due to the recurring droughts in the country since 2000. Therefore, more

rainfall will be beneficial and crucial for successful farming in most parts of the country. The elasticity results show that net farm revenues are highly sensitive to changes in climate and this is relatively high for both summer temperature and precipitation. This is the main cropping season and changes in climate variables in this season have relatively high impacts on net farm revenues compared to the other seasons. It is important to also note that dryland farms are highly sensitive to changes in temperature and precipitation and they are affected most due to these changes as they have relatively high elasticities.

Conclusion

The study measured the economic impacts of climate change on agriculture in Zimbabwe based on a cross-section survey of over 700 farming households. We applied the Ricardian approach to analyse the response of net revenue from crop and livestock agriculture across various farm types and systems in the country to changes in climate normals (that is, mean rainfall and temperature). The sensitivity of net farm revenues was used to make inferences on the food security implications of climate change in the country. The analyses controlled for effects of key socioeconomic, technology, soil and hydrological factors influencing agricultural production. Results show that net farm revenues are affected negatively by increase in temperature and positively by increase in precipitation.

The results from sensitivity analysis suggest that agricultural production in Zimbabwe's smallholder farming system is significantly constrained by climatic factors (high temperature and low rainfall). Farms with irrigation are more resistant to changes in climate, indicating that irrigation is an important adaptation option to help reduce the impact of further changes in climate. Dryland farming predominantly typical in Zimbabwe is the most vulnerable to warming and lower rainfall, whereas the irrigated systems are the most tolerant. These results have important policy implications especially for the need to support dryland smallholder adaptation strategies for agricultural development in the country in light of expected climate changes. For example, irrigation offered better adaptation options for farmers against further warming and drying predicted under various future climate scenarios.

Conflict of Interest

The author(s) have not declared any conflict of interests.

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Full Length Research Paper

Parametric stability analyses for green forge yielding traits in oats (*Avena sativa* L.)

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The objectives of this research were to assess genotype environment interaction and determine stable oat (*Avena sativa* L.) cultivar in Kashmir division over three locations for fodder yield and its components in 10 genetically diverse genotypes using randomized block design. There was considerable variation in forage yield within and across environments. Stability analysis for forage yield was conducted to check the response to Genotype × Environment (G × E) interactions. The mean squares due to G × E (linear) were significant depicting genetic differences among genotypes for linear response to varying environments. Mean squares due to pooled deviations were highly significant, reflecting considerable differences among genotypes for nonlinear response. Out of 10 genotypes, only 4 oats lines, that is, Sabzaar, SKO-211, SKO-212 and SKO-213 showed non-significant deviation from regression and their regression coefficient values were close to unity classified were desirable for forge and dry matter yield across the environments. The cultivar, "SKO-212" with respective regression coefficient value of 1.001, the smallest deviations from regressions (S^2di) value and the highest green forage yield could be considered the most widely adapted cultivar. The other test cultivars were sensitive to production-limiting factors, their wider adaptability, stability and general performance to the fluctuating growing conditions within and across environments being lowered.

Key words: Genotype × Environment (G × E) interaction, stability analysis, forage yield, oats.

INTRODUCTION

Oats occupies a prominent place among rabi fodders in India. It provides a high tonnage of nutritious green fodders. In India, oats have a wider adaptability, particularly in western and north western regions of the country because of its excellent growing habitat's, quick re-growth and better nutritional value. As per national estimate, by 2015 and 2025 A.D., 60 corers animals will need 1097 and 1170 million tonnes of green fodder, respectively. Deficiency of green fodder will be about 64.9% and for dry fodders it may go to up to 24.9% in 2025 A.D. (Government of India, Planning Commission, 2001). The yielding ability of a variety is the result of its interaction with the prevailing environment. Environmental factors such as soil characteristics and types, moisture, sowing time, fertility and temperature and day length vary over the years and locations. There

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Table 1. Genotypes used in the study with their accession number.

S/N	Genotype	EC number/Place of collection
1.	SKO-204	EC-529089
2.	SKO-205	EC-529090
3.	SKO-207	EC-529092
4.	SKO-208	EC-529093
5.	SKO-209	EC-529094
6.	SKO-210	EC-529095
7.	SKO-211	EC-529096
8.	SKO-212	EC-529097
9.	SKO-213	EC-529098
10.	Sabzaar	Released variety (SKUAST-Kashmir)

is strong influence of environmental factors during various stages of crop growth (Bull et al., 1992), thus genotypes differ widely in their response to environments. The development of cultivars or varieties, which can be adapted to a wide range of diversified environments, is the ultimate goal of plant breeders in crop improvement program. The adaptability of a variety over diverse environments is usually tested by the degree of its interaction with different environments under which it is planted. A variety or genotype is considered to be more adaptive or stable one if it has a high mean yield but low degree of fluctuations in yielding ability when grown over diverse environments (Arshad et al., 2003). The phenotypic performance of a genotype may not be the same under diverse agro-climatic conditions. This variation is due to Genotype × Environment (G × E) interactions, which reduces the stability of a genotype under different environments (Ashraf et al., 2001). Many models have been developed to measure the stability of various parameters and partitioning of variation due to G × E interactions. The most widely used model (Eberhart and Russell, 1966) was followed to interpret the stability statistics in different crops.

Many research workers are of the view that average high yield should not be the only criteria for genotype superiority unless its superiority in performance is confirmed over different types of environmental conditions (Qari et al., 1990). Therefore, in the present investigation an attempt has been made to evaluate oat genotypes for forage yield and its component characters under different environments to identify genotypes with suitable performance in variable environments.

MATERIALS AND METHODS

The basic material for the present study consisted of 10 diverse genotypes of Oats (*Avena sativa* L.) viz: SKO-204, SKO-205, SABZAAR, SKO-207, SKO-208, SKO-209, SKO-210, SKO-211, SKO-212, and SKO-213 collected from National Bureau of Plant Genetic Resource (NBPGR) New Delhi and Sabzaar (Released variety) (Table 1) were evaluated at three locations viz., Experimental Farm of the Division of Plant Breeding and Genetics, SKUAST-K, Shalimar district Srinagar, Mountain Research Centre for Field Crops, Khudwani district Anantnag and FOA, Wadura district Baramula (Jammu and Kashmir, India) during rabi 2010 to 2011 in a randomized block design with three replications at each location and each treatment was sown in 2 rows each of 4 m length. Row to row and plant to plant spacing was maintained at 30 and 10 cm. The observations were recorded on 5 quantitative characters viz. number of leaves plant^{-1}, number of tillers metre^{-1} row, leaf stem ratio, green fodder yield plant^{-1}(g), and dry matter yield plant^{-1}(g). Data were subjected to stability analysis according to Eberhart and Russel (1966).

RESULTS AND DISCUSSION

The analysis of variance (ANOVA) for stability analysis (Table 2) indicated the presence of Genotype (G), Environment (E), G × E (Interaction), Environment + (Genotype × Environment), Environment (Linear) and Genotype × Environment (Linear) significant for all the characters under study.

The partioning of mean squares (environments + genotype × environments) (Table 2) showed that environments (linear) differed significantly and were quite diverse with respect to their effects on the performance of genotypes for forage yield and majority of yield components. Further, higher magnitude of mean squares due to environments (linear) as compared to genotype × environments (linear) exhibited that linear response of environments accounted for major part of total variation for most of the characters studied. The significance of mean squares due to genotype × environment (linear) component against pooled deviation for green fodder yield suggested that the genotypes were diverse for their regression response to change with the environmental fluctuations. Similarly, the significant mean squares due to pooled deviation observed for all the characters under study suggested that the deviation from linear regression also contributed substantially to words the difference in stability of genotypes. Thus, both linear (predictable) and nonlinear (un-predictable) components significantly contributed to genotype × environment interactions

Table 2. Analysis of variance for stability for forage yield and its attributing traits in oats.

Source of variation	df	Mean square				
		Number of tillers m^{-1}	No of leaves $plant^{-1}$	Leaf stem ratio	Green fodder yield $plant^{-1}$(g)	Dry matter yield $plant^{-1}$ (g)
Genotype (G)	9	219.935**	41.022**	33.916**	11.706**	0.468**
Environment (E)	2	56.207*	30.048**	272.657**	1.110*	0.044*
G × E (Interaction)	18	54.483**	21.233**	14.724**	210.647**	0.095*
Pooled error	54	1.016	0.081	0.0736	0.015	0.006
Environment + (G × E)	20	54.655**	51.114**	40.517**	0.694*	0.027*
E (Linear)	1	112.414**	20.096**	545.314**	112.220**	0.988**
G × E (Linear)	9	95.240**	71.773**	26.865**	1.024*	0.040*
Pooled deviation	10	12.353**	0.623**	2.325**	0.244**	0.009**

**, * significance at 1 and 5% level respectively.

observed for all the characters. This suggested that predictable as well as un-predictable components were involved in differential response of stability. Similar results were reported by (Wani et al. (1999), Rasul et al. (2006) and Akcura and Ceri (2011). Higher magnitude of mean squares due to environments indicates considerable difference between environments for all the characters suggesting large difference between environments along with greater part of genotypic response, that is, the environments created by locations were justified and had linear effects (Nehvi et al., 2007). By partitioning G × E interaction into linear and nonlinear (pooled deviation) components, differences between environments (environment linear) were highly significant, which indicated the genetic control of genotypic response to environments (Zubair and Gafoor, 2001).

The stability parameters for all cultivars are given in Table 3. Eberhart and Russell (1966) emphasized the need of considering both linear (bi) and nonlinear (S^2di) components of genotype-environment interactions in judging the stability of a genotype. A wide adaptability genotype was defined as one with bi = 1 and high stability as one with S^2di = 0. In this study, values for the regression coefficient (bi) ranged from -3.491 (SKO-210) to 1.773 (SKO-204) for number of tillers m^{-1}, -9.615 (SKO-205) to 16.538 (SKO-208) no of leaves $plant^{-1}$, 0.272 (SKO-209) to 2.199 (SKO-208) for leaf stem ratio, -1.012 (SKO-207) to 4.372 (SKO-204), -0.001 (SKO-205) to 0.029 (SKO-2013) for dry matter yield.

The regression coefficient of genotypes viz., Sabzaar, SKO-2012, SKO-2011 and SKO-2013 for green fodder yield was non-significant and almost approaching unity (bi = 1) and it had the lowest and non-significant deviation from regression and was most suitable for green forage yield over all the locations. Genotypes SKO-207, SKO-208, SKO-209 and SKO-2010 gave below average performance besides deviation from regression were significant hence the performance of these cultivars seems to be unpredictable. Accordingly, “SKO-2012” was the most stable cultivar for green forage yield, since its regression coefficient was almost equal to the unity and it had the lowest deviation from regression.

Conclusion

The cultivar “SKO-2012” was the most stable cultivar for green forage yield over all the locations. Hence, this cultivar is recommended for cultivation in different environmental conditions. The cultivar “SKO-2013” had regression coefficient significantly greater than 1.0 would be recommending for cultivation under favorable conditions only. “SKO-204” had regression coefficient significantly less than 1.0 low in green forage yield; this cultivar is, therefore, insensitive to environmental changes and adapted only to poor environments. The performance of cultivar “SKO-209” was poor; it produced below average green forage yield. This cultivar had high deviations from regression indicating sensitivity to environmental changes.

This cultivar cannot be recommended due to its overall poor performance. Genotypes Sabzaar,

Table 3. Stability parameters for forage yield and its attributing traits in oats.

S/N	Genotype	Number of tillers m^{-1}			No of leaves plant^{-1}			Leaf stem ratio			Green fodder yield plant^{-1}(g)			Dry matter yield plant^{-1} (g)		
		Mean	bi	S^2 di	Mean	bi	S^2 di	Mean	bi	S^2 di	Mean	bi	S^2 di	Mean	bi	S^2 di
1	SKO-204	69.000	1.773	14.203**	4.444	3.077	0.342*	26.465	0.954	5.229**	12.128	0.372	0.097**	2.465	4.372	0.003**
2	SKO-205	68.444	0.216	15.314**	4.444	-9.615	0.876**	28.323	2.033	8.9366**	14.822	1.916	-0.013	2.964	1.916	-0.001
3	SABZAAR	86.222	1.010	0.001	4.666	1.030	0.002	22.512	1.074	0.039	11.576	1.022	0.007	2.315	1.012	0.0053
4	SKO-207	86.666	1.522	11.058**	6.000	2.308	0.530**	25.036	0.747	0.976**	12.211	-0.012	0.079**	2.442	-0.012	0.003**
5	SKO-208	73.666	-1.381	4.332*	5.555	16.538	0.021	31.768	2.199	1.390**	11.316	0.617	0.175**	2.263	0.617	0.007**
6	SKO-209	64.777	-2.826	11.853**	4.555	-6.538	1.799**	24.505	0.272	1.397**	10.596	-1.281	0.595**	2.119	-1.281	0.008**
7	SKO-210	65.000	-3.491	19.433**	4.222	-0.769	-0.016	21.407	0.591	1.720**	12.080	2.167	0.885**	2.416	2.167	0.035**
8	SKO-211	72.555	1.051	0.034	4.777	1.098	0.046	24.270	1.031	0.019	15.238	1.051	0.062	3.047	1.051	0.006
9	SKO-212	83.555	1.042	0.102	5.333	1.049	0.085	20.992	1.069	0.006	16.111	1.001	0.005	3.222	1.012	0.007
10	SKO-213	80.777	1.057	0.083	4.444	1.023	0.042	22.280	1.030	0.048	13.183	1.066	0.026	3.036	1.064	0.029
Population mean			75.066			4.844			24.756			13.146			2.629	
SE ±			2.485			0.558			1.078			0.349			0.699	

Bi, Regression coefficient; S^2di, deviation from regression (Eberhart and Russell, 1966).

SKO-2011 and SKO-2013 showing better performance under unfavorable environmental environment are best candidates for evaluating this performance under marginal environments through participatory varietal selection programmes.

Conflict of Interest

The author(s) have not declared any conflict of interests.

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Full Length Research Paper

Genetic diversity of citrus (Rutaceae) in Iraq based on random amplified polymorphic DNA (RAPD) markers

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Citrus is an economically important fruit crop with a long history of cultivation worldwide. A great number of varieties of citrus are extensively grown in the Middle East including Iraq for domestic consumption and exports. However, the genetic diversity of this genus in Iraq has not been reported. Therefore, the objective of this study was to evaluate genetic relationships of Iragi citrus genotypes to provide useful information for germplasm conservation and planning of breeding strategies. Twenty decamer primers were used to generate RAPD markers to evaluate genetic relationship among 16 genotypes (14 species and hybrids) of cultivated Citrus in Iraq. Based on RAPD polymorphisms, the citrus genotypes were classified into two main groups; the first consisted of citron (*Citrus medica*) and its hybrids (lime and lemon). The second group contained the remaining genotypes including three sub-groups; the first being formed of sour orange (*Citrus aurantium*), sweet orange (*Citrus sinensis*) and grapefruit (*Citrus paradisi*), the second the mandarins (*Citrus reticulata*) and the third the pummelo (*Citrus grandis*). The RAPD-based classification was consistent with previous studies based on other types of molecular markers.

Key words: Citrus, genetic diversity, Iraq, oranges, random amplified polymorphic DNA (RAPD) markers.

INTRODUCTION

Among the most important fruit crops in the world is Citrus, where the international production has reached 122 million tons (FAO, 2008). The taxonomy and phylogeny of the genus Citrus is very complicated and confusing and many hypotheses have been formulated. Two most widely accepted classifications of Citrus were proposed based on morphological traits. The Swingle system (Swingle and Reece, 1967) recognized 16 species in the genus Citrus. On the other hand, the Tanaka system (Tanaka, 1977) has identified as many as

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162 species. Scora (1975) suggested that there are only three 'basic true species' of Citrus within the subgenus Citrus as defined by Swingle, that is, Citron (*Citrus medica*), Mandarin (Citrus reticulate Blanco) and Pummelo (*Citrus maxima* (Burm.) Merr. or Citrus grandis Osbeck). Other cultivated Citrus species within the subgenus Citrus are believed to be hybrids derived from these true species, species of the subgenus Papeda, or closely related genera. Additionally, Mabberley (2004) also indicated *Citrus australis* (Mudie) Planch. (Australian lime) and *C. australasica* F. Muell. (finger lime) as Australian wild parental species of many commercial hydrids. This idea has recently been supported by data derived from molecular markers (Barkley et al., 2006). High level of genetic variations exists among cultivated species of Citrus due to frequent bud mutations, wide sexual compatibility between Citrus genus and related genera, the long history of cultivation and the worldwide dispersion (Scora, 1988). Phylogeny and taxonomy for certain Citrus cultivars have been somewhat debatable in the past; however, results from molecular marker technologies are helping to clarify some of these relationships. A variety of DNA markers is available and has been used to study the classification of Citrus genus and phylogenetic relationships within Citrus and with related genera (Yamamoto et al., 1993; Federici et al., 1998).

Several molecular marker systems including Random Amplified Polymorphic DNA (RAPD), microsatellites (SSR), amplified fragment length polymorphism (AFLP) and inter-simple sequence repeats (ISSR) have been used to evaluate genetic diversity of various collections of Citrus and related genera in different countries. The monophyletic origin of Citrus was clearly confirmed by AFLP molecular analysis of 59 genotypes, six genera of the True Citrus Fruit Trees Group (Xie et al., 2008). Analysis based on 262 RAPD and 14 SCAR markers revealed that Fortunella is phylogenetically close to Citrus while the other three related genera (Poncirus, Microcitrus and Eremocitrus) are distant from Citrus and from each other (Nicolosi et al., 2000). However, molecular data based on two regions of chloroplast DNA supported a clade constituted by Citrus, Poncirus, Fortunella, and Microcitrus (Araújo et al., 2003). Data based on 119 RAPD and 48 SSR markers were used to classify 31 genotypes of Syrian Citrus and trifoliate orange into two main groups, the first consisted of Trifoliate orange (*Poncirus trifoliata*), and the other contained members of the genus Citrus. The Citrus genotypes were divided into 5 distinct groups; Sour orange, Mandarin, Rough lemon, Volkamer lemon and Sweet lime (EL-Mouei et al., 2011a). Furthermore, the same authors (EL-Mouei et al., 2011b) found that among the four Citrus groups, Lemon is distant from the others (Mandarins, Grapefruits and Sweet orange) and the highest genetic diversity was detected in the Mandarin and the lowest in the Grapefruit group. Several molecular studies supported *C. maxima*, *Citrus medica* and *Citrus reticulata* as the basal species of edible Citrus and identified probable hybrid origins of several commercial cultivars (Jena et al., 2009; Pessina et al., 2011; Ramadugu et al., 2013). In an attempt to identify the paternal and maternal origins of 30 accessions of cultivated Citrus, Li and Xie (2010) has employed three marker systems; the chloroplast DNA and internal transcribed spacer sequences and AFLP fingerprints. Molecular markers (SSR and mitochondrial DNA) have been used to characterize 201 accessions of Tunisian citrus rootstock germplasm and found that the clustering was generally consistent with varietal group classification and a core sample of accessions were identified for further use in a breeding program (Snoussi et al., 2012).

The objective of this study was to evaluate the genetic relationships among 16 taxa including 14 species and hybrids of Citrus cultivated in the central part of Iraq using RAPD markers. The genetic characterization of Citrus germplasm in Iraq has not yet been reported. The information obtained from this study is expected to provide a basis for future studies for characterization and preservation of agro-biodiversity of Citrus germplasm collection in Iraq, inferring the hybrid origin of species or cultivar identification among others.

MATERIALS AND METHODS

Plant materials

Sixteen taxa of Citrus (Rutaceae) representing 14 species/hybrids (Table 1) were collected during 2010-2012 from different geographical locations covering 4 Eastern provinces of Iraq (Figure 1). The collected plants were identified and herbarium specimens were prepared from the plant parts (stems, leaves, flowers and fruits) and deposited at the National Herbarium, Baghdad, Iraq.

Isolation of genomic DNA

Total genomic DNA was isolated from dry leaves of 16 taxa of Citrus species using the modified cetyltrimethyl ammonium bromide (CTAB) method (Doyle and Doyle, 1987). The RNA was removed by the treatment with 10 µg μl^{-1} RNase at 37°C for 30 min. The quality of the DNA were tested by staining with ethidium bromide after electrophoresis in 1% agarose gel at 100V for 45 min in 1XTBE buffer and the image was visualized with an ultraviolet transilluminator. The amount of DNA was determined by measuring the absorbance at 260 nm and the concentration was adjusted to 50 ng μl^{-1} and stored at -20°C.

RAPD analysis

Twenty different 10-mer oligonucleotide RAPD primers (Operon Technologies Inc., USA) (Table 2) were used. Each polymerase chain reaction (PCR) was carried out in a 25 µl volume containing 8 µg µl-1 template DNA, 1XPCR buffer, 1.5 mM $MgCl_2$, 0.32 mM dNTP, 1.0 µM primer and 0.16 unit μl^{-1} Taq DNA polymerase (iTaq DNA polymerase Kit). Amplification was performed in a thermal

Table 1. List of 16 *Citrus* taxa included in the study.

S/N	Species	Locality	Parentage	Common name
1	*C. aurantifolia* var. *Acidica* (Christm.) Swingle	Salah aldeen	*C. medica* x *C. Limon* X *C. micrantha*	Mexican lime
2	*C. aurantium* L.	Diyala	*C. grandis* x *C.reticulata*	Sour orange
3	*C. deliciosa* Ten.	Babel	-	Willow leaf Mandarin
4	*C. grandis* Osbeck	Baghdad	Female parent	Pummelo
5	*C. japonica* Thunb.	Salah aldeen	-	Margarita
6	*C. latifolia* Tanaka	Babel	*C. sinensis* x *C. aurantifolia*	Persian lime
7	*C. limetta* Risso	Baghdad	-	Sweet lemon
8	*C. limon* (L.) Burm.f.	Karbala	*C. medica* x *C. aurantium*	Lemon
9	*C. medica* L.	Diyala	Male Parent	Citron
10	*C. paradisi* Macfad	Baghdad	*C. sinensis* x *C. grandis*	Grape fruit
11	*C. reshni* Hort. ex Tanaka	Diyala	-	Cleopatra (Egyptian mandarin)
12	*C. reticulata* var. *Clementine* Blanco	Karbala	*C. reticulate* x *C. sinensis*	Mandarin
13	*C. sinensis* Osbeck	Baghdad	*C. grandis* x *C. reticulata*	Sweet orange
14	*C. aurantium* L. x *C. trifoliata* (L.) Raf.	Diyala	*C.aurantium* x *C. trifoliata*	Citradia
15	*C. sinensis* var. *moro* (L.) Osbeck	Diyala	*C. grandis* x *C. reticulata*	Red orange
16	*C. volkameriana* Pasq.	Babel	*C. medica* x *C. aurantium*	Volkamer lemon

cycler (Corbett Research, Australia) using the following conditions: denaturation at 95°C for 3 min; 40 cycles of 1 min denaturation at 94°C, 1 min annealing at 40°C and 2 min extension at 72°C; and a final extension at 72°C for 7 min. The RAPD-PCR products were analyzed directly on 1.5% agarose gel in 1XTBE buffer. The DNA was stained with 0.5 mg ml^{-1} ethidium bromide, visualized and photographed under a UV transilluminator.

Data analysis

The amplified bands were scored for each RAPD primer based on the presence (1) or absence (0), on the basis of size. RAPD matrix was then analyzed using the NTSYS-pc statistical package version 2.1. The data matrix was used to calculate the genetic similarity within and among species based on Jaccard's similarity coefficients, and a dendrogram displaying relationships among the 16 genotypes of Citrus was constructed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA).

RESULTS AND DISCUSSION

A total of 143 amplified RAPD bands ranging from 100 bp to 1.8 kb in size were observed from the 16 Citrus genotypes. The number of RAPD bands varied from 2 (primer OPX16) to 13 (primers OPA-04 and OPW-06), with an average of 7.15 bands per primer. One hundred and twenty-two polymorphic bands (85.15% of the total amplified bands) were obtained, with an average of 6.1 bands per primer. Some representative polymorphisms revealed by RAPD primers are presented in Figure 2.

The dendrogram showing the genetic relationships among the 16 Citrus genotypes (Figure 3) showed that Citrus species were basically divided into 2 main clusters, the first (Cluster I) consisted of citron, lime and lemon; the second (Cluster II) contained pummelo, mandarin, grapefruit, sweet orange, sour orange and sweet lemon. The two main clusters separated at the similarity value of 0.67. Similar clustering was reported by Uzun et al. (2009) who divided 83 accessions of the genus Citrus into two large groups based on sequence related amplified polymorphism markers (SRAP). The first group included citron, lemon, lime and rough lemon; and the second group consisted of pummelo, grapefruit, sour orange, mandarins, sweet oranges and their hybrids. Using nine cpDNA sequences Bayer et al. (2009) showed that Citrus contained two lineages; the largely "southern clade" contains primarily wild species from New mandarin group, the lime group and the pummello group. Recently, Luro et al. (2011) also organized 87 citrus varieties into two main groups based on single strand conformation polymorphism (SSCP). The first group contained mandarins, sour

Figure 1. Geographic localization of sampling sites of Iraqi *Citrus* in this study (No. 1- 16 = *Citrus* taxa listed in Table 1).

oranges, sweet oranges, pummelo and grapefruits; and the second group included citrons, lemons, and limes and lemon hybrids.

Furthermore, an assessment of genetic diversity and population structure of a citrus germplasm collection of 370 accessions using simple sequence repeat markers (SSR) revealed five main populations which supported the hypothesis that there are only a few naturally occurring species of Citrus and most other types of Citrus arose through various hybridization events and mutations. The ancestral groups included citron which was separated from the cluster containing mandarins, pummelos and papedas (Barkley et al., 2006).

The first major Cluster (I) which included citron (*C. medica*) as the basic true species consisted of 2 sub-clusters; IA included two genotypes, that is, Mexican lime (*Citrus aurantifolia* var. acidic) and Citrus japonica var. margarita which showed similarity coefficient of 0.79. The second sub-cluster (IB) contained lemon (*Citrus limon*) and Persian lime (*Citrus latifolia*). This sub-cluster linked with citron (*C. medica*) by similarity value of 0.78. Nicolosi et al. (2000) also placed *C. medica*, *C. aurantifolia* and *C. limon* in the same group based on RAPD and SCAR markers. Both lemons and limes were proposed to be hybrids with citron contributing most of the alleles as the male parent (Barrett and Rhodes, 1976; Federiciet al., 1998; Nicolosi et al., 2000; Barkley et al., 2006). Recently, Li and Xie (2010) analyzed plastid genomes, nuclear ITS sequences and AFLP fingerprints of 30 citrus accessions in an attempt to infer into the origin of cultivated citrus. Such detailed molecular analysis Guinea, Australia, New Caledonia, New Ireland and two (*C. indica* and *C. medica*) historically considered to have arisen from India. The “northern clade” contained most of the economically important citrus species and cultivars which can be separated into the kumquat group, the demonstrated that sour orange was the maternal and citron the paternal parent of *C. limon.*

Moreover, it was strongly supported that *C. aurantifolia* was a hybrid of Papeda (maternal parent) and citron (paternal parent). Using the combined molecular, morphological and cytometric parameters Pessina et al.

Table 2. The codes and sequences of twenty RAPD primers used for PCR amplification of genomic DNA from 16 *Citrus* genotypes.

Primer	Primer sequence (5´-3´)	AN	Size range of bands (bp)	PM	%	MM	%
OPA-04	AATCGGGCTG	13	100->1500	13	100	0	0
OPA-05	AGGGGTCTTG	6	270-1500	5	83.3	1	16.7
OPA-09	GGGTAACGCC	7	300->1500	5	71.4	2	28.6
OPA-12	TCGGCGATAG	6	250-1400	5	83.3	1	16.7
OPB-06	TGCTCTGCCC	9	250-1600	8	88.8	1	11.2
OPB-12	CCTTGACGCA	6	150-610	5	83.3	1	16.7
OPW-06	AGGCCCGATG	13	250-1400	11	84.6	2	15.3
OPW-07	CTGGACGTCA	8	250-1400	6	75	2	25
OPW-09	GTGACCGAGT	11	100-1100	11	90.9	0	0
OPW-19	CAAAGCGCTC	4	300-900	3	75	1	25
OPX-02	TTCCGCCACC	5	400-1300	5	100	0	0
OPX-03	TGGCGCAGTG	5	100-1000	4	80	1	20
OPX-07	GAGCGAGGCT	6	200-1500	5	83.3	1	16.7
OPX-08	CAGGGGTGGA	5	100-800	5	100	0	0
OPX-09	GGTCTGGTTG	7	200-900	6	85.7	1	14.3
OPX-11	GGAGCCTCAG	9	200->1500	5	75	4	25
OPX-12	TCGCCAGCCA	9	100-1000	8	88.8	1	11.2
OPX-15	CAGACAAGCC	5	400-900	5	100	0	0
OPX-16	CTCTGTTCGG	2	200-1000	2	100	0	0
OPX-17	GACACGGACC	7	250-1000	6	85.7	1	14.3
Total		143	100->1500	123	-	20	-

Total number and size range of amplified bands and the number of polymorphic and monomorphic bands obtained for each primer. AN = alleles number; PM = Polymorphic bands; MM = Monomorphic bands.

(2011) confirmed the hybrid origin of *C. limonimedica* from *C. medica* and *C. limon*.

Sweet orange, mandarin, sour orange, pummel and grapefruit nested in the same large Cluster (II). Within this group, the mandarin (*Citrus reticulata*) and pummelo *(C. grandis*) were considered the parental species and the remaining genotypes were hybrids derived from mandarin, pummelo and citron (Barett and Rhodes, 1976). This group separated into three subgroups; the orange-grapefruit, the mandarin and the pummelo. The first sub-group contained sour orange (C. aurantium), grapefruit *(C. paradisi*), two genotypes of sweet orange (*C. sinensis*), Citradia (*C. aurantium* x *C. trifoliata*) and *C. volkameriana*. Different marker systems have been used to support the clade containing sour orange, grapefruit and sweet orange. The marker systems used included RAPD and SCAR (Nicolosi et al., 2000), SRAP (Uzun et al., 2009), AFLP (Li and Xie, 2010), and SSCP (Luro et al., 2011). Barett and Rhodes (1976) suggested that sweet orange, sour orange and grapefruit were all were all hybrids of pummelo and mandarin. Molecular evidence recently confirmed that pummelo and mandarin were the maternal and paternal origins, respectively of sweet orange and sour orange. Whereas grapefruit was a hybrid of pummelo and sweet orange which acted as female and male parents, respectively (Barkley et al., 2006; Li and Xie, 2010).

The sweet orange (*Citrus sinensis*) group has high similarity coefficient value of about 0.82 indicating their close relationship. Fang et al. (1998) reported similar results while working on 41 samples of 33 cultivars, belonging to 3 sweet orange groups, that is, Valencia, blood and navel, based on fruit traits. All of these cultivars had almost the same DNA fingerprints, isozymes and RFLP profiles. The two taxa of sweet orange, *C. sinensis* and *C. sinensis* var. moro (red orange) were grouped together with similarity value of 0.84 which is the highest value among any two citrus taxa in this study. This result further supported the suggestion that these two varieties were hybrids between pummelo and mandarin (Barett and Rhodes, 1976). Analysis of chloroplast DNA demonstrated that pummelo was the maternal parent of the sweet orange (Li and Xie, 2010). The other member of orange (citradia; *C. aurantium* x C. trifoliata) was separated from the others and formed a group with C. volkameriana. This close relationship supported the suggestion that citradia was a hybrid between trifoliate orange and sour orange (Swingle and Reece, 1967) and Citrus volkameriana was a hybrid between citron and sour orange (Nicolosi et al., 2000).

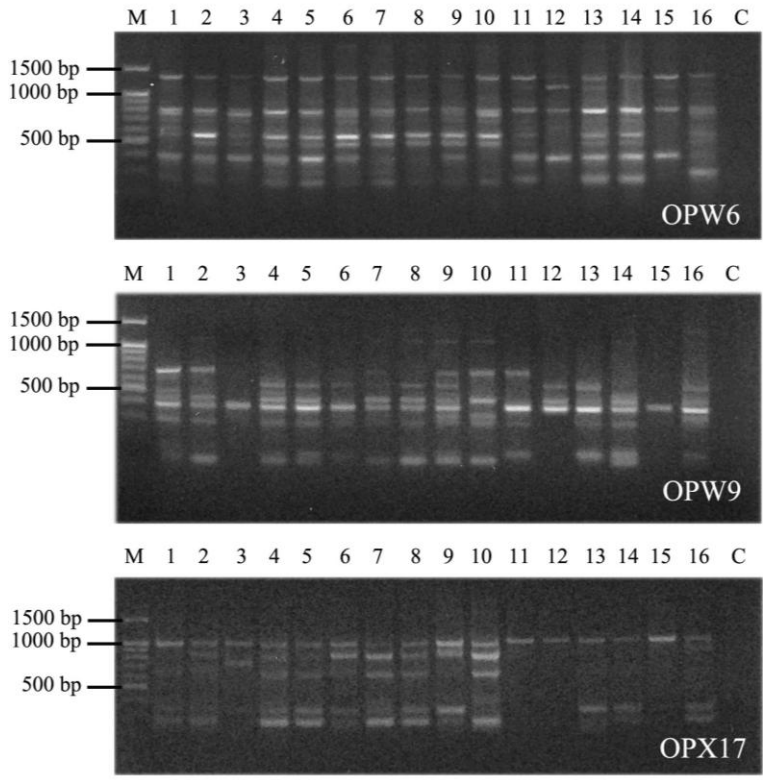

Figure 2. RAPD profiles amplified from genomic DNA of 16 *Citrus* taxa using primer OPW-06, OPW-09 and OPX-17. M = 100-bp DNA ladder, 1-16 = *Citrus* taxa described in Table 1, C = negative control without template DNA.

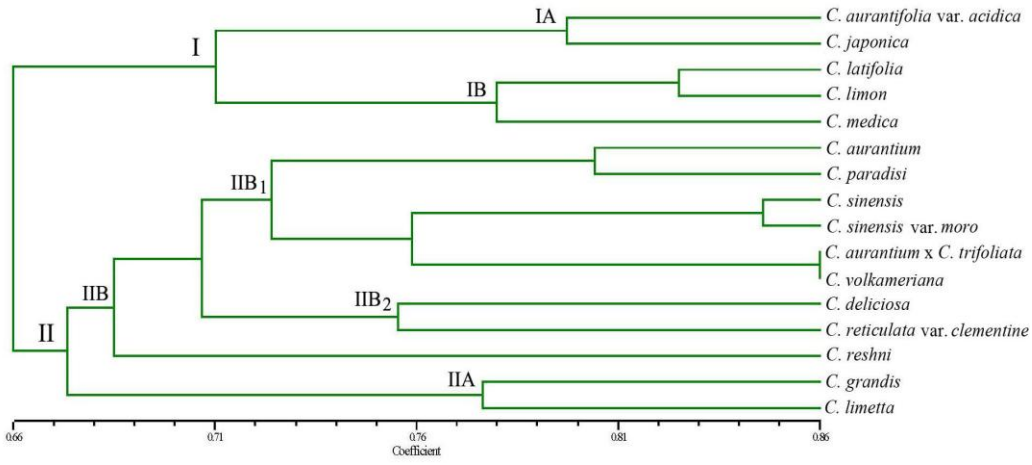

Figure 3. Dendrogram showing genetic relationships among 16 taxa of *Citrus* in Iraq.

Although, most molecular analysis placed *C. volkameriana* in the citron group, this particular type of *C. volkameriana* cultivated in Iraq seemed to have more shared alleles from sour orange than citron.

Three mandarins were included in this study, that is, *Citrus deliciosa* (willow leaf mandarin), *C. reticulata* var. clementine and *Citrus reshni* (cv. Cleopatra). In this RAPD-based analysis, willow leaf mandarin and clementine formed a group with similarity value of 0.75 which separated from the orange-grapefruit group at the

similarity value of 0.71. *C. reshni* was more distantly related and separated from the orange-mandarin group at the similarity value of 0.68. The observation that C. deliciosa was more closely related to *C. reticulata* and *C. reshni* was more distantly related was earlier shown by Filho et al. (1998) also based on RAPD markers. This molecular characterization confirmed the earlier classification by Tanaka (1954) who recognized 36 species of mandarins in five taxonomic groups; C. reticulata and *C. deliciosa* were placed in Group III whereas *C. reshni* in Group IV. The pummelo (*C. grandis*) was a member of Cluster II which was separated from all remaining genotypes. Pummelo was reported as one of the three true citrus species by Barett and Rhodes (1976) and most of the molecular studies were in agreement with this statement (Nicolosiet al., 2000; Barkley et al., 2006; Uzun et al., 2009).

Preservation of the genetic diversity of crop species throughout the world has become a major issue of international concern. Reduction in agro-biodiversity often increases vulnerability of crops to climatic stresses and diseases (Thrupp, 2000). Notably, the outbreak of citrus canker disease in Florida in 1984 leading to an eradication of twenty million citrus plants was in part due to genetic uniformity of the citrus crops (Schubert et al., 2001). Understanding of genetic diversity of citrus using both morphological and molecular data is essential forgermplasm management, planning and application of breeding programs in Iraq.

Conflict of Interest

The author(s) have not declared any conflict of interests.

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