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Full Length Research Paper

In vitro inhibitory effect of selected fungicides on mycelial growth of ambrosia fungus associated with the black coffee twig borer, *Xylosandrus compactus* Eichhoff (Coleoptera: Curculionidae) in Uganda

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Black coffee twig borer is a new but rapidly spreading insect pest of coffee in Uganda. Female beetles bore into primary branches/twigs and cultivate an ambrosia fungus for feeding their larvae. Thus, controlling the fungus means depriving the brood a source of food. Three fungicides, chlorothalonil (Glider), tebuconazole (Orius 25EW) and dimethomorph + mancozeb (Volar) were evaluated *in vitro* for their effectiveness in inhibiting mycelial growth of ambrosia fungus associated with the beetle. The pathogen was exposed to four concentrations (1.5x, 1.25x, 1.0x and 0.5x times the manufacturer recommended rate) incorporated into potato dextrose agar using inhibition and food poisoning techniques. The three fungicides inhibited fungal growth to some extent, even at the lowest concentration (0.5x) and percentage inhibition was significantly different (P≤0.05) from each other. Tebucozanole caused 100% growth inhibition irrespective of concentration and technique used while chlorothalonil and dimethomorph + mancozeb caused less than 40% inhibition for both techniques. Therefore, research should determine effectiveness of tebucozanole for suppressing fungal growth under field conditions for diminishing beetle incidence and fungal pathogenic effects in infested branches. This will pave way for integration of use of tebucozanole into overall Integrated Pest Management package (IPM) for the beetle in Uganda.

Key words: Ambrosia-fungus, black-coffee-twig-borer, chlorothalonil, dimethomorph + mancozeb, fungicides, tebuconazole, *Xylosandrus-compactus*.

INTRODUCTION

Coffee plays a vital role in the economy of Uganda, being the main export crop and a major source of

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foreign currency. It is estimated that the coffee sub-sector in Uganda provides livelihood to over 1.3 million households and about 5 million people in various businesses along the coffee value chain (UCDA, 2010). Despite its importance, coffee production has been declining for over a decade due to a number of constraints, in particular, the Coffee Wilt Disease (CWD) (Adipala-*Ekwamu et al.,* 2001). Just as the sustained effort to manage CWD led to recent release of 7 CWD resistant Robusta coffee varieties (Musoli et al., unpublished), the threat of the black coffee twig borer (hereafter abbreviated as BCTB), *Xylosandrus compactus* Eichhoff (Coleoptera: Curculionidae) has emerged (Egonyu et al., 2009). BCTB is a highly invasive and damaging pest that spreads far and wide over a short period of time. A survey carried out in 2011/2012 shows that the pest is rapidly spreading countrywide causing severe damage on coffee particularly in central, Busoga and southwestern regions (Kagezi et al., unpublished data). These regions produce the bulk of the country's Robusta coffee (Musoli et al., 2001).

The female beetle bores into the primary branches (twigs), causing them to wilt and die within a few weeks (Egonyu et al., 2009). The beetle cultivates an ambrosia fungus in the bored coffee galleries for feeding its larvae (Hara and Beardsley, 1976; Ngoan et al., 1976). The ambrosia fungus found in the BCTB is *Fusarium solani* (Martius) Saccardo (Egonyu et al., 2009). However, little is known about the actual cause of the wilting and eventually death of the attacked twigs exists. This could be due to either disruption of water and nutrient movement across the galleries made by the pest or pathogenicity of the ambrosia fungus to coffee. *Fusarium* spp. are among the most important plant pathogens in the world (Nelson et al., 1983). *F. solani* has been isolated from coffee plant tissues (Serani et al., 2007; Tshilenge et al., 2010) and has been reported to cause cankers, root rot, wilt and dieback symptoms on coffee (Baker, 1972; Venkatasubbaiah et al., 1984; Dudley et al., 2008). Thus, in addition to being a source of food for the young beetles (Hara and Beardsley, 1976; Ngoan et al., 1976), it is most probable that the ambrosia fungus might be pathogenic to the coffee. Fungal pathogens are normally controlled by use of fungicides (Murdoch and Wood, 1972) and a number of *in vitro* studies have demonstrated that some fungicides may restrict or prevent mycelial growth of many Fusaria. For example, Mancozeb gave 100% inhibition of mycelia growth of *F. solani* at 0.2 and 0.3% concentrations *in vitro* (Chavan et al., 2009). However, sensitivity of the ambrosia fungus associated with *X*. *compactus* in Uganda to fungicides has not yet been determined. This information will provide a baseline for evaluating the potentiality of these fungicides for field use to suppress the ambrosia fungus, hence control the twig borer. Consequently, successful candidate fungicides shall be incorporated into the overall IPM strategy for *X*. *compactus* in Uganda, since controlling the fungus means depriving the young beetles their exclusive source of food. Pursuant to the above therefore, we conducted *in vitro* experiments to evaluate the effect of three fungicides *viz* chlorothalonil (Glider), tebuconazole (Orius) and dimethomorph + mancozeb (Volar) on the mycelial growth of ambrosia fungus isolated from female beetle mycangium, and from its associated coffee galleries.

MATERIALS AND METHODS

Study site

The study was conducted at the Coffee Research Center (COREC), Kituza located about 40 km from Kampala in Mukono district, south-central Uganda. Kituza lies on the longitude 32°45'0" E and latitude 0°22'0" N. Mukono district experiences two rainy seasons (March-May and September-December) with a mean annual rainfall of 1400 to 1600 mm. The mean annual maximum temperature is 25 to 27.5°C and the mean annual minimum is 15 to 17.5°C. The predominant soils types are mainly ferralic with sandy clay- loams as the main constituents (DSER, 1997).

Isolation of the ambrosia fungus, media preparation and fungicides used

The ambrosia fungus used in this study were isolated from the mycangium of the female beetles and the debris in the associated coffee galleries collected from coffee fields at COREC in 2011. To isolate the ambrosia fungus, the beetles were chopped into small pieces and plated on tap water agar (2% Agar technical-Oxoid in 1000 ml tap water). The debris from the galleries was also scrapped with surgical blades and sprinkled on solidified tap water agar (TWA). Both cultures (from the beetle and the galleries) were incubated separately at 25°C for three days and then sub-cultured on synthetic nutrient agar (SNA) (Nirenberg, 1976) and potato dextrose agar to reveal the characteristic conidia shapes and pigmentation respectively. The cultures were incubated under 12 h fluorescence light and dark cycles at room temperature for 10 days. Evaluation of fungicide against the fungus was done by two methods, namely: inhibition techniques (Meah et al., 2002) and food poisoning (Borum and Sinclair, 1968) as described below.

Three fungicides, chlorothalonil (Glider), 720 g/L with a dosage of 2 to 2.5 ml/L, tebuconazole (Orius 25 EW) 250 g/L with a dosage of 70 ml/L and dimethomorph + mancozeb (Volar), 690 g/kg with a dosage of 2.7 g/L at four concentrations (1.5x, 1.25x, 1.0x and 0.5x: where x is the field rate recommended by the manufacturer) were used in this study. The fungicides were mixed into 10 ml of sterile water before application and allowed to set to obtain concentrations of 3.6, 7 and 2.6 mg/L for chlorothalonil, tebuconazole and dimethomorph + mancozeb respectively.

The inhibition technique

A plug of the ambrosia fungus (4 x 4 mm) from a 10 day-old culture was placed in the middle of a Petri dish containing potato dextrose agar (PDA). Three sterile pieces of filter paper squares (5 x 5 mm) were placed on the surface of solidified agar at a distance of about 30 mm from the inoculum in a radial pattern in each Petri dish (Plates 1a, 2a, 3a). The fungicides were then applied on the filter paper in order to defuse into the agar. The experiment was laid in completely randomized design (CRD) with the fungicide concentration as the treatment. Each treatment was replicated 4 times. The radial growth of the fungus was measured after 14 days

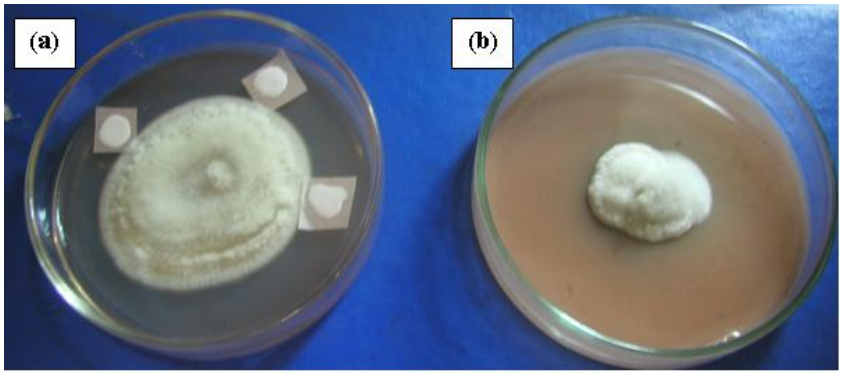

Plate 1. *In vitro* effect of fungicide chlorothalonil (Glider) on the mycelial growth of ambrosia fungus isolated from mycangium of female beetles and debris in the associated coffee galleries using the inhibition technique (a) and food poisoning technique (b).

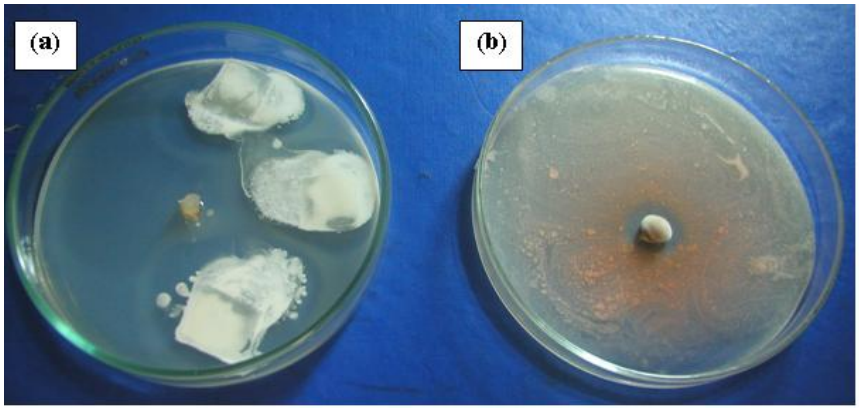

Plate 2. *In vitro* effect of fungicide tebuconazole (Orius 25 EW) on the mycelial growth of ambrosia fungus isolated from mycangium of female beetles and debris in the associated coffee galleries using the inhibition technique (a) and food poisoning technique (b).

of growth (when the fungus completely covered the plates in the control). The percentage growth inhibition (I) for the different fungicides was assessed as follows (Fokkema, 1973):

$$I=\frac{r1-r2x100}{r1}$$

Where: I=percentage growth inhibition, *r1*=radius of the pathogen away from the fungicide (mm/cm) and *r*2=radius of the pathogen towards the fungicide (mm/cm).

Food poisoning technique

This was done by incorporating the fungicides into the medium before setting in Petri dishes. A 5 mm diameter agar disk of the test fungus was extracted from a 10 day-old PDA culture plate using a sterile surgical blade and placed in the centre of Petri plates that contained the fungicides incorporated into the PDA at the various concentrations levels (Plates 1b, 2b, 3b). The plate without fungicides served as the control (Plate 4). The plate without fungicides served as the control (Plate 4). The experiment was laid in a completely randomized design (CRD) with fungicide type and concentration as the treatments. These were replicated four times. The radial fungal growth was recorded after 45 to 50 days (when the fungus completely covered the plates in the control). The percentage inhibition (PI) of the fungus over control was calculated using the following formula:

$$PI=\frac{A-B}{A}x100$$

Where: A=colony growth of the fungus in control plate and B=colony growth of the fungus in treated plate.

Data analysis

Before the analysis, the percentage inhibition data were arcsine-transformed to reduce non-normality and heterogeneity of variances. The analysis of variance (ANOVA) was performed with the general linear model (GLM) procedure of the Statistical Analysis System (SAS) software (SAS Institute, 2008). Means were

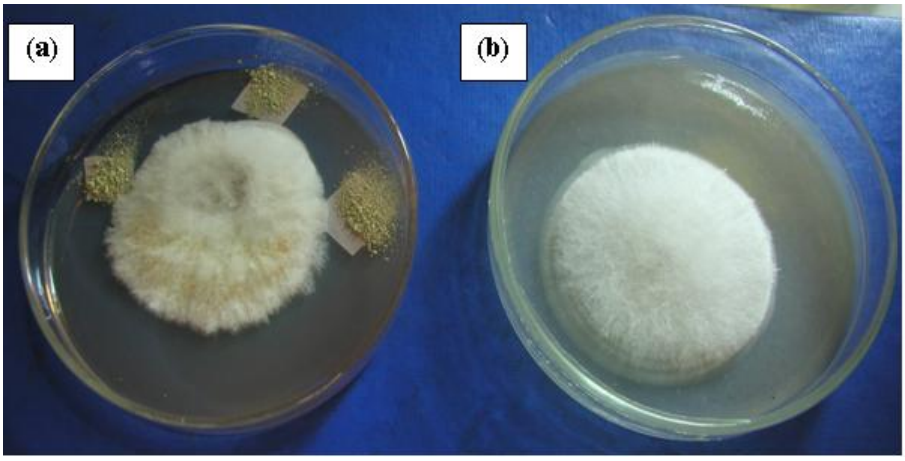

Plate 3. *In vitro* effect of fungicide dimethomorph + mancozeb (Volar) on the mycelial growth of ambrosia fungus isolated from mycangium of female beetles and debris in the associated coffee galleries using the inhibition technique (a) and food poisoning technique (b).

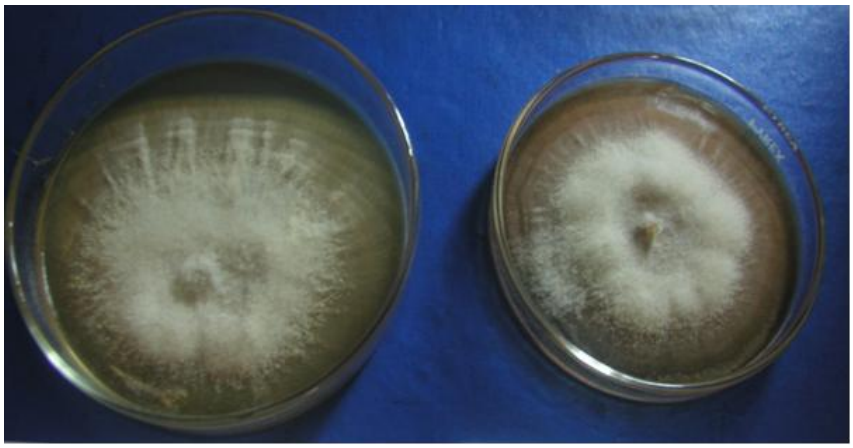

Plate 4. *In vitro* effect of the control (without fungicide) on the mycelial growth of ambrosia fungus isolated from mycangium of female beetles and debris in the associated coffee galleries using the food poisoning technique.

separated by the Tukey test at 5%. Note that the data for fungicide tebucozanole (Orius) were omitted when analysing for the effect of fungicidal concentration on mycelial growth of the fungus because 100% inhibition was attained for all the concentrations irrespective of the technique used.

RESULTS

Our results clearly show that irrespective of the concentration level and technique used, all the three fungicides tested in the study were able to inhibit growth of the ambrosia fungus to some extent (at least >20%; Tables 1 and 2). The percentage fungal growth inhibition varied across the fungicides and they were highly significantly different (P≤0.05) from each other for both techniques (food poisoning and inhibition). Of the three fungicides, tebucozanole (Orius) was the most effective in inhibiting the mycelial fungal growth, causing 100% inhibition in both techniques (Table 1). Overall, for both techniques, fungicides chlorothalonil (Glider) and dimethomorph + mancozeb (Volar) caused less than 40% inhibition of mycelial fungal growth (Table 1). Further, the inhibitory effect of fungicides, chlorothalonil (Glider) and dimethomorph + mancozeb (Volar) increased significantly with increasing fungicidal concentrations in both techniques. However, the inhibitory effect of chlorothalonil (Glider) did not differ significantly for concentrations 1.5x and 1.25x, and also for 1.25x and 1.0x. Similarly, the inhibitory effect of dimethomorph + mancozeb (Volar) did not differ significantly for concentrations 1.5x and 1.25x, and, 1.0x and 0.5x (Table 2).

Table 1. Percentage inhibition of radial mycelial growth of ambrosia fungus isolated from mycangium of female beetles and debris in the associated coffee galleries by fungicides, tebucozanole (Orius), chlorothalonil (Glider) and dimethomorph + mancozeb (Volar) using food poisoning and inhibition techniques. Same letters within a column indicate means (after arcsine transformation) are not significantly different by Tukey's test (*P≤0.05). Values in parenthesis are the untransformed means.

Fungicide	Food poisoning technique	Inhibition technique
Orius	7.9 (100.0)[a]	7.9 (100.0)[a]
Glider	4.4 (31.8)[b]	4.8 (37.4)[b]
Volar	3.7 (23.3)[c]	4.3 (29.6)[c]
F value	361.74**	234.53**
CV	8.78	9.0

Table 2. Percentage inhibition of radial mycelial growth of ambrosia fungus isolated from mycangium of female beetles and debris in the associated coffee galleries using food poisoning and inhibition techniques by fungicides, chlorothalonil (Glider) and dimethomorph + mancozeb (Volar) at varying concentration using food poisoning and inhibition techniques. Same letters within a column indicate means (after arcsine transformation) are not significantly different by Tukey's test (*P≤0.05). Values in parenthesis are the untransformed means.

Concentration	Food poisoning technique		Inhibition technique	
	Glider	Volar	Glider	Volar
0.5X	3.6 (22.5)[d]	2.8 (12.5)[d]	3.8 (23.6)[c]	3.7 (21.4)[b]
1.0X	4.2 (28.4)[c]	3.5 (19.7)[c]	4.7 (36.2)[b]	3.8 (22.5)[b]
1.25X	4.6 (34.4)[b]	4.0 (25.9)[b]	5.2 (42.6)[ab]	4.8 (36.7)[a]
1.5X	5.1 (41.9)[a]	4.4 (31.3)[a]	5.5 (48.3)a	4.9 (37.6)[a]
F value	156.51**	208.10**	34.88**	597.56**
CV	2.07	2.58	5.05	1.28

DISCUSSION

This study evaluated the efficacy of three fungicides, chlorothalonil (Glider), tebuconazole (Orius 25 EW) and dimethomorph + mancozeb (Volar) *in vitro* for inhibiting radial mycelial growth of ambrosia fungus using the inhibition and food poisoning techniques. Our data show that all the three fungicides tested were able to inhibit mycelial growth of the fungus, even at the lowest concentration (0.5x) irrespective of the technique used. These results are in agreement with a number of earlier *in vitro* studies which have demonstrated that various fungicides may restrict or prevent growth of *F. solani* and other Fusaria (Tepper et al., 1983; Chavan et al., 2009; Sultana and Ghaffar, 2010). Tebucozanole (Orius) had the greatest inhibitory effect, causing 100% inhibition irrespective of the fungicide concentration or techniques employed. These results are in line with earlier research studies which reported very strong *in vitro* inhibition effects of tebuconazole-based fungicides on fungal mycelial growth of several *Fusarium* species. For example, *F. avenaceum* (Simpson et al., 2001; Ivić et al., 2011), *F. culmorum* (Simpson et al., 2001), *F. graminearum* (Ramirez et al., 2004 ; Ivić et al., 2011) and *F. verticillioides* (Ivić et al., 2011) among others.

The high effectiveness of tebucozanole (Orius) is of great importance in the management of the ambrosia fungal gardens and thus the twig borer. First of all, tebucozanole-based products are is highly systemic with protective, curative, and eradicant action (Shtienberg and Dreishpoun, 1991; Labrinos and Nutter, I993). The fungicide is absorbed rapidly into the vegetative parts of the plants by the leaves and stems and is translocated acropetally upward in the plants. Thus, the fungicide can easily reach the ambrosia fungal gardens located deep inside the galleries/tunnels in the coffee twigs (Hara and Beardsley, 1976; Ngoan et al., 1976). Secondly, the fact that tebucozanole is equally effective even at half the manufacturer's recommended rate implies that future research can explore the possibilities of using even lower dosages that would minimize costs. One of the major limitations of using tebucozanole in disease management is its cost. Currently, a liter of tebucozanole costs 150,000 Uganda shillings (approximately US$ 60) on the market which is definitely too high for the small scale coffee farmers who produce more than 80% of the coffee in the country (Musoli et al., 2001).

Our data further show that chlorothalonil (Glider) and dimethomorph + mancozeb (Volar) caused far less fungal growth inhibitory effect (<40%) compared to tebucozanole

(Orius) irrespective of the inhibition technique used. These results are in agreement with earlier *in vitro* studies which reported low fungal mycelial inhibitory effect (>25%) by chlorothalonil-based fungicides on several *Fusarium* species including *F. oxysporum* f. sp. *cumini* (Bardia and Rai, 2007) and *Fusarium avenaceum* (Kopacki and Wagner, 2006). Similarly, Kim et al. (2005), Cha et al. (2007) and Mamza et al. (2008) reported low inhibitory effect of dimethomorph- and mancozeb-based fungicides on mycelial growth of *F. pallidoroseum* and *F. oxysporum* respectively. However, our results contradict studies by Tepper et al. (1983) who reported complete (100%) *in vitro* inhibition of mycelial growth of *F. solani* by chlorothalonil-based fungicides at 1000 mg/L. Similarly, Tepper et al. (1983), Chavan et al. (2009) and Sultana and Ghaffar (2010) reported that mancozeb-based fungicides caused complete (100%) *in vitro* inhibition of mycelial growth of *F. solani* at 100 mg/L, 0.2 and 0.3% concentrations respectively. The contradiction in the results could have been due to the difference in the content of the fungicides in final commercial products and also the source of the fungicide. Incidentally, counterfeit or adulterated chemicals including fungicides are very common on Ugandan market (MAAIF, 2009).

Further, the inhibitory effect of chlorothalonil (Glider) and dimethomorph + mancozeb (Volar) increased significantly with increasing fungicidal concentrations in both techniques. These results are in agreement with earlier studies which reported that the fungal inhibitory effect of the chlorothalonil-, mancozeb- and dimethomorph-based fungicides increased with increasing concentration of the fungicide (Kopacki and Wagner, 2006; Bardia and Rai, 2007; Cha et al., 2007; Mamza et al., 2008).

In conclusion, our results clearly show that fungicide tebucozanole (Orius25 EW) caused 100% inhibition of the mycelial growth of the ambrosia fungus. Thus, further research should concentrate on determining the effectiveness of this fungicide for controlling ambrosia under field conditions. Secondly, this fungicide should be combined with candidate insecticides (tank mixture) and integrated it into the chemical control option and the overall Integrated Pest Management (IPM) strategies for managing the ambrosia fungus and thus its associated beetle.

Conflict of Interest

The authors have not declared any conflict of interest.

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REFERENCES

Adipala-Ekwamu Opio O, Kyetere D, Hakiza G, Tushemereirwe W, Bua A, Kisanzi D, Koyobyo G (2001). Background and importance of Coffee Wilt Disease in Uganda. National Agricultural Research Organisation (NARO) research progress report (1997-2000). pp. 1-16.

Baker CJ (1972). *Fusarium solani* associated with a wilt of *Coffea arabica* in Kenya. East Afr. Agric. For. J. 38:137-140.

Bardia PK, Rai PK (2007). *In vitro* and field evaluation of biocontrol agents and fungicides against wilt of cumin caused by *Fusarium* oxysporum f. sp. cumini. J. Spices Aromatic Crop 16(2):88-92.

Borum DF, Sinclair JB (1968). Evidence of systemic fungicides protection against *R. solani* with Vitavax in cotton seedlings. Phytopathology 58:976-980.

Cha S-D, Jeon Y-J, Ahn G-R, Han JI, Han K-H, Kim SH (2007). Characterization of *Fusarium oxysporum* isolated from Paprika in Korea. Mycobiology 35(2):91-96.

Chavan SC, Hegde YR, Prashanthi SK (2009). Management of wilt of patchouli caused by *Fusarium solani.* J. Mycol. Plant Pathol. 39:32-34.

DSER (1997). District State of Environment Report. Mukono district. http://www. unep.org/Pearl/Login/OP/BLOBS/FullText/PEARLFullTextBLOB_1534_642.pdf. Last accessed on April 02, 2012.

Dudley N, Stein JD, Jones T, Gillette N (2008). Semiochemicals provide a deterrent to the black twig borer, *Xylosandrus compactus* (Coleoptera: Curculionidae, Scolytidae). In: Gottschalk KW (ed) Interagency research forum on gypsy moth and other invasive species. Proceedings of the 17th U.S. Department of Agriculture interagency research forum on gypsy moth and other invasive species held at Newtown Square, Pennsylvania, USDA, Forest Service, Northern Research Station. P. 34.

Egonyu JP, Kucel P, Kangire A, Sewaya F, Nkungwa C (2009). Impact of the black twig borer on Robusta coffee in Mukono and Kayunga districts, central Uganda. J. An. Plant Sci. 2(4):163-169.

Fokkema NJ (1973). The role of saprophytic fungi in antagonism against *Dreschlera sorokiniana* (*Helminthosporium sativum*) on agar plates and rye leaves with pollen. Physiol. Plant Pathol. 3:15-105.

Hara AH, Beardsley JW Jr (1976). The biology of the black twig borer, *Xylosandrus compactus* (Eichhoff), in Hawaii. Proc. Hawaiian Entomol. Soc. 13:55–70.

Ivić D, Sever Z, Kuzmanovska B (2011). In vitro sensitivity of *Fusarium graminearum*, *F. avenaceum* and *F. verticillioides* to carbendazim, tebuconazole, flutriafol, metconazole and prochloraz. Pesticidi i fitomedicina 26(1):35-42.

Kim GH, Hur JS, Choi W, Koh YJ (2005). Fusarium wilt of winter daphne (*Daphne odora* Thumb.) caused by *Fusarium oxysporum*. Plant Pathol. J. 21(2):102-105.

Kopacki M, Wagner A (2006). Effect of some fungicides on mycelium growth of *Fusarium avenaceum* (Fr.) Sacc. Pathogenic to chrysanthemum (*Dendranthema grandiflora* Tzvelev). Agron. Res. 4:237-240.

Labrinos JL, Nutter FW Jr (I993). Effects of a protectant versus a systemic fungicide on disease components of peanut late leaf spot. Plant Dis. 77:837-845.

MAAIF (2009). Ministry of Agriculture, Animal Industry and Fisheries (MAAIF), Uganda. The New Vision newspaper, Monday, March 23, 2009. http://www. newvisionuganda.info/D/9/579/675811. Last accessed on April 01, 2012.

Mamza WS, Zarafi AB, Alabi O (2008). *In vitro* evaluation of six fungicides on radial mycelial growth and regrowth of *Fusarium pallidoroseum* isolated from castor (*Ricinus communis*) in Samaru,

Nigeria. Afr. J. Gen. Agric. 4(2):65-71.
Meah MB, Hossain MD, Islam MR (2002). Development of an integrated approach for management of Phomopsis blight and fruit rot of eggplant in Bangladesh: Annual research report (2001-2002), Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh, Bangladesh. P. 25.
Murdoch AW, Wood RKS (1972). Control of *Fusarium solani* rot of potato tubers with fungicides. Ann. Appl. Biol. 72(1):53-62.
Musoli PC, Hakiza GJ, Birinkunzira JB, Kibirige-Sebunya I, Kucel P (2001). Coffee (*Coffea spp*). In: Mukiibi JK (ed), Agriculture in Uganda Vol. II. Fountain Publishers/CTA/NARO. pp. 376-436.
Nelson PE, Toussoun TA, Marsas WFU (1983). *Fusarium* species. An Illustrated Manual for Identification. The Pennsylvania State Univ. Press. P. 193.
Ngoan ND, Wilkinson RC, Short DE, Moses CS, Mangold JR (1976). Biology of an introduced ambrosia beetle, *Xylosandrus compactus*, in Florida. Ann. Entomol. Soc. Am. 69(5):872-876.
Nirenberg HI (1976). Untersuchungen über die morphologische und biologische Differenzierung in der Fusarium-Section Liseola. Berlin-Dahlem: Mitt. Biol. Bundesanst. Land-Forstwirtsch. 169:1–117.
Ramirez ML, Chulze S, Magan N (2004). Impact of environmental factors and fungicides on growth and deoxinivalenol production by *Fusarium graminearum* isolates from Argentinian wheat. Crop Prot. 23:117-125.
SAS Institute Inc. (2008). SAS/STAT Software: Reference, Version 9.2, Cary, NC: SAS Institute Inc.
Serani S, Taligoola HK, Hakiza GJ (2007). An investigation into *Fusarium* spp. associated with coffee and banana plants as potential pathogens of Robusta coffee. Afri. J. Ecol. 45:91-95.
Shtienberg D, Dreishpoun J (1991). Suppression of *Alternaria* leaf spot in Pima cotton by systemic fungicides. Crop Prot. 10:381-385.
Simpson DR, Weston GE, Turner JA, Jennings P, Nicholson P (2001). Differential control of head blight pathogens of wheat by fungicides and consequences for mycotoxin contamination of grain. Eur. J. Plant Pathol. 107:421-431.
Sultana N, Ghaffar A (2010). Effect of fungicides, microbial antagonists and oilcakes in the control of *Fusarium solani*, the cause of seed rot, seedling and root infection of bottle gourd, bitter gourd and cucumber. Pak. J. Bot. 42(4):2921-2934.
Tepper BL, Raju BC, Semer CR (1983). Fusarium stem rot of chrysanthemums (*Chrysanthemum* x *morifolium* ramat.) caused by *Fusarium solani* (mart.) Appel & wr: *in vitro* fungicide efficacy and disease control studies. Proceedings of the Florida State Hort. Soc. 96:300-303.
Tshilenge L, Kalonji A, Tshilenge P (2010). Determination of cultural and biometrical characters of *Fusarium* species isolated from plant material harvested from coffee (*Coffea canephora* Pierre.) infected with CWD in Democratic Republic of Congo. Afr. J. Agric. Res. 5(22):3145-3150.
UCDA (2010). Uganda Coffee Development Authority (UCDA) http://www.uganda. coffee.org. Last accessed on Jane 22, 2011.
Venkatasubbaiah P, Shekar Shetty H, Safeeulla KM (1984). Seed-borne nature of *Fusarium solani* in *Coffea arabica* and its role in seedling rot. Indian Phytopath. 37:158-160.

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Alternative methods of soybean inoculation to overcome adverse conditions at sowing

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Soybean growth in Brazil relies solely on biological fixation for nitrogen nutrition, However, the effective establishment of the symbiosis between plants and elite strains of *Bradyrhizobium* is jeopardized by current agricultural practices, such as seed treatment with pesticides that can be toxic to the bacteria. In addition, global climatic changes have altered temperature and rainfall patterns, which, in turn, may affect the early stages of the symbiosis and, consequently, nodulation, N2 fixation, and yield, especially when drought and high temperatures occur right after sowing. New technologies to improve nodulation and N_2 fixation must be developed. In this study, we evaluated the effects of spraying diluted inoculants towards the seeds at sowing, or on the soil-root interface after seedling emergence on attributes relative to soybean N_2 fixation and yield. Field experiments were set up at different locations, in a randomized block design according to standard Brazilian protocols. Inoculant application in the soil resulted in benefits for both nodulation and yield when plants faced adverse conditions at the initial stages of growth, and the inclusion of *Azospirillum* in co-inoculation with *Bradyrhizobium* also helped plants bypass initial adverse situations. The results also revealed that when adverse situations to nodulation occur, it may be possible to perform corrective inoculation by spraying diluted inoculant at sowing or after seedling emergence, even though some degree of yield loss may be expected. However, more information is necessary to establish inoculation frames.

Key words: Spray-inoculation, plant growth-promoting rhizobacteria (PGPR), *Azospirillum*, *Bradyrhizobium.*

INTRODUCTION

Many legumes can establish symbiotic relationships with specific soil bacteria collectively referred as rhizobia, which possess the dinitrogenase enzyme complex capable of capturing atmospheric nitrogen (N_2) and fixing it into ammonium, which is incorporated into carbon skeletons to form nitrogenous organic acids that can be readily assimilated by plants (Ormeño-Orrillo et al., 2013). Brazil stands as a model country in benefiting from biological N_2 fixation (BNF), especially from the inoculation of soybeans [*Glycine max* (L.) Merr.] with elite strains of the genus *Bradyrhizobium*, a symbiotic combination capable of fully supply the crop demand for nitrogen (Hungria et al., 2005a, 2006a,b; Hungria and Mendes, 2014). Another group of beneficial soil microorganisms comprises plant growth-promoting rhizobacteria (PGPR). These may produce plant growth

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hormones (auxins, giberellins, cytokinines, and ethylene), induce the plant's systemic resistance to diseases and or stresses, act as biocontrol agents, and solubilize phosphates, besides performing non-symbiotic biological N_2 fixation (Hartmann and Zimmer, 1994; Compant et al., 2005; Cassán et al., 2008; Bassan et al., 2012; Bhattacharyya and Jha, 2012; Fibach-Paldi et al., 2012). Bacteria belonging to the genus *Azospirillum* are the best studied and most employed worldwide as PGPR inoculants for agriculture (Okon and Labandera-Gonzalez, 1994; Bashan and Holguin, 1997), including Brazil (Hungria et al., 2010). Co-inoculation of rhizobia and *Azospirillum* results not only in soybean grain yield increase (Hungria et al., 2013), but also in pathogen control as satisfactory as the action of fungicides (Cassán et al., 2008).

The soybean crop is of utmost economic importance for Brazil. The fixation of over 300 kg of N per hectare, in every cropping season, and the delivery, to the soil, of about 20 to 30 kg of N per hectare, which remain available for the following crop, are certainly key elements for the success of soybean in the country (Hungria et al., 2005a, 2006a, b; Hungria and Mendes, 2014). However, more investigation is necessary to extend these benefits to the new soybean cultivars, as well as to make them compatible with modern soil and crop management techniques (Hungria and Mendes, 2014).

The global climatic changes also threaten the contribution of BNF to agriculture, as longer periods of drought and high temperatures have become more frequent, and Brazil is not an exception (Zullu Jr et al., 2008). Environmental stresses have marked negative effects on nodulation and BNF (Hungria and Franco, 1993; Hungria and Vargas 2000; Hungria and Kaschuk, 2014). Moreover, climatic changes increase plant susceptibility to diseases and pests, demanding more intensive seed treatment with chemicals that may be highly toxic to rhizobia, such as fungicides, insecticides, and nematicides; the use of pesticides is a common practice for over 90% of the soybeans grown in Brazil (Campo et al., 2009).

Any factor that reduces the population of inoculum rhizobia on the seeds and, consequently, nodulation, may decrease the contribution of BNF. Therefore, scientists must develop new technologies that minimize the negative impacts of seed treatment with chemicals on inoculated rhizobia. One technology is in-furrow delivery of *Bradyrhizobium* inoculants. This technology has long been proposed (Brockwell et al., 1988) as an effective possibility to release soybean rhizobia into the soil. This alternative is currently under successful utilization, although still by few farmers in Brazil, as long as larger doses of inoculant than those recommended for seed inoculation are employed (Vieira Neto et al., 2008; Campo et al., 2010).

Another palliative technology, especially in cases of failure or limitations to seed inoculation, is spray inoculation, which can promote, at least, partial recovery of nodulation and BNF (Zilli et al., 2008). However, this technology must be analyzed very carefully, especially if performed after seedling emergence. The successful establishment of the legume-rhizobia symbiosis starts with an intricate exchange of molecular signals between the partners (Hungria et al., 1996; Hungria and Stacey, 1997; Geurts and Bisseling, 2002; Desbrosses and Stougaard, 2011), triggered by seed germination, resulting in root hair deformations that are visible right after exposure of bradyrhizobia to soybean root exudates (Hungria et al., 1996). As new root segments are only transiently susceptible to rhizobial infection (Bhuvaneswari et al., 1981), delayed contact between the bacteria and the roots may result in poor nodulation and BNF. Therefore, it is essential to gain information about doses and timeframe for delayed inoculation, as well as about when such practice would be viable.

The increasing use of pesticides for soybean seed treatment at sowing jeopardizes the maintenance of adequate numbers of viable rhizobial cells on the seed surface (Campo et al., 2009). This situation is even more critical in the case of sowing pre-inoculated seeds, a practice that is becoming more common and popular in South America (Hungria and Mendes, 2014). The objectives of this study were to evaluate soybean spray inoculation as an alternative to traditional seed or in-furrow inoculation, and investigate the effects of co-inoculation with *Azospirillum* on soybean growth and yield.

MATERIALS AND METHODS

Site description and procedures before sowing

Four field experiments were conducted in the 2012/2013 cropping season. Geographic information about each experimental location is presented in Table 1.

At each location, 20 soil subsamples were collected at 0-20 cm soil layer about 40 days before experimental setup. Subsamples were combined and one composite sample from each location was analyzed for chemical, granulometry and microbiological characteristics. For chemical analysis (Pavan et al., 1992), samples were oven-dried (60°C, 48 h) and sieved (2 mm). Soil pH was determined in 0.01 M $CaCl_2$ (1:2.5; soil:solution) after 1 h shaking. Ca, Mg, and Al contents were determined in the extract obtained with 1 M KCl (1:10; soil:solution) after 10 min shaking. P and K contents were determined en Mehlich-1 extract (0.05 M HCl + 0.0125 M H_2SO_4; 1:10 soil:solution) after shaking for 10 min. Al was determined by titration with 0.015 N NaOH, with bromthymol blue as indicator. Ca and Mg concentrations were determined in an atomic absorption spectrophotometer, K in a flame photometer, and P by colorimetry, by the molybdenum blue/ascorbic acid method. C was determined by dichromate oxidation. Soil chemical characteristics are presented in Table 2.

Soil granulometry at each experimental site was determined according to Embrapa (1997), and soil rhizobial populations were estimated by the plant-infection most probable number technique (Vincent 1970), with soybean cultivar BMX Potência RR as the trapping host, using statistical tables based on Andrade and

Table 1. Geographic and climatic information about the locations where experiments were conducted.

Location	Coordinates[a]	Altitude (m)	Climatic Classification[b]
Rio Verde	17°47' S; 50°54' W	730	Aw
Cachoeira Dourada	18°29' S; 49°28' W	450	Aw
Luiz Eduardo Magalhães	12°05' S; 45°48' W	720	Aw
Ponta Grossa	25°05′ S; 50°09′ W	950	Cfa

[a] Latitude and longitude; [b] According to the Köppen-Geiger system of classification.

Table 2. Chemical characteristics of the soils (0-20 cm) at the locations where experiments were conducted. All analyses were performed before sowing.

Location	pH	Al	H + Al	K	Ca	Mg	P	C	B	S	Sum of bases	V	Zn	Cu	Mn	Fe
	$CaCl_2$	$cmol_c\ dm^{-3}$					$g\ dm^{-3}$		$mg\ dm^{-3}$		$cmol_c\ dm^{-3}$	%	$mg\ dm^{-3}$			
Rio Verde	5.14	0.00	3.64	0.80	1.65	1.78	9.56	22.55	0.29	5.87	4.23	53.75	2.95	2.21	115.85	32.42
Cachoeira Dourada	5.40	0.00	3.07	0.37	3.55	1.73	1.71	18.55	0.20	7.23	5.65	64.79	1.41	8.27	146.84	38.09
Luiz Eduardo Magalhães	5.57	0.00	1.03	0.03	2.94	0.77	10.36	5.72	0.05	1.68	3.74	78.41	0.24	0.25	4.57	49.99
Ponta Grossa	4.60	0.26	7.89	0.15	2.02	1.30	0.80	30.50	0.30	5.80	3.47	30.55	1.10	1.40	41.00	56.00

Table 3. Soil granulometry and soybean rhizobial population of the soils at the locations where experiments were conducted.

Location	Soil granulometry (%)			No. of rhizobia g^{-1} soil
	Clay	Silt	Sand	
Rio Verde	36.35	9.55	54.10	< 10
Cachoeira Dourada	57.75	18.20	24.05	< 10
Luiz Eduardo Magalhães	13.55	1.00	88.45	< 10
Ponta Grossa	58.45	15.70	25.85	1×10^2

Hamakawa (1994). Soil granulometry and rhizobial populations at each location are shown in Table 3.

Lime was applied to the soil at each location about 50 days before sowing. The amounts of lime to be applied were determined on the basis of soil base saturation as specified by Embrapa Soja (2011), so as to obtain 70%. Still before sowing, all sites received 300 kg ha^{-1} of N-P-K (0-28-20) fertilizer, applied in-furrow. No N fertilizer was applied, except where specified (in the control with N-fertilizer).

Treatments, inoculation and field management

The experimental protocol adopted for the experiments reported here followed the guidelines established by the Ministry of Agriculture, Livestock and Food Supply (MAPA) in all tests of agronomic efficiency of new products or technologies making use of biological nitrogen fixation with legumes (MAPA, 2011). All experiments had 12 treatments, and treatments 1, 2, and 3 are mandatory in Brazil, as controls required by guidelines mentioned above. The treatments were:

Treatment 1 (T1) = Non-inoculated control;
T2 = T1 + 200 kg N ha^{-1} (100 kg N ha^{-1} at sowing + 100 kg N ha^{-1} as side dressing around 35 days after seedling emergence);
T3 = Standard peat-based *Bradyrhizobium* inoculant applied to seeds at sowing to provide 1.2×10^6 cells $seed^{-1}$ (1 dose);
T4 = Liquid *Bradyrhizobium* inoculant applied to seeds at sowing (1 dose);
T5 = Liquid *Bradyrhizobium* inoculant applied in-furrow at sowing (3 doses);
T6 = Liquid *Bradyrhizobium* inoculant sprayed close to the sowing line at sowing (3 doses);
T7 = T6, but with 5 doses;
T8 = Three doses of liquid *Bradyrhiobium* inoculant + two doses of liquid *Azospirillum* inoculant, sprayed close to the sowing line at sowing;
T9 = T8, but with five doses of liquid *Bradyrhizobium* inoculant + two doses of liquid *Azospirillum* inoculant;
T10 = Liquid *Bradyrhizobium* inoculant sprayed towards the root/stem interface region between VC and V1 (3 doses);
T11 = T 10, but with 5 doses;
T12 = T10, but with 10 doses

All bacterial inoculants employed in this study were analyzed for purity and cell concentration. Inoculants with *Bradyrhizobium* were counted by spread-plating on yeast extract-manitol agar with Congo red (Vincent, 1970), while inoculants containing *Azospirillum* were counted by spread-plating on RC agar medium (Cassán et al., 2010). In all cases, bacterial colony morphology was compared to expected patterns to confirm the absence of contaminants. The results of cell concentrations and purity of the inoculants are presented in Table 4.

Standard peat-based inoculant was prepared at a concentration of 5×10^9 cells g^{-1}, and contained the commercial strains *B.*

Table 4. Composition, concentration[a], and purity[b] of the inoculants used in the experiments.

Inoculant	Bacterial species	Strains	Concentration[a]	Contaminants[b]
Standard peat-based	*B. japonicum/B. diazoefficiens*	5079 + 5080	4.14×10^9	Absent
Standard liquid	*B. japonicum/B. diazoefficiens*	5079 + 5080	7.33×10^9	Absent
Standard liquid	*A. brasilense*	Ab-V5 + Ab-V6	2.97×10^8	Absent

[a] Number of colony forming units (CFU) per g or mL of product. [b] Characterized as presence (present) or absence (absent) of detectable contaminants at the 10^5 dilution of the products spread on plates with appropriate media.

Table 5. Agronomic information about the experiments.

Location	Cultivar	Sowing	Harvest	Plot size (m^2)	Area for yield evaluation (m^2)
Rio Verde	BMX-Potência (RR)	28/11/2012	19/03/2013	24.3	6
Cachoeira Dourada	BRS-GO-8360 (Conv.)	22/11/2012	22/03/2013	24	5.6
Luiz Eduardo Magalhães	BMX-Potência (RR)	06/12/2013	no harvest	24	6
Ponta Grossa	BMX-Potência (RR)	05/12/2012	08/05/2013	24	6

japonicum SEMIA 5079 (=CPAC 15) and *B. diazoefficiens* SEMIA 5080 (=CPAC 7) (Table 4). The doses of both liquid and peat-based *Bradyrhizobium* inoculants were adjusted to provide 1.2×10^6 viable cells of bradyrhizobia per seed, according to Brazilian regulations (Embrapa, 2011), and the peat-based inoculant was applied to the seeds with 10% sucrose solution to improve adherence, as described before (Hungria et al., 2006b). For *Azospirillum* liquid inoculant, one dose was considered as 1.2×10^5 viable cells per seed (10-fold less than bradyrhizobia)

The non-inoculated control treatment with N fertilizer (T2) received 200 kg N ha^{-1} as urea, split in two broadcast applications of 100 kg N ha^{-1} at sowing, and 100 kg N ha^{-1} as side dressing around 35 days after seedling emergence.

For in-furrow inoculation at sowing, the liquid inoculant was diluted in water to make up a final volume of 150 L ha^{-1}, and the mixture was applied directly over the seeds in the sowing furrow. For spray inoculations, the appropriate amounts of inoculants were mixed with water to make up a final volume of 150 L ha^{-1}, and the mixtures were applied by spraying either towards the sowing line (at sowing), or towards the root/stem interface region between stages VC and V1 (Fehr and Caviness, 1977), both with a coastal sprayer.

Information about cultivars, sowing dates, and sampling dates at each location are shown on Table 5. At all locations, row spacing was 50 cm, with 18 plants m^{-1}, and a final population of approximately 300,000 plants ha^{-1}. All experiments were set in a completely randomized block design with six replicates. Plot sizes varied from 24 m^2 to 24.3 m^2 (Table 5). At all locations the plots were separated by 0.5 m-wide rows and 1.5 m-wide terraces to avoid cross contamination from surface flushes containing bacteria or fertilizers that may occur in consequence of heavy rainfall.

All plants received leaf sprays of Mo (20 g ha^{-1}) and Co (2 g ha^{-1}) at the V4 stage (Fehr and Caviness, 1977). Weeds were controlled with herbicides in all treatments. Glyphosate was employed when the transgenic cultivar was grown, whereas conventional herbicides were employed with the non-transgenic cultivar. Insect control was accomplished by means of biological and chemical insecticides (Embrapa, 2011).

Sampling, harvest and analyses performed

Thirty-five to 50 days after sowing, five plants were collected from each plot for evaluation of nodulation (nodule number and dry weight), plant biomass, and N content and accumulation in shoots. Roots and shoots were separated in the laboratory, carefully washed and oven-dried at 65°C for approximately 72 h. Nodules were then removed from roots and allowed to dry for another 72 h before counting and weighing. Dry shoots were also weighed and then employed for determination of N content and accumulation by the Kjeldahl technique.

At harvest, the central area from each plot (Table 5) was harvested to estimate grain yield. Seeds from the harvested plants were collected, cleaned, weighed, and seed moisture was determined and adjusted to 13%.

All data from each experiment were first tested for normality and for variance homogeneity. If necessary, data were transformed to the square root of (x+1) before analysis of variance (ANOVA) (SAS, 1999). In cases where statistical significance was detected, a *post hoc* test with $p < 0.1$ was performed. For multiple comparisons, Duncan test was employed.

RESULTS

In areas cropped for the first time with soybean, in Rio Verde (Table 6), Cachoeira Dourada (Table 7) and Luiz Eduardo Magalhães (Table 8) nodulation evaluated during vegetative growth was, in general, low, especially when liquid inoculants were employed. These areas suffered from drought immediately after sowing and between sowing and early flowering, which may have reflected negatively on the process of root infection and nodule development.

In Rio Verde the number and dry weight of nodules from plant samples collected at 40 DAS was far superior when peat-based inoculant was employed, compared to the liquid inoculant (Table 6). The application of a triple dose of inoculant in-furrow at sowing also promoted nodule dry weight, even though no significant differences relative to the non-inoculated control were observed

Table 6. Nodule number (NN) and dry weight (NDW), shoot dry weight (SDW), nitrogen content (NC) and total nitrogen accumulated in shoots (TNS) 40 days after sowing, and grain yield at final harvest of the soybean in Rio Verde.

Treatments [a]	NN plant^{-1} [b]		NDW (mg plant^{-1})		SDW (g plant^{-1})		NC (g kg^{-1})		TNS (mg N plant^{-1})		Yield (kg ha^{-1})	
T1	0.5	bc [c]	12.5	c	6.6	a	27.1	d	177	A	2762	ab
T2	0.2	c	2.4	c	6.7	a	31.0	bc	200	A	3035	a
T3	16.1	a	148.7	a	6.6	a	28.2	cd	186	A	3074	a
T4	1.7	bc	21.0	bc	6.6	a	29.1	cd	191	A	2842	ab
T5	2.2	b	37.6	b	6.3	a	28.1	cd	177	A	2705	b
T6	0.3	c	6.7	c	6.2	a	29.3	cd	182	A	2678	b
T7	1.4	bc	12.8	c	6.8	a	33.6	ab	231	A	2698	b
T8	0.8	bc	17.0	bc	6.2	a	34.4	a	215	A	2655	b
T9	0.4	bc	8.0	c	6.0	a	30.7	bc	188	A	2532	b
T10	0.2	c	2.8	c	6.4	a	28.8	cd	182	A	2825	ab
T11	0.9	bc	26.4	bc	6.3	a	33.1	ab	207	A	2507	b
T12	1.0	bc	15.8	bc	5.5	a	33.6	ab	189	A	2824	ab
p value	<0.001		<0.001		0.9841		<0.001		0.7869		0.0369	
Mean	2.13		25.97		6.36		30.60		193.7		2761	
CV (%)	77.8		85.0		24.3		9.1		26.2		10.6	

[a] T1 = Non-inoculated control; T2 = T1 + 200 kg N ha^{-1}; T3 = Standard peat-based *Bradyrhizobium* inoculant applied to seeds at sowing to provide 1.2 x 10^6 cells seed^{-1} (1 dose); T4 = Liquid *Bradyrhizobium* inoculant applied to seeds at sowing (1 dose); T5 = Liquid *Bradyrhizobium* inoculant applied in-furrow at sowing (3 doses); T6 = Liquid *Bradyrhizobium* inoculant sprayed close to the sowing line at sowing (3 doses); T7 = T6, but with 5 doses; T8 = Three doses of *Bradyrhizobium* inoculant + two doses of *Azospirillum* inoculant, sprayed close to the sowing line at sowing; T9 = T8, but with five doses of *Bradyrhizobium* inoculant + two doses of *Azospirillum* inoculant; T10 = Liquid *Bradyrhizobium* inoculant sprayed towards the root/stem interface region between VC and V1 (3 doses); T11 = T 10, but with 5 doses; T12 = T10, but with 10 doses. [b] Analyzed after transformation to square root of (x + 1). [c] Means (n=6) on the same column which are followed by different letters are significantly different ($p \leq 0,10$, Duncan test).

Table 7. Nodule number (NN) and dry weight (NDW), shoot dry weight (SDW), nitrogen content (NC) and total nitrogen accumulated in shoots (TNS) 50 days after sowing, and grain yield at final harvest of the soybean in Cachoeira Dourada.

Treatments [a]	NN plant^{-1} [b]		NDW (mg plant^{-1})		SDW (g plant^{-1})		NC (g kg^{-1})		TNS (mg N plant^{-1})		Yield (kg ha^{-1})	
T1	1.5	a [c]	8.4	a	8.6	a	23.0	a	195	a	3000	a
T2	0.9	a	3.2	a	8.9	a	26.6	a	237	a	3054	a
T3	2.7	a	17.7	a	6.7	a	24.1	a	172	a	2543	a
T4	1.1	a	13.6	a	8.1	a	22.4	a	182	a	2563	a
T5	1.5	a	7.6	a	7.7	a	23.3	a	179	a	2880	a
T6	1.1	a	5.8	a	7.1	a	22.5	a	155	a	2662	a
T7	1.0	a	9.9	a	7.3	a	25.0	a	185	a	2499	a
T8	1.4	a	10.1	a	7.0	a	22.7	a	159	a	2792	a
T9	1.1	a	7.8	a	6.3	a	26.2	a	164	a	2767	a
T10	0.8	a	6.6	a	7.3	a	23.4	a	171	a	2991	a
T11	1.3	a	7.9	a	7.1	a	26.4	a	184	a	2726	a
T12	1.7	a	18.3	a	8.6	a	21.6	a	182	a	2826	a
p value	0.2971		0.2523		0.9050		0.6134		0.9346		0.4100	
Mean	1.344		9.73		7.55		23.94		180.45		2775	
CV (%)	85.4		110.3		38.3		19.3		43.7		12.9	

[a] The same as Table 6; [b] Analyzed after transformation to square root of (x + 1); [c] Means (n=6) on the same column which are followed by different letters are significantly different ($p \leq 0,10$, Duncan test).

(Table 6).

A probable more intense effect of nodulation-limiting factors was observed in Cachoeira Dourada, where treatments did not differ from the non-inoculated control when sampled at 50 DAS (Table 7). When ten doses of *Bradyrhizobium*-containing inoculant were sprayed during

Table 8. Nodule number (NN) and dry weight (NDW), shoot dry weight (SDW), nitrogen content (NC) and total nitrogen accumulated in shoots (TNS) 38 days after sowing, and grain yield at final harvest of the soybean in Luiz Eduardo Magalhães.

Treatments [a]	NN plant^{-1} [b]		NDW (mg plant^{-1})		SDW (g plant^{-1})		NC (g kg^{-1})		TNS (mg N plant^{-1})		Yield (kg ha^{-1})
T1	3.6	defg [c]	14.7	de	4.6	a	12.4	c	58.1	a	nd [d]
T2	0.4	h	2.5	e	4.6	a	18.4	a	85.9	a	nd
T3	42.1	a	106.2	a	3.5	a	18.0	ab	62.8	a	nd
T4	15.7	b	57.2	b	4.9	a	15.1	c	73.9	a	nd
T5	6.1	cde	33.8	bcd	4.4	a	14.7	c	65.1	a	nd
T6	11.9	bc	40.0	bc	4.4	a	13.2	c	58.0	a	nd
T7	5.7	cdef	22.4	cde	4.3	a	15.4	bc	64.1	a	nd
T8	8.6	bcd	35.5	bcd	4.1	a	18.2	ab	71.3	a	nd
T9	12.1	bc	53.3	b	3.8	a	15.1	c	56.1	a	nd
T10	1.9	efg	11.6	de	4.0	a	13.8	c	56.0	a	nd
T11	1.0	h	5.0	e	4.6	a	18.1	ab	83.8	a	nd
T12	1.3	fg	8.9	e	4.0	a	13.9	c	54.5	a	nd
p value	<0.001		<0.001		0.9515		<0.001		0.3421		-
Mean	9.2		32.6		4.3		15.5		65.8		-
CV (%)	80.8		69.0		34.4		17.6		37.3		-

[a] The same as Table 6. [b] Analyzed after transformation to square root of (x + 1). [c] Means (n=6) on the same column which are followed by different letters are significantly different ($p \leq 0,10$, Duncan test). [d] Yield was not determined (nd) due to drought effects on plant development.

VC-V1, or when seeds received either liquid or peat-based inoculant, there was an improvement in nodule dry weight, but with no statistical difference from the other treatments.

Seed inoculation with peat-based inoculant promoted significantly more nodulation at 38 DAS in Luiz Eduardo Magalhães too (Table 8). In addition, significant gains in nodulation were also obtained by inoculating seeds with liquid inoculant, and by spraying *Bradyrhizobium* alone or in combination with *Azospirillum* in-furrow at sowing, in comparison with the non-inoculated controls (Table 8).

In Ponta Grossa, soybean had been cropped before the experiment, thus the soil had a naturalized population of *Bradyrhizobium* (Table 3). At 35 DAS, no differences in nodule number were observed relative to the non-inoculated control, except when 10 doses of *Bradyrhizobium* inoculant were sprayed in VC-V1, and no differences whatsoever were observed in nodule mass (Table 9). The use of N fertilizer, however, caused a significant decrease in both nodule number and dry weight (Table 9). Negative effects of N fertilizer on nodulation were also observed in the other sites (Tables 6, 8 and 9).

Plant biomass and total N accumulated in shoots were not significantly affected across treatments and locations (Tables 6 to 9), and the differences observed at some locations (Rio Verde, Luiz Eduardo Magalhães, and Ponta Grossa) in N content (%) reflect the dilution caused by variations in plant growth.

No significant differences in grain yield were observed at any of the locations. In Rio Verde, however, the standard practice of seed inoculation with peat-based inoculants resulted in a 292 kg ha^{-1} gain in grain yield (Table 6). N fertilizer had no effect on grain yield either. The same situation was observed in Cachoeira Dourada (Table 7). No grains were produced in Luiz Eduardo Magalhães, where soybean was mostly affected by water deficit. The Ponta Grossa location also suffered the effects of recurrent dry spells, resulting in low yields. However, although not showing statistical difference, in Ponta Grossa the combined inoculation of *Bradyrhizobium* and *Azospirillum* by spraying at sowing (T9) promoted more gain in grain yield than did soil rhizobia (T1) or N fertilizer (T2) (Table 9).

DISCUSSION

The ever-increasing world population and the awareness of potential impacts of human activities on global weather changes demand that agriculture becomes more efficient. Technologies must be developed that guarantee production and food supply, but cause the least, if none, alterations on the natural landscape, and make the area already claimed by agricultural activities more productive. The increasing use of chemicals to protect seeds and seedlings from pests and diseases (Campo et al., 2001, 2009), and the demand for seeds with anticipate inoculation challenge scientists to develop technologies that not only do not affect survival of inoculated bacteria (Ferreira et al., 2011), but also contribute to yield increase.

Fertilization has long been known to increase efficiency in agriculture, as a means to improve plant nutrition. N deficiency is a limiting factor in many places of the world, demanding heavy fertilization, but the supply of N to

Table 9. Nodule number (NN) and dry weight (NDW), shoot dry weight (SDW), nitrogen content (NC) and total nitrogen accumulated in shoots (TNS) 35 days after sowing, and grain yield at final harvest of the soybean in Ponta Grossa.

Treatments [a]	NN plant^{-1} [b]		NDW (mg plant^{-1})		SDW (g plant^{-1})		NC (g kg^{-1})		TNS (mg N plant^{-1})		Yield (kg ha^{-1})	
T1	26.3	a [c]	55.9	a	0.9	a	34.6	A	29.7	a	1796	a
T2	5.6	c	7.6	b	0.9	a	34.8	a	28.8	a	1881	a
T3	24.2	a	53.8	a	1.0	a	31.3	b	32.9	a	1685	a
T4	23.9	a	51.7	a	1.0	a	32.3	ab	30.9	a	1741	a
T5	23.3	a	50.9	a	0.8	a	34.6	a	26.9	a	1858	a
T6	22.2	a	55.5	a	0.9	a	31.7	b	29.7	a	1607	a
T7	27.6	a	39.3	a	0.8	a	32.5	ab	24.5	a	1759	a
T8	27.1	a	53.2	a	0.7	a	31.1	b	22.7	a	1844	a
T9	21.3	ab	53.2	a	0.7	a	32.7	ab	22.0	a	2088	a
T10	21.4	ab	44.9	a	2.6	a	34.6	a	92.9	a	1771	a
T11	23.0	a	53.6	a	0.8	a	32.3	ab	26.4	a	1759	a
T12	15.5	b	40.2	a	0.9	a	32.5	ab	28.0	a	1643	a
p value	<0.001		0.001		0.4273		0.0334		0.3954		0.6172	
Mean	21.8		44.2		1.00		32.9		33.0		1786	
CV (%)	26.3		41.2		127.2		7.0		137.1		19.1	

[a] The same as Table 6; [b] Analyzed after transformation to square root of (x + 1); [c] Means (n=6) on the same column which are followed by different letters are significantly different ($p \leq 0{,}10$, Duncan test).

plants can be increased by using BNF, especially when legumes are the main crop or take part in rotations with cereals (Ormeño-Orrillo et al., 2013). Successful soybean crops in Brazil rely solely on BNF as N source, but yield gains are still possible by the development of new cultivars and of technologies which increase either nodulation (number and mass) or its efficiency (Hungria et al., 2006a, b; Hungria and Mendes, 2014). The inoculation of a combination containing the traditional soybean BNF partner, *Bradyrhizobium*, and the plant growth-promoting *Azospirillum* may improve such benefits (Hungria et al., 2013), so that we tested this approach for soybean inoculation in comparison with traditional seed coating with peat-based or liquid inoculants.

Three of our experiments were carried in central Brazil (Rio Verde, Cachoeira Dourada and Luiz Eduardo Magalhães) which were severely affected by drought before and right after sowing. The hydric stress at sowing and at such an early growth stage affects root infection and nodule formation, and does not favor survival of inoculated rhizobia in the soil. In addition, all were first crop areas, and no naturalized population of rhizobia was present to guarantee a secondary nodulation after stress cessation. This explains why so few nodules were observed at the three locations. Indeed, hydric and thermic stresses are amongst the most limiting factors to BNF in the tropics, seriously affecting stages such as root infection, nodule formation and N fixation (Hungria and Franco, 1993; Hungria and Vargas, 2000; Hungria and Kaschuk, 2014).

Although without statistical difference, in Rio Verde the application of triple doses of the inoculant in-furrow at sowing, in comparison to seed inoculation, resulted in positive effects and, in addition to previous reports (Vieira Neto et al., 2008; Campo et al., 2010), reinforces its usefulness as an alternative inoculation procedure. Indeed, this alternative inoculation method has been recommended and used by Brazilian farmers, especially when pesticides are used in seed treatment in areas without naturalized bradyrhizobia population (Embrapa, 2011).

In our studies, we dedicated more attention to nodulation parameters because it has been shown that especially nodule dry weight is most well-correlated to symbiotic behavior and performance of soybean-*Brayrhizobium* associations in the field (Souza et al., 2008a, b). Nodulation results from the three locations, all first crop areas and under hydric stress, confirmed the superiority of peat-based inoculants under adverse conditions, evidencing the protective effect of peat (Hungria et al., 2005b). These results highlight that although the liquid inoculant market represents most of the commercialized products, e.g. about 80% of the 27 million doses for the soybean crop in Brazil in the last crop season, there is still much to be improved in liquid formulations to achieve the high and secure standards of using peat inoculants. Noteworthy is to reanalyze pioneer studies and to observe that the same observations and concerns date from decades ago (e.g. Burton and Curley, 1965; Burton, 1975). For example, Burton and Curley (1965) report that higher soybean yields and superior nodulation were obtained when peat-base inoculum was used, in comparison to liquid inoculum containing 2.5 times as many rhizobia. The authors speculate that the superiority of peat-inoculant could be because of

sheltering rhizobia from toxic substances, or to some protective action of peat after the seeds are in the soil (Burton and Curley, 1965). Almost half a century later, we are still not aware of the peat properties which lead to its superior performance, such that the technology can be transferred to liquid inoculants.

When the same treatments were evaluated in Ponta Grossa in a soil with naturalized population of *Bradyrhizobium* established by previous inoculations and soybean cropping, no differences were observed in nodulation. Even though the crop was subjected to some degree of water stress, soil bradyrhizobia were able to nodulate plants satisfactorily. A decrease in nodulation was observed only when inoculation, even with a high dose of *Bradyrhizobium*, was performed after seedlings had emerged. In this case nodulation was probably hampered by the transient susceptibility of roots to infection (Bhuvaneswari et al., 1981). It is well known that the first steps of the symbiosis rely on the existence of molecules present in seed exudates, responsible for turning on important genes in both partners (Hungria et al., 1996; Hungria and Stacey, 1997; Geurts and Bisseling, 2002; Desbrosses and Stougaard, 2011). Therefore, it is crucial that rhizobia be in contact with the plant roots at the right place, at the appropriate time.

Experiments performed in first crop locations such as in Rio Verde, Cachoeira Dourada and Luiz Eduardo Magalhães allow us to identify and confirm positive effects of new products and technologies, which may not be observed when there is a naturalized population of rhizobia in the soil. Indeed, the importance of evaluating strains, inoculants and inoculation in soils void of compatible rhizobia has also been long recognized (Burton and Curley, 1965) and our study reinforces this importance. However, in such areas very frequently no differences are detected in parameters such as plant biomass and N accumulation in the shoots. This might be attributed to the intense mineralization of organic N in these areas, which are generally covered with grass pastures or forests prior to experiment set up. Such process may be able to supply almost all N plants demand during a first year crop.

Due to the water stresses that affected negatively all experiments, no treatment improved grain yield significantly. In Rio Verde, however, considerable increase (292 kg ha^{-1} = ca. 5 bags) was observed when peat-based inoculant was applied to seeds, relative to non-inoculation. It is worth mentioning that no effect of N fertilizer on yield was observed, and plants responded better to inoculation, with an emphasis on peat inoculants, than to mineral N fertilization. These findings support the statement that under the experimental conditions at Rio Verde, the BNF accomplished by the bradyrhizobia is able to supply all N demanded by the soybean crop.

No significant differences among treatments were observed in Cachoeira Dourada either, and no positive effects of N fertilizer occurred. In Ponta Grossa periods of drought occurred recurrently during the plant growth cycle, also affecting grain yield. It is well known that the occurrence of adverse conditions to both plant and bacteria at sowing or during early stages of the plant developmental cycle will reflect on yield. In this case, the negative effect of adverse conditions affected nodulation, which, in turn, was defective and could not supply proper amounts of N to reach higher yields.

The availability of technologies that alleviate adverse conditions for plants and bacteria at early stages of the growth cycle would be welcome. For example, placement of rhizobia in the soil nearby the seed could avoid contact between the sensible bacteria and the pesticides, micronutrients and other chemical used in seed treatment, especially if drougth occurs. Inoculants could also be sprayed at sowing, or later on after seedling emergence. However, the delay between sowing and spray inoculation may affect nodulation, especially if inoculated bacteria reach infection sites on the roots after they are no longer susceptible to infection (Bhuvaneswari et al., 1981). Zilli et al. (2008) have demonstrated that spray inoculation up to 18 days after sowing can partially promote recovery of soybean nodulation and grain yield, but longer delays may seriously compromise the crop.

Alternatively, the inclusion of one or more plant growth-promoting species in the inoculant or in the soil might help plants bypass the initial negative effects, form more nodules, and increase yield. In our experiments, the benefits of including *A. brasilense* strains produced good results. In Ponta Grossa, when five doses of *Bradyrhizobium* inoculant were combined with two doses of *Azospirillum* and applied by spraying at sowing, yield was superior, although not significantly, than that of the non-inoculated (nodulated by soil rhizobia), and of the non-inoculated + N (200 kg ha^{-1}) controls. These findings agree with recent reports (Hungria et al., 2013) of increased yield resulting from combined inoculation of soybeans with *Bradyrhizobium* (seeds) and *Azospirillum* (in-furrow) in soils containing naturalized rhizobia.

Even though our results have confirmed previous reports that peat-based inoculants are by far superior to liquid formulations (e.g. Campo et al., 2001), especially in first year crops or when situations adverse to nodulation establishment occur, it has been shown that in some situations it may be possible to remedy inoculation by spraying diluted inoculant after sowing or after seedling emergence, even though some degree of yield compromise may be expected. However, more information is necessary in order to establish inoculation frames so as to take the most benefits from the new technology.

Conflict of Interest

The authors have not declared any conflict of interest.

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REFERENCES

Andrade DS, Hamakawa PJ (1994). Estimativa do número de células de rizóbio no solo e inoculantes por infecção em planta. In: Hungria M, Araujo RS (ed) Manual de métodos empregados em estudos de microbiologia agrícola. Embrapa-SPI, Brasília pp. 63-94.

Bassan LE, Hernandez J-P, Bashan Y (2012). The potential contribution of plant growth-promoting bacteria to reduce environmental degradation – A comprehensive evaluation. Appl. Soil Ecol. 61: 171–189.

Bashan Y, Holguin G (1997). *Azospirillum*-plant relationship: environmental and physiological advances (1990-1996). Can. J. Microbiol. 43:103-121.

Bhattacharyya PN, Jha DK (2012). Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. World J. Microbiol. Biotechnol. 28:1327–1350.

Bhuvaneswari TV, Bhagwat AA, Bauer WD (1981). Transient susceptibility of root cells in four common legumes to nodulation by rhizobia. Plant Physiol. 68:1144-1149.

Brockwell J, Gault RR, Herridge DF, Morthorpe LJ, Roughley RJ (1988). Studies on alternative means of legume inoculation: microbiological and agronomic appraisals of commercial procedures for inoculating soybeans with *Bradyrhizobium japonicum*. Aust. J. Agric. Res. 39:965-972.

Burton JC (1975). Methods of inoculating seeds and their effect on survival of rhizobia. In: Nutman PS (ed) Nitrogen fixation in plants. Cambridge University Press, Cambridge pp. 175-189.

Burton JC, Curley RL (1965). Comparative efficiency of liquid and peat-base inoculants on field-grown soybean (*Glycine max*). Agron. J. 57: 379-381.

Campo RJ, Araujo RS, Hungria M (2009). Nitrogen fixation with the soybean crop in Brazil: compatibility between seed treatment with fungicides and bradyrhizobial inoculants. Symbiosis 48:154-163.

Campo RJ, Araujo RS, Mostasso FL, Hungria M (2010.) In-furrow inoculation of soybeans as alternative for fungicides and micronutrients seed treatment and inoculation. Rev. Bras. Ci. Solo 34:1103-1112.

Campo RJ, Hungria M, Albino UB, Moraes JZ, Sibaldelli RNR (2001). Compatibilidade de aplicação conjunta nas sementes, de fungicidas, micronutrientes e inoculantes, sobre a sobrevivência do *Bradyrhizobium* e a eficiência da fixação biológica do nitrogênio. In: Resultados de Pesquisa da Embrapa Soja 2000 – Microbiologia do Solo. Embrapa Soja, Londrina, pp. 29-39. (Embrapa Soja. Documentos, 163).

Cassán F, Penna C, Creus C, Radovancich D, Monteleleone E, Salamone IG, Salvo LD, Mentel I, Garcia J, Pasarello MCM, Ltt L, Puente M, Correa O, Puunschke VK, Massa R, Roosi A, Diaz M, Catafesta M, Righes S, Carletti S, Cáceres ER (2010). Protocolo para el control de calidad de inoculantes que contienen *Azospirillum* sp. Associación Argentina de Microbiología, Buenos Aires. CD-ROM. (Documento de Procedimientos de la REDCAI, 2).

Cassán F, Sgroy V, Perrig D, Masciarelli O, Luna V (2008). Producción de fitohormonas por *Azospirillum* sp. Aspectos fisiológicos y tecnológicos de la promoción del crecimiento vegetal. In: Cassán FD, Garcia de Salamone I (ed) *Azospirillum* sp.: cell physiology, plant interactions and agronomic research in Argentina. Asociación Argentina de Microbiologia, Buenos Aires pp. 59-84.

Compant S, Duffy B, Nowak J, Clément C, Barka EA (2005). Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. Appl. Environ. Microbiol. 71:4951–4959.

Desbrosses GJ, Stougaard J (2011). Root nodulation: a paradigm for how plant-microbe symbiosis influences host development pathways. Cell Host Microbe 10:348-358.

Embrapa (Centro Nacional de Pesquisa de Solos) (1977). Manual de métodos de análise de solo, 2nd edn. Centro Nacional de Pesquisa de Solos, Rio de Janeiro.

Embrapa (Empresa Brasileira de Pesquisa Agropecuária). (2011). Tecnologias de produção de soja – Região Central do Brazil 2012 e 2013. Embrapa Soja, Londrina (Sistemas de Produção, 15).

Fehr WR, Caviness CE (1977). Stages of soybean development. Iowa State University, Ames (Special Report, 80).

Ferreira E, Nogueira MA, Fukami J, Conceição RB, Hungria M (2011). Nova legislação, recomendação de doses de inoculantes e pré-inoculação: riscos ao sucesso da contribuição da fixação biológica do nitrogênio para a cultura da soja. In Reunião de Pesquisa da Soja na Região Central do Brasil, 23, São Pedro. Anais... Embrapa Soja, Londrina.

Fibach-Paldi S, Burdman S, Okon Y (2012). Key physiological properties contributing to rhizosphere adaptation and plant growth promotion abilities of *Azospirillum brasilense*. FEMS Microbiol. Lett. 326:99–108.

Geurts R, Bisseling T (2002). *Rhizobium* Nod factor perception and signaling. Plant Cell S239-S249.

Hartmann A, Zimmer W (1994). Physiology of *Azospirillum*.In: OkonY (ed) *Azospirillum/*Plant associations. CRC Press, Boca Raton pp. 15-39.

Hungria M (2011). Inoculação com *Azospirillum brasilense*: inovação em rendimento a baixo custo. Londrina, Embrapa Soja. (Embrapa Soja. Circular Técnica, 325).

Hungria M, Franco AA (1993). Effects of high temperatures on nodulation and N_2 fixation by *Phaseolus vulgaris* L. Plant Soil 149:95-102.

Hungria M, Kaschuk G (2014). Regulation of N_2 fixation and NO_3^-/NH_4^+ assimilation in nodulated and N-fertilized *Phaseolus vulgaris* L. exposed to high-temperature stress. Environ. Exp. Bot. 98:32-39.

Hungria M, Mendes IC (2014). Nitrogen fixation with soybean: the perfect symbiosis? In: De Bruijn F (ed) Biological nitrogen fixation. Wiley-Blackwell. (in press).

Hungria M, Stacey G (1997). Molecular signals exchanged between host plants and rhizobia: Basic aspects and potential application in agriculture. Soil Biol. Biochem. 29:819-830.

Hungria M, Vargas MAT (2000). Environmental factors impacting N_2 fixation in legumes grown in the tropics, with an emphasis on Brazil. Field Crops Res. 65:151-164.

Hungria M, Campo RJ, Mendes IC, Graham PH (2006a). Contribution of biological nitrogen fixation to the N nutrition of grain crops in the tropics: the success of soybean (*Glycine max* L. Merr.) in South America. In: Singh RP, Shankar N, Jaiwa PK (ed) Nitrogen nutrition and sustainable plant productivity. Studium Press, LLC Houston, pp. 43-93.

Hungria M, Campo RJ, Souza EM, Pedrosa FO (2010). Inoculation with selected strains of *Azospirillum brasilense* and *A. lipoferum* improves yields of maize and wheat in Brazil. Plant Soil 331:413-425.

Hungria M, Franchini JC, Campo RJ, Crispino CC, Moraes JZ, Sibaldelli RNR, Mendes IC, Arihara J (2006b). Nitrogen nutrition of soybean in Brazil: contributions of biological N_2 fixation and of N fertilizer to grain yield. Can. J. Plant Sci. 86:927-939.

Hungria M, Franchini JC, Campo RJ, Graham PH (2005a). The importance of nitrogen fixation to soybean cropping in South America. In: Newton WE (ed) Nitrogen fixation: origins, applications and research progress, v.4, Werner W, Newton WE (ed) Nitrogen fixation in agriculture, forestry, ecology and the environment. Springer, Dordrecht, Amsterdam pp. 25-42.

Hungria M, Loureiro MF, Mendes IC, Campo RJ, Graham PH (2005b). Inoculant preparation, production and application. In: Newton WE (ed) Nitrogen fixation: origins, applications and research progress,

v.4, Werner W, Newton WE (ed) Nitrogen fixation in agriculture, forestry, ecology and the environment. Springer, Dordrecht, Amsterdam pp. 223-254.
Hungria M, Nishi CYM, Cohn J, Stacey G (1996). Comparison between parental and variant soybean *Bradyrhizobium* strains with regard to the production of lipo-chitin nodulation signals, early stages of root infection, nodule occupancy, and N_2 fixation rates. Plant Soil 186:331-341.
Hungria M, Nogueira MA, Araujo RS (2013). Co-inoculation of soybeans and common beans with rhizobia and azospirilla: strategies to improve sustainability. Biol. Fertil. Soils 49:791-801.
Mapa (Ministério da Agricultura, Pecuária e Abastecimento). Instrução Normativa Nº 30, de 12/11/2010. http://extranet.agricultura.gov.br/sislegis. Acessed 13 June 2011.
Mapa (Ministério da Agricultura, Pecuária e Abastecimento). Instrução Normativa Nº 13, de 24/03/2011. http://extranet.agricultura.gov.br/sislegis. Acessed 13 June 2011.
Okon Y, Labandera-Gonzalez C (1994). Agronomic applications of *Azospirillum*: an evaluation of 20 years worldwide field inoculation. Soil Biol. Biochem. 26:1591-1601.
Ormeño-Orrillo E, Hungria M, Martínez-Romero E (2013). Dinitrogen-fixing prokaryotes. In: Rosemberg E, De Long EF, Lory S, Stackebrandt E, Thompson F (ed) The Prokaryotes - prokaryotic physiology and biochemistry. Springer-Verlag, Berlin Heidelberg pp. 427-451.
Pavan MA, Bloch MF, Zempulski HD, Miyazawa M, Zocoler DC (1992). Manual de análise química do solo e controle de qualidade. Instituto Agronômico do Paraná, Londrina (Circular, 76).
SAS Institute (1999). Proprietary of software: version 6. 4th edn. SAS Institute, Cary.
Souza RA, Hungria M, Franchini JC, Chueire LMO, Barcellos FG, Campo RJ (2008a) Avaliação qualitativa e quantitativa da microbiota do solo e da fixação biológica do nitrogênio pela soja. Pesq. Agropec. Bras. 43:71-82.
Souza RA, Hungria M, Franchini JC, Maciel CD, Campo RJ, Zaia DAM (2008b). Conjunto mínimo de parâmetros para avaliação da microbiota do solo e da fixação biológica do nitrogênio pela soja. Pesq. Agropec. Bras. 43:83-91.
Vieira Neto AS, Pires FR, Menezes CCE, Menezes JFS, Silva AG, Silva GP, Assis RL (2008). Formas de aplicação de inoculante e seus efeitos sobre a nodulação da soja. Ver. Bras. Ci. Solo 32:861-870.
Vincent JM (1970). Manual for the practical study of root nodule bacteria. Blackwell, Oxford (International Biological Programme Handbook P. 15.
Zilli JE, Marson LC, Marson BF, Gianluppi V, Campo RJ, Hungria M (2008). Inoculação da soja com *Bradyrhizobium* através de pulverização em cobertura. Pesq. Agropec. Bras. 43:541-544.
Zullu Jr J, Pinto HS, Assa ED, Evangelist SRM (2008). Potential economic impacts ofglobal warming on two Brazilian commodities according to IPCC prognostics. Terrae 3:28-39.

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Full Length Research Paper

Tourism pattern of Alwar district of Rajasthan: A case study

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Every state always motivate tourism to revenue and also exchange of culture. A huge biodiversity across the world exploring by the peoples in last few decades. And this trends is more in developing countries like India. India is having vast cultural, social, religious immense diversity. It is intermixing of various cultures like British, Mugals and local kings for a long period of time. Nowadays these situations transform in to tourism hub and it is a key development strategy. The survey was carried out in the area of Sariska Tiger Project area and also secondary data collected from Alwar tourist department of Rajasthan, India. From the results showing that the total number of tourist decline 2005 to 2009 were 70% in Alwar district whereas in Rajasthan it was increased to 33%. If we analyzed the domestic and foreign tourist there will be a huge declined in domestic tourist at Alwar region compared to Rajasthan.

Key words: Agriculture, biodiversity, India, tourism.

INTRODUCTION

Alwar district of Rajasthan famous for lakes, old forts and mix culture of adjoining state Haryana and national capital of India. Apart from this Sariska tiger reserve also located, and it is main attraction for tourist in Alwar, having vast biodiversity. India is agricultural state beginning of the days, its nature and diversity in religion, ethnicity, and a huge mass of peoples living in villages adding the essence of flavor in biodiversity. The biodiversity means variety of life found on earth and all of the natural processes. This includes ecosystem, genetic and cultural diversity, and the connections between these and all species. Biodiversity conservation in India, and across the globe, is complex and often contentious (Torri, 2010; Torri and Herrmann, 2010). It decreased with the speedy rate due to fast growing population and industrial growth. Dense forest areas shrinking with faster rates. More urbanization and agricultural practices dominating in natural habitat of animals (Yadav and Gupta, 2006). It created the disturbance for the animals which affected the animal's routine and specially breeding activities in that habitat. In many national parks, reserved forest was facing the declining rate of breeding and population of animals. One way local governmental agencies popularized the tourism, to get state revenue with harmonization in nature. A number of law and official policies governing the wildlife and conservation of ecosystem, but it hardly taken consideration of local population and their environment.

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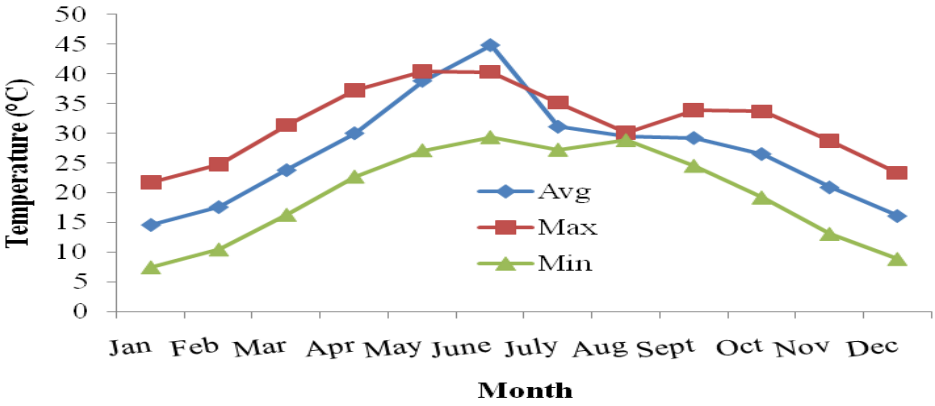

Figure 1. Temperature variation during the study period.

Table 1. Total number of tourist came to Rajasthan.

Year	Indian	Foreigner	Total
2005	18787298	1131164	19918462
2006	23483287	1220164	24703451
2007	25920529	1401042	27321571
2008	28358918	1477646	29836564
2009	25558691	1073414	26632107

The insite living poor people's degradation of forest and land erosion due to poor alternatives source of income, which forced them to over exploitation of natural resources. The increasing population in rural villages associated significant cost with the adjoin national park or natural habitat. It may be loss of animals, forest, reduced social and political. Saberwal (2001) described in the book 'Peoples, Parks and Wildlife: Towards coexistence' that decline in biodiversity and change in land use pattern are due to diversion of ground and surface water, resulting in the drying up streams and other water bodies from saltation, and pollution from pesticides and other chemicals. In another case, biodiversity decline trends published by Vasan in 2005 in the 'Journal of Economic and political weekly' and told that some areas biodiversity decline due to invasion of exotic species of plants that is, *Prosopis juliflora* in dry parts of North India replaced the species *Acacia nitotica*. So in this backdrop, we conducted a primary survey to find out the people's views regarding biodiversity of the Sariska and tourist effect on their economic growth.

MATERIALS AND METHODS

The field work took place January to April, 2011 in the Alwar district of Rajasthan. Data collected from individual and group interview as well as from participative observation and informal interviews, government agencies like district tourism office etc. The effect of geographical location on tourism and agricultural production system are also affecting the state revenue generation (Lata, 2013b). Alwar is having all amenities and well connected to country capital Delhi. It is having many historical and tourist places that is, Neelkant, Bhangarh, Jain temple and Kakadi fort. Apart from it is famous only for Sariska (Chandel, 2011). Sariska Tiger Reserve was created in 1978, situated at Alwar district of Rajasthan. The present area of the reserve is 866 sq. km. Having three core area and buffer zone. Forest mainly tropical dry deciduous and tropical thorn. It 180 various birds sp., 23 types of mammals, 33 types of grasses and more than 200 tree species including shrubs and herns, in other side according to the 1991 census 24 village with 10, 344 population identified inside the core area and 246 village with 243667 population were identified in the buffer zone (Sultana, 2013). Alwar in a hilly mountain of natural beauty called Singh Door of Rajasthan. Geographically situated in North east, having 27°4' - 28°4' N to 76°7 to 77°13 E. It is located around 160 km south of Delhi, and about 150 km north of Jaipur, the capital of Rajasthan. Alwar is part of National Capital Region (NCR). During the study period was fluctuation in temperature was observed (Figure 1). Data analyzed with the help of central tendency parameters.

RESULTS AND DISCUSSION

The number of tourist in Rajasthan during the course of study was increasing 24703451 in the year 2006, which is 24% higher than the year 2005, and this trends were increased 10 and 9% in the years 2007 and 2008 respectively (Table 1). But if we see the tourist growth year 2008 to 2009, was decreased 10%, which showing

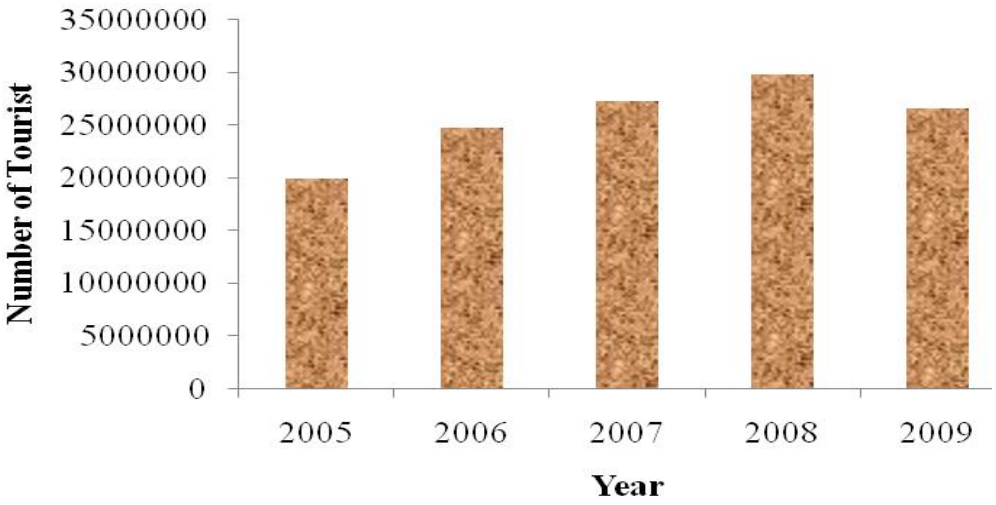

Figure 2. Number of tourist visited Rajasthan.

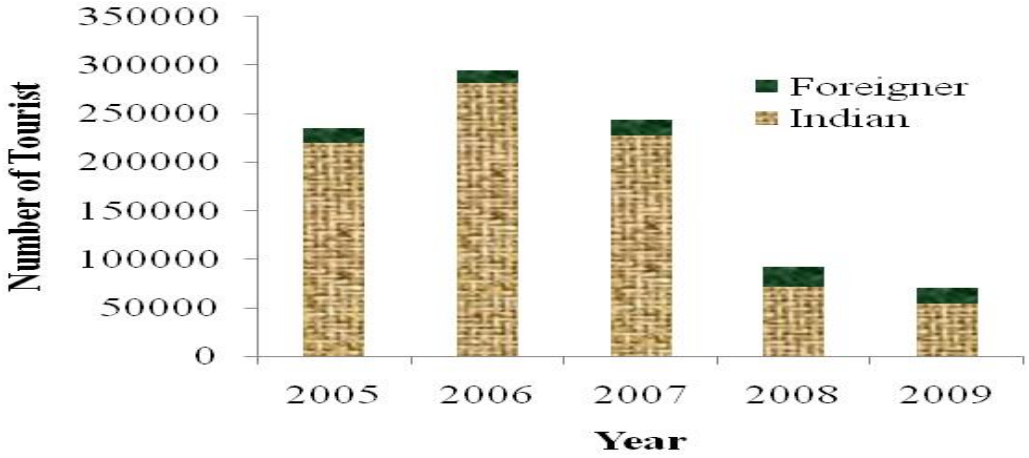

Figure 3. Number of tourist in Alwar, Rajasthan.

the drastic deduction in the tourist number. Indian tourists were increased from 2005 to 2006, 2006 to 2007, 2007 to 2008 were 4695987, 2437242 and 2438389, whereas decreased 2008 to 2009 was 2800227 (Figure 2). The increasing growth of tourist was due to favorable condition for the tourist and enormous interest by the global mass. At Alwar tourist population was also positively increased since 2005 to 2007 , which was 234846 to 244221 but after it decreasing with 60 and 75% from the base year of 2005 (Figure 3).

In the primary survey the view of the core area peoples saying that the biodiversity of the region was decline due to more interfere by the villagers. In 2000, Kumar et al., wrote a book ' Setting Biodiversity Conservation priorities for India' and described that threats to species are principally due to a decline in the area of their habitats, fragmentation of habitats and declines in habitats quality, and in the case of some mammals, hunting. Fragmentation raises the extinction risks because isolated subpopulation can go extinct one by one without being repopulated (Lata, 2013a; Kevin, 2000). Stochastic declines in small subpopulation make it more likely that they will go extinct, and this is further exacerbated by the reduction of genetic variability in sub population resulting from isolation species with already restricted range are particularly vulnerable to these threats (Clay, 1988). Conversion of lands to agriculture in the Canada prairies has resulted in the loss of 87% of native short grass prairie habitat, 81% of native mixed-grass prairie habitat, almost all the tall grass prairie habitat and 84% of the native aspen parkland habitat (CBIN, 1998; Behera and Nayak, 2013). Unfortunately, increased demands for food production are further accelerating the rate of conversion of lands with

moderate agricultural value to farmland.

Conclusion

Biodiversity is the degree of variation of life. This can refer to genetic variation, species variation, or ecosystem variation within an area, biome, or planet. It is proving the ample source of revenue for the region so that a region can economically developed. From the study the interference of human activities, affected the biodiversity of Sariska Tiger Reserve, which is directly affected by the number of tourist. With the help of awareness among the peoples which is living in the core area, can save the biodiversity of Sariska and tourist scope in Alwar.

Conflict of Interest

The authors have not declared any conflict of interest.

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REFERENCES

Behera RN, Nayak DK (2013). Population growth, agricultural land use change and implication for food security in Meghalaya Plateau, India. http://www.iussp.org /en/event/ 17/ programme/paper/5600.

CBIN (1998). Impacts on biodiversity. http: //redpath-museum. mcgill.ca /Qbp/3. Conservation /impacts.htm# 2.1.

Chandel A (2011). Sariska Tiger Reserve: Approach to sustainable development. conference on inclusive & sustainable growth. doi www.new health.in/ ojs/index. php/ISG /article/ download/4/4.

Clay JW (1988). Indigenous peoples and tropical forests, cultural survival, Cambridge, Massachusetts.

Kevin GJ (2000). Global patterns in biodiversity. Nature 405(6783):220–227. doi:10. 1038/35012228.

Kumar A, Walker S, Molur S (2000). Prioritasation of endangered species setting. In: Singh S, Sastry ARK, Mehta R, Uppal V (Ed) Setting biodiversity conservation priorities for India. WWF-India, New Delhi pp. 341-425.

Lata M (2013a). Conserve biodiversity and save the earth. Readersshelf. 9(7):10-11.

Lata M (2013b). Role of geography in agriculture. Readersshelf 9(7):18-19.

Saberwal V, Rangarajan M, Kothari A (2001). Peoples, parks and wildlife: Towards coexistence. Orient Longman, New Delhi.

Sultana A (2013). An updated checklist of birds of Sariska Tiger Reserve, Rajasthan, India. J. Threatened Taxa. 5(13):4791–4804.

Torri MC (2010). Conservation approaches and development of local communities in India: debates, challenges and future perspectives. Intl. J. Environ. Sci. 1(5):871-833.

Torri MC, Herrmann TM (2010). Biodiversity conservation versus rural development: what kind of possible harmonization? the case study of alwar district, rajasthan, India. J. Hum. Ecol. 31(2):93-101.

Vasan S (2005). In the name of law, legality, illegality and practice in Jharkhand forest. Eco. Political Weekly 40(41):4447-4450.

Yadav AS, Gupta SK (2006). Effect of micro environment and human distribution on the diversity of wood species in the Sariska Tiger Project in India. Rajasthan University pp. 25-126.

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Full Length Research Paper

The contribution of farmers' organizations to smallholder farmers' well-being: A case study of Kasulu district, Tanzania

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Farmers' Organizations' (FOs) play a significant role as an institutional vehicle for promoting agricultural development through helping farmers solve common problems in relation to agricultural inputs, credit, technical knowledge and marketing of produce. All these services aim at improving farming activities and enabling them to gain economic benefits to sustain their well-being. Based on the above, this paper assessed the contribution of FOs to smallholder farmers' well-being in Kasulu district. Specifically, the study assessed farmers' perception towards FOs, identified goods and services accrued by farmers from the organizations, and the contribution of goods and services from the same to farmers' well-being. A cross-sectional research design was employed whereby data was collected from 160 randomly selected farm households. Primary data was collected using a pre-structured questionnaire with both open and close-ended questions. Both quantitative and qualitative information was collected. Observations from the study showed that FOs contributed positively to their members' well-being. Generally, FO's members had a relatively higher income compared to the non-members, based on t-test analysis; the difference was shown to be statistically significant. Generally, the results indicated that extension services and the use of inorganic fertilizers and pesticides were positively associated with a household's income and assets ownership. Therefore, it is recommended that, rural farm households be encouraged to form or join farmers' organizations as these have a great potential of solving their problems.

Key words: Farmers' Organization, smallholder farmers, well-being.

INTRODUCTION

Farmers' Organizations (FOs) emerged in the world due to farmer-felt needs such as sharing of local resources (land, labour, water) and market pressures (prices and access to markets). Other needs are access to services (credit, input supply, and advisory services) or for purely social reasons (social security, food security) (Wennink et al., 2007). Before the era of liberalization, cooperatives were the main farmers' organizations in Sub-Saharan

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Africa (SSA). However, most of these cooperatives were created and managed under government directives (Chilongo, 2005). Due to state control, these cooperatives lacked accountability, became dependent on state subsidies and hence were uneconomically viable. Nonetheless, due to the withdrawal of the state from being a provider of many services through privatization, democratization and liberalization, most cooperatives failed to compete in the open-market economies, and eventually collapsed (World Bank, 1995 cited by Abaru et al., 2006). The decline of cooperatives and other FOs, lead to farmers' lack of a collective voice. Consequently, farmers cannot access inputs and technologies at affordable prices. Subsequently, a number of small-scale farmers remain poor and cannot influence policies that affect their well-being: hence, the need for formulation of farmers' organizations.

Generally, cooperation among farmers in search for common solutions to their problems is seen as one of the major ways in promoting the well-being of small-scale farmers, even if cooperatives encounter shortcomings (Grigoryan et al., 2008). Accordingly, during the 1990s developing countries, Tanzania included, encouraged formulation of farmers' organizations at different levels in order to enable their incorporation into research, extension system and other services (Carney, 1996). The formation of FOs is an important tool of assuring smallholder farmers improve their standard of living. FOs provide a wide range of services such as sourcing of agricultural inputs, access to knowledge and information, reducing transaction costs associated with marketing, allow collective lobbying for desired changes and as such they have the potential to positively influence agricultural policy outcome (Hellin et al., 2007, cited by Mapila et al., 2010). Furthermore, FOs might be a good vehicle for donors to reach small-scale farmers, as a group living in sparsely populated rural areas with weak infrastructure; this could in turn facilitate assistance in terms of grants or loans that can enable these farmers improve their well-being (Bachke, 2009).

Despite the fact that FOs play a crucial role in the development of rural agriculture and farmers' well-being, there is nonetheless a lack of clear indication on their contribution to the well-being of individual farmers, especially for Kasulu District, the study area. The paper therefore aims to; assess farmers' perception of FOs; identify goods and services accrued by farmers from FOs and to assess the contributions of goods and services obtained from FOs towards farm households' well-being.

METHODOLOGY

A cross-sectional research design was used to generate data for the study on which the paper is based. The study was conducted in Kasulu district, which is divided into 7 divisions. The divisions are further sub-divided into 30 wards and 90 villages. To obtain respondents, both purposive and simple random sampling techniques were used. Purposive sampling was used to select wards and villages in the study area. The selected wards were Munanila and Nyakitonto. The villages selected from the two wards were Mkatanga, Kibwigwa, Nyakitonto and Kitagata. From each village, 40 respondents were randomly selected, out of whom 20 respondents were FOs members and 20 were non-members. The study also involved five key informants (Ward Extension officer (WEO), District Cooperative Officer (DCO), and village leaders) selected purposively to explain or further clarify issues related to the FOs in the study area. Purposive sampling was also used to select Focus Group Discussion (FGDs) participants, 4 FGDs each involving 10 participants (5 males and 5 females) were conducted.

The study involved 160 respondents (80 FOs members and 80 non-members). In order to address the specific objectives, both primary and secondary data were collected. A structured questionnaire and interview checklist/guide were used for primary data collection. Qualitative data was analysed using content analysis. Quantitative information from the questionnaires was coded and analysed using Statistical Package for Social Science (SPSS) software. Descriptive statistics such as frequencies, percentages, mean and standard deviation were determined in order to answer objectives one and two. Inferential statistics, t-test, Chi-square and multiple linear regressions were carried out to answer objective three. The study's unit of analysis was the household. The regression model used is shown below:

$$Y = \beta_o + \beta_1 Xi_1 + \beta_2 Xi_2 + \beta_3 Xi_3 + \beta_4 Xi_4 + \beta_5 Xi_5 + \beta_6 Xi_6 + \beta_7 Xi_7 + \beta_8 Xi_8 + \beta_9 Xi_9 + e_i$$

Y = dependent variable (farmers well-being determined by income and asset ownership)
B_o = constant
$\beta_1 - \beta_9$ = are regression coefficient which is a determinant of change to Y.
$X_1 - X_9$ = Independent variables e_i = error term
X_1 = Access to market information (information on price and demand), X_2 = Access to extension service, X_3 = Use of inorganic fertilizers, X_4 = Use of pesticides, X_5 = Access to credit, X_6 = Use of herbicide, X_7 = Use of Improved seeds, X_8= Respondents education level, X_9= Respondents marital status.

RESULTS AND DISCUSSION

Respondents' socio-economic aspects such as age, sex, marital status, education level, household size and respondent's occupation are as shown in Table 1. Generally, these characteristics have some influence on farmers' involvement in farming and decision to join Farmers' Organization (FOs). For example, age is a variable, which can determine the period of one's entry into agricultural production and other activities. In addition, one's age can influence an individual's preferences of whether or not to participate in certain activities. Results from the study (Table 1) show that more than half of the respondents (both FOs members and non-members) were above 35 years; 83.8 and 77.5% of both FOs members and non-members were males. Additionally, more than 84% of both FOs members and non-members were married and had completed primary school education. Table 1 also shows that farming was the main economic activity of all (100%) the respondents (FOs members and non-members).

Table 1. Demographic and socio-economic characteristics of the respondents (n = 160).

Characteristic		FOs members ($n_{fo = 80}$)		Non-members ($_{nm =80}$)	
		Frequency	Percent	Frequency	Percent
Age of respondents	23 – 35	31	38.8	38	47.5
	36 – 60	47	58.8	42	52.5
	61and above	2	2.5	-	-
Respondents' Sex	Male	67	83.8	62	77.5
	Female	17	16.2	18	22.5
Respondents' Marital status	Married	72	90.0	73	91.2
	Widow	7	8.8	7	8.8
	Single	1	1.2	-	-
Respondents' Education level	Adult education	6	7.5	4	5.0
	Primary education	68	85.0	71	88.8
	Secondary education	6	7.5	5	6.2
	Diploma and above	-	-	-	-
Respondents' Household size	Below 3	3	3.8	2	2.5
	3 - 5	30	37.5	35.	43.8
	6 - 9	46	57.5	41	51.2
	10 and above	1	1.2	2	2.5
Respondents' main occupation	Farming	80	100	80	100
Respondents other activities	Petty trade	32	40.0	39	48.8
	Livestock keeping	40	50.0	37	46.2
	Wage employment	6	7.5	4	5.0
	Carpentry	2	2.5	-	-

NB: n_{fo} = sample size for FOs members and n_{nm} = sample size for the non-members. Source: Field data 2012.

Table 2. Farmers' perception of FOs (n=160).

Statements	FOs members (n_{fo}=80)		Non-members (n_{nm}=80)	
	Disagree	Agree	Disagree	Agree
1.FOs helps farmers to seek agricultural service e.g. credit	3(3.8)	75(93.8)	12(16.7)	67(83.7)
2.FOs members access market information through FOs	6(7.4)	4(92.6)	14(17.5)	66(82.5)
3. New agricultural technology disseminated through group approach	18(22.5)	62(77.5)	31(38.8)	49(61.2)
4. Farmers join FOs gain experience and knowledge	4(5.0)	76(95.0)	16(20.0)	64(80.0)
5. Working in FOs is better than working individually	3(3.7)	77(96.3)	40(25.0)	60(75.0)
6.Through FOs members get agricultural training through farmers field school	15(18.7)	65(81.3)	24(30.0)	56(70.0)
7. Farmers in FOs were access more to extension services than non-members	6(7.6)	74(92.4)	14(17.5)	66(82.5)
8. Individual farmers have low bargaining power enabling traders impose low price to their products	38(45)	52(65.0)	39(48.8)	41(51.2)
9. There is no difference between FOs members and non-members in accessing services (e.g. Loan and extension services	69(83.2)	11(16.8)	46(57.5)	34(42.5)
10.Farmers working in FOs access agricultural inputs i.e. fertilizers, pesticides and improved seeds compared to non-members	0	80(100)	8(11.3)	72(88.7)

Numbers in brackets indicate percentage. Source: Field data 2012.

Farmers' perception of FOs

A Likert scale type of statements as shown in Table 2 determined farmers' perception. Observations from the study (Table 2) show that more than 70% of both FOs members and non-members agreed with the statements

that favour services offered by FOs to its members. Mapila et al. (2010) and Kassam et al. (2011) reported a similar observation. As regards with the services offered to farmers from other development partners through FOs, more than three quarters of the respondents (both FOs members and non-members) agreed with the statement, that development partners such as NGOs (Non-governmental Organizations) reach farmers through FOs; hence, farmers in such organizations are more likely to get more services than those with no affiliation. This finding is in line with Nshimirimana (2009) and Jason (2008) who reported that farmers in FOs were linked to development partners such as NGOs, and these had access to agricultural services. Also 83.3 and 57.5% of FOs members and non-members respectively disagreed with the statement that there was no difference between FOs members and non-members in accessing services through FOs. These results imply that most of the respondents had a positive perception towards services provided by FOs to members.

Goods and services obtained from FOs

Goods and services accessed by the FOs members interviewed include; inorganic fertilizers, pesticides, improved seeds, herbicides, credit, extension services, and market information. Generally, literature has shown that use of these goods and services has an influence on crop production, hence increased crop yield. Results show that 62.5 and 45% of both FOs members and non-members received between 50 to 200 kg of fertilizers. These results imply that there were more FOs members than non-member who used inorganic fertilizer. The above observation seems to be in line with a study by Alemayehu (2008) which reported that FOs provide credit for agricultural inputs such as fertilizers; hence, members are more likely to use fertilizers in their production thus increasing their yield or productivity in terms of product per unit of land used (kgs/ha). Non-members use less fertilizer due to high costs despite the Tanzanian government subsidizing input prices.

Observations from the study further show that 68.8 and 48.8% of both FO's members and non-members used pesticides in the range of 1 to 5 L to spray their crops in particular coffee trees. As regards access to the extension services, observations from the study show that most (93.8%) of the FOs members and a few (12.5%) of non-members use extension services. This observation generally conforms to the stated benefits of farmers organizations that, FOs enable integration of farmers with extension services (Carney, 1996). Observations from the study further show that 66% of the FOs members and 27.5% of the non-members received credit in the range of 50 000 – 250 000 Tanzanian Shillings (TZS). These findings generally suggest that FOs members had more access to goods and services in comparison to non-members. Therefore, this observation implies that the FOs members have better chances of raising their crop productivity and income if the goods and service offered are put into use (Demaine, 2008).

Contribution of goods and services from FOs on farm production and income

Access to goods and services from FOs has a positive impact on farmers' production and productivity (Demaine, 2008). Access to goods and services enables FOs members to increase the area (acreage) under cultivation. This is justified by the results of the t- test (Table 3) which show that there was a significant difference ($P<0.05$) in the acres the farmers cultivated before and after joining FOs.

Observations on estimated income levels from both farm production and off-farm activities show that, income of 67.5% of the FOs members' had increased after joining FOs as compared to before joining. The results further show that 67.5% of the FOs members earned an income of above 2 000 000 TZS, while non-members 45% earned incomes of between 1 000 000 and 1 500 000 TZS per annum. This result suggests that goods and services received by farmers from FOs contributed positively to farmers' incomes. Similar observations have been reported by Bachke (2009) in Mozambique, Jason (2008) in Malawi and by Mushi (2000) in Mvomero district. In addition, the results of a t-test (Table 4) show a significant difference in the income earned ($p < 0.05$) before and after joining FOs, and among the groups.

Improvement of respondents' well-being

Well-being was determined by a household's ability to meet its children's education costs, its asset ownership, and a households' food security status. The results (Table 5) of the study reveal that 67.8 and 83.3% of both members and non-members of FOs had children in primary school. This result implies that the respondents interviewed were able to meet the costs of education offered in public schools and not in private schools. This can be attributed to school fees paid in public schools and the selection criterion for joining secondary education in public school. In addition, Table 5 shows that almost all the children attending secondary schools went to public schools. Generally, the fees in public schools are lower than those in the private sector hence many parents/guardians with limited resource will pay for public education. Moreover, the Chi-square test results (Table 6) show a lack of a significant association ($P> 0.05$) between children's attendance to both public and private schools and parents' memberships to FOs.

Household assets are the components of a household's physical capital and can be used to measure a

Table 3. t-test results on acres allocated to coffee and tobacco farming before and after joining FOs.

Characteristic	Mean	Std.Dev	P- value
Acres for coffee before joining FOs	0.3750	0.45138	0.001*
Acres for coffee after joining FOs	1.000	1.8271	
Acres for tobacco before joining FOs	0.3175	0.480	0.001*
Acres for tobacco after joining FOs	1.6250	1.190	

*Significant at the 5% level, Source: Field data 2012.

Table 4. t-test results on income (TZS) earned by farmers before and after joining FO's and between FOs members and non-members.

Characteristic	Mean ('000)	Std.Dev ('000)	P- value
Income before joining FOs	2,645	2,410	0.001*
Income after joining FOs	6,306	1,104	
Income of FOs members	2,325	1,105	0.001*
Income of non-member	1,130	5,822	

*Significant at the 5% level, Source: Field data 2012.

Table 5. Distribution of respondents by type of school attended by children (n=160).

Type school	FO members		Non members	
	Frequency	Percent	Frequency	Percent
Public primary school	59	67.8	65	83.3
Private primary school	2	2.3	-	-
Public secondary school	24	27.6	13	16.7
Private secondary school	2	2.3	-	-

Source: Field data 2012.

Table 6. Chi-square test results on food security, houses owned and children's education based on FOs membership.

Characteristic	Chi-Square value	P- value
Number of meals consumed	15.185	0.001*
Education attained by children	1.096	0.296
Types of house owned	42.977	0.001*

*Significant at the 5% level.

household's well-being. According to Komba (2008), assets provide people with the opportunities and options in the face of impoverishing forces. Moreover, being asset poor limits people's capacity to improve and safeguard their well-being. The study's findings show that the majority (85%) of FOs members owned a house with walls made of burnt bricks, mud floor and corrugated iron sheets (CIS) roofing after joining the FOs. These results imply that after joining the FOs, members were in a good position to improve their houses. A similar study by Pinto (2009) shows that farmers in organizations have been able to register improved production and access to marketing, which enables them, build modern houses. This is further reflected by Chi-square test results (Table 6) which shows a significant association ($P<0.05$) existed between the types of the house owned and membership to FOs.

Food security is critical for peace and social stability; and according to FAO (2011), a household's food security is more than food production. Generally, a

Table 7. Multiple linear regression results for respondents' income and asset ownerships after joining FOs.

Variables	Income				Assets			
	Coefficient				Coefficient			
	Beta	Std.E	t	Sig. level	Beta	Std.E	t	Sig. level
Constant		0.991	6.240	000		0.344	6.240	0.000
Fertilizers	0.204	0.544	2.455	**0.015***	0.204	0.544	2.455	**0.015***
Pesticides	0.235	0.633	2.154	**0.033***	0.258	0.612	2.201	**0.029***
Improved seeds	- 0.067	0.295	-0.972	0.333	0.083	0.034	1.162	0.247
Herbicides	-0.121	0.425	-1.199	0.232	-0.226	0.862	-2.118	**0.036***
Credit services	-0.051	0.212	0.716	0.475	0.073	0.238	0.994	0.322
Extension services	0.538	0.311	6.707	**0.000***	0.291	0.354	3.505	**0.001***
Market information	-0.003	0.361	-0.038	0.970	0.065	0.062	0.880	0.380
Marital status	0.171	0.670	2.200	**0.029***	0.051	0.540	0.648	0.518
Education level	-0.025	0.281	-0.384	0.702	0.072	0.868	1.044	0.298
	Adjusted R. Square (R^2) = 0.346, F-value = 8.010*				Adjusted R. Square (R^2) = 0.297, F-value = 6.610*			

* = statistically significant at the 0.05 significance level.

household is food secure if it has the ability to access and utilize sufficient quantities and quality of food to support a healthy and active lifestyle. Findings from the study show that 62.5 and 3.8% of FOs members and non-members respectively are able to consume three meals per day. This result implies that being a member of a farmer's organization enables one to have the opportunity to be food secure because the income obtained from commodities produced is used to sustain other household requirements such as construction of modern houses and paying for school fees. In addition, where a household produces both food crops and cash crops it then becomes easy for the household to retain all or most of the food produced for own consumption. Therefore, food produced by the household can then be used for own consumption. As stated earlier, membership to FOs enables easy access to inputs, which are important in raising crop productivity and eventually households' income. Generally, the extra income from crop sales can allow a household to buy enough food or other food stuffs not produced by the household. Furthermore, the results of the Chi-square test (Table 6) shows a significant (P<0.05) association existed between the number of meals consumed by households and membership to FOs.

Results of the multiple linear regression analysis on membership to FOs and households' well-being

A multiple linear regression model was employed to determine the contribution of goods and services accrued by FOs members to their well-being. The well-being of members was determined by considering income and assets ownership before and after joining FOs; two separate models were run using the same set of variables (Table 7). Results in Table 7 show that extension services, use of inorganic fertilizers and pesticides were positively associated with a household's income and assets ownership. Extension services had a regression coefficient of 0.538 (significant P<0.05). This implies that an increase in access to extension services by FOs members enables farmers to improve farming which leads to increased crop yields as well as income and assets ownership by 53.8%. Generally, access to extension services by FOs members created awareness particularly of modern farming techniques, which helped them to improve agricultural productivity and increase income and assets ownership. This observation conforms to what was reported by Mushi (2000) that access to extension services assists farmers to solve farming problems. Based on the regression analysis results (Table 7), a household's use of pesticides and fertilizers were positively related to FOs members' assets ownership, with regression coefficients of 0.258 and 0.204 respectively (significant at P<0.05). These results imply that an increase in the use of pesticides and fertilizers would increase agricultural productivity as well as FOs members' ownership of assets. This is consistent with FAO's (2002) observation that use of fertilizers would supply the nutrients needed by the crops and thereby increases crop yields. Moreover, pest management techniques (both conventional and the integrated pest management practice (IPM) learned or obtained through FO's could lead to a reduction of incidences of diseases and pests and thereby improve the quality and quantity of agricultural produce.

Conclusion

Farmers' Organizations' (FOs) are important in farming

households' agricultural development. The paper therefore aimed at assessing farmers' perception of FOs; identify goods and services accrued by farmers from FOs and to assess the contributions of goods and services obtained from FOs towards farm households' well-being. Based on the findings from the study it can be concluded that FOs members access more services than is the case with non-members, as a result this enables them to raise their productivity. It can also be concluded that goods and services farmers obtained through FOs contributed positively to increasing farm production as is proven by t-test analysis whereby crop yields of FOs members were significantly ($P < 0.05$) higher compared to non-members. Generally, the higher yields were a result of a combination of factors, these include, easy access to agricultural inputs, extension services and marketing information, which are core objectives of farmers organizations. Lastly, it is concluded that, membership to FOs brought positive changes in the well-being of its members and that access to extension services, use of pesticides and inorganic fertilizers were positively and significantly associated with FOs members' income and assets ownership.

Based on the study's observations and conclusions it is recommended that, rural farm households be encouraged to form or join farmers organizations as these have a great potential of increasing farmer's income and asset ownership. It is also recommended that village/ward agricultural extension officers and village/ward community development officers do their best to ensure farmers join farmers' organization. Doing this will not only allow farming households to have a common voice but will also allow them to improve their productivity based on the various services provided by FOs.

Conflict of Interest

The authors have not declared any conflict of interest.

REFERENCES

Abaru M, Nyakuni A, Shone G (2006). Strengthening farmer's organizations: RELMA's experience in Eastern and Southern Africa. [http://www.worldagroforestry.org/projects/relma/pdfs/strengthening - farmers - organisations. Pdf] site visited on 25/4/2012.

Alemayehu MS (2008). Farmers' Perception on the Effectiveness of Cooperatives in Disseminating Agricultural Technology in Ethiopia. Dissertation for Award of MSc Degree at Sokoine University of Agriculture, Morogoro, Tanzania. P.102.

Bachke ME (2009). Are farmers' organizations a good tool to improve small-scale farmers' welfare? [http://www.umb.no/statisk/ncde-2009/maren-e-bachke.pdf] site visited on 8/6/2012.

Carney D (1996). Formal Farmers Organizations in the Agricultural Technology System: Current Roles and Future Challenges. ODI Natural Resource Perspectives, London. P.121.

Chilongo T (2005). Tanzanian Agricultural Co-operatives: An Overview. A Draft Report. Moshi University College of Co-operative and Business Studies, Moshi, Tanzania. P. 69.

Demaine H (2008). Formal Farmers Organizations in the Agricultural Technology System: Current roles and future challenges. ODI Natural Resource Perspectives, Johannesburg, South Africa. P.14.

FAO (2002). Farmers' organizations in central and eastern European countries and their role. In: Provision of Input-output Service in the Context of Accession of the European Union Proceeding FAO Workshop, 11 – 14 June 2001, Prague, Czech Republic. pp. 187 – 190.

FAO (2011). The State of Food Insecurity in the World: How does international price volatility affect domestic economies and food security? http://www.fao.org/docrep/014/i2330e] site visited on14 / 7/ 2012. P. 55.

Grigoryan A, Hakhnazaryan T, Kwapong NA (2008). Farmers organization in the development of agriculture in the South Caucasus*:* Case of Armenia Paper Prepared for Presentation at the ICA Research Conference, Riva del Garda, Trento, Italy, 15 – 18 October, 2008. P. 23.

Jason A (2008). Farmers' Oganizations in Malawi for Southern Cross/Oxfam GB Final Report. Southern Africa Trust (SAT), Lilongwe, Malawi. P. 72.

Kassam L, Subasinghe R, Phillips M (2011). Aquaculture farmer organizations and cluster management: Concepts and experiences. Fisheries and aquaculture technical paper [http://wwwfao.org/docrep/014/i2275e/i2275e.pdf] site visited on 6/8/2012.

Komba MP (2008). The contribution of Victoria farming and fishing project in poverty reduction in Musoma rural District, Mara region, Tanzania. Dissertation for Award of MSc Degree at Sokoine University of Agriculture, Morogoro, Tanzania. P. 81.

Mapila M, Makwenda B, Chitete D (2010). Elitism in the Farmers Organizations Movement in Post-colonial Malawi. Putting people first. National Smallholder Farmers Association, Lilongwe, Malawi. P.10.

Mushi C (2000). The role of farmer' groups in rural development in Mvomero district, mountains agricultural development Project. Dissertation for Award of MSc Degree at Sokoine University of Agriculture, Morogoro, Tanzania. P. 99.

Nshimirimana R (2009). Appraisal of existing farmer organizations and linkages with development partners in Burundi. Dissertation for Award of MSc Degree at Universite Espoir D'afrique, Department of Business Administration and Economics, Bujumbura, Burundi. P. 69.

Wennink B, Nederlof S, Heemskerk W (2007). *Access of the Poor to Agricultural Services:* The role of farmers' organizations in social inclusion. Bulletin No. 376. KIT Publishers, Amsterdam. P. 160.

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Correlation analysis for various grain contributing traits of *Zea mays*

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For the study of genetic variability and correlation analysis among grain yield and its contributing traits an experiment was conducted in the research area of Department of Plant Breeding and Genetics, University of Agriculture Faisalabad, Pakistan during crop season of 2011. The heritability was found 96.06 to 99.99% while genetic advance was from 15.939 to 63.439%. Significant genotypic correlation was found for grain yield per plant with stem diameter, cob diameter, cob length, cob weight, 100-seed weight, dry matter yield, leaves per plant, chlorophyll contents, grain rows per cob and cobs per plant. It was accomplished that higher heritability and genetic advance was found. It was suggested that selection of higher grain yielding genotypes may be helpful to enhance crop yield and productivity.

Key words: *Zea mays*, heritability, genetic advance, genotypic, phenotypic, correlation.

INTRODUCTION

Maize (*Zea mays* L) is an imperative cereal food crop all over the world with extra impact for developing countries like Pakistan. Maize is the third essential cereal in Pakistan following to wheat and rice. It contributes 5.67% in the worth of agriculture outputs. It was grown on 1083 thousands hectares with annual production of 4271 thousands tons and average yield 3940 kg/ha (Anonymous, 2011-12). Maize is dilapidated as food for human while feed for livestock and also worn as an industrial raw material to produce various types of by-products. It has highest 9.9% crude protein at early and at full blooming stages that lower down to 7% at milk stage (grain formation stage) and to 6% at maturity. It contains 72% starch, 10% protein, 4.80% oil, 9.50% fiber, 3.0% sugar, 1.70% ash, 82% endosperm, 12% embryo, 5% testa bran and 1% tip cap (Chaudhary, 1983; Bureau of Chemistry, U.S., 2010). Maize production of Pakistan is lower as compared to other maize growing countries due to non-availability of quality inputs and timely availability. Grain yield is related with diverse physiological, morphological and agronomic traits of maize. By improving these traits the production of maize genotypes may be improved. Heritability, genetic advance and genotypic correlation provide a great prospect to a plant breeder to select genotypes on the basis of strong correlation among grain yield and its contributing traits (Mehdi and Ahsan, 1999; Mehdi and Ahsan, 2000a; Grzesiak et al., 2007; Ali et al., 2011; Ali et al., 2012a, b, 2013a, b). On the basis of above said views, present study was conducted to evaluate maize accessions for morphological and physiological traits of maize for grain yield.

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Table 1. Genetic components for various grain yielding and its contributing traits of maize.

Source of variation	Cobs per plant	Grain rows per cob	Cob length (cm)	Cob diameter (cm)	Cob weight (g)	100-seed weight (g)	Stover weight (g)	Grain yield per plant (g)	Leaves per plant	Stem diameter (cm)	Chlorophyll contents (mgg^{-1}fr.wt.)	Total dry matter (g/m^2)
M.S	0.53629*	7.283**	21.786**	0.0409**	3029.114**	17.773**	160.501**	2118.833**	2.661*	0.0491*	79.655**	241213.392**
G.M	1.989	14.113	18.848	1.515	121.845	31.141	33.823	90.185	11.333	0.856	45.735	1091.501
S.E	0.05477	0.09747	0.1049	0.03162	5.244	0.5177	0.3536	0.7071	0.1936	0.0413	1.00	4.703
G.V	0.265	3.632	10.887	0.0197	1514.816	8.599	80.175	1059.381	1.293	0.0244	39.291	120584.6
GCV	25.89	13.50	17.51	9.26	31.94	9.42	26.47	36.09	10.03	18.25	13.71	31.81
PV	0.268	3.642	10.898	0.0205	1514.856	8.867	80.251	1059.417	1.330	0.0245	39.827	120606.7
PCV	26.03	13.52	17.51	9.44	31.94	9.56	26.49	36.09	10.180	18.30	13.80	31.82
EV	0.0031	0.00938	0.011	0.0008	0.0393	0.268	0.0756	0.0357	0.038	0.000133	0.537	22.115
ECV	2.79	0.69	0.56	1.87	0.16	1.66	0.81	0.21	1.71	1.35	1.60	0.43
$h^2_{bs\ \%}$	98.88	99.72	99.89	96.09	99.99	96.97	99.90	99.99	97.20	99.50	98.65	99.98
S.E h^2_{bs}	0.2157	0.0583	0.0337	0.7917	0.002855	0.03787	0.0124	0.003414	0.0977	0.711	0.017724	0.00032
GA%	45.167	23.667	30.712	15.939	56.059	16.274	46.439	63.439	17.362	31.960	23.891	56.465

** = Significance at 5% level, * = Significance at 1% level, mean sum of squares (M.S), grand mean (G.M), standard error (S.E), genotypic variance (GV), genotypic coefficient of variance (GCV %), phenotypic variance (PV), phenotypic coefficient of variance (PCV %), environmental Variance (EV), environmental coefficient of variance (ECV %), broad sense heritability ($h^2_{bs\ \%}$), Standard error for broad sense heritability (S.E h^2_{bs}), genetic advance (GA).

MATERIALS AND METHODS

The current study was carried out in the experimental field area of the Department of Plant Breeding and Genetics, University of Agriculture Faisalabad to levy the maize genotypes for fodder yielding traits for the period of the crop season in February 2011. The experimental material was comprising of 80 accessions including ten check varieties namely: F-150, F-142, EV-334, EV-330, EV-343, BF-248, EV-338, B-314, F-147, BF-212, B-308, F-118, B-304, F-143, F-113, F-111, F-105, F-121, F-130, F-140, F-128, EV-347, F-96, F-134, F-135, F-117, B-326, BF-236, B-312, EV-344, E-352, F-148, E-341, E-351, E-349, B-121, E-336, F-122, B-316, EV-324, EV-335, EV-310, EV-323, B-321, F-151, Pop/209, B-306, B-303, B-313, EV-342, B-305, Sh-139, F-114, F-136, BF-238, B-15, E-322, Sh-213, F-98, B-96, F-146, B-303, B-327, BF-337, VB-06, EV-329, EV-340, E-346, B-11, SWL-2002, Pak-Afgoee, Islamabad W, EV-7004Q, EV-1097, Raka-Poshi, VB-51, Gold Isalamabad, Sawan-3, BS-2 and Pop/2007). The accessions were grown in the field following three replications incompletely randomized block design. The plant-to-plant and row-to-row distances were kept 25 and 75 cm, respectively. The data of 10 randomly selected plants were recorded for stem diameter, cob diameter, cob length measured by vernier caliper (Model RS232), plant height measured by using meter rod, cob weight, 100-seed weight, grain yield per plant, dry matter yield, stover weight measured with the help of electronic balance (OHAUS-GT4000, USA), chlorophyll contents by using chlorophyll meter, grain rows per cob, leaves per plant and cobs per plant. The data was statistically analyzed by using analysis of variance technique (Steel et al., 1997). The genotypic and phenotypic correlations were calculated as given by Kwon and Torrie (1964). The genetic advance was calculated as described by Falconer (1989). Heritability was computed according to Burton (1951).

RESULTS AND DISCUSSION

It was suggested from Table 1 that significant differences were found for all traits. It was persuaded that higher heritability (98.88%) and genetic advance (45.167%) was found for leaves per plant, cob length (h^2=99.89%, GA=30.712%), cob weight (h^2=99.99%, GA=56.059%), stover weight (h^2=99.90%, GA=46.439%), grain yield per plant (h^2=99.99%, GA=63.439%) and total dry matter (h^2=99.98%, GA=56.465%), respectively. Higher heritability and genetic advance indicated that selection of higher grain and fodder yield may be helpful to perk up crop yield and production. The genotypes that showed higher cob weight, cob length and cob diameter indicated that grain may be improved by selecting such genotypes. Higher leaves per plant, stover weight and total dry matter indicated that the genotypes may be selected for the improvement of fodder yield of maize. The findings were similar as reported by Mehdi and Ahsan (1999), Mehdi and Ahsan (2000a, 2000b), Afarinesh et al. (2005), Ali et al.

Table 2. Genotypic correlations of various morphological and physiological and grain yielding traits of maize.

Traits	NGRPC	CL	CD	CW	HSW	SW	SD	TDM	Chl.C	NLP	PH	GYP
NCP	0.2215**	0.3269*	0.1959*	0.3543*	-0.1199	0.1063*	0.1454	0.1179*	0.2706*	0.1754	0.2923*	0.3150*
NGRPC		0.0333	0.5933*	0.4712*	-0.2172*	-0.0246	0.1019	0.1705*	0.3182*	0.1437*	0.2096*	0.4245*
CL			0.2022*	0.4497*	0.1831*	0.2962*	0.1815*	0.1364*	0.1676*	0.1772*	0.3014*	0.3061*
CD				0.7592*	0.0640	0.3262*	0.1699*	0.1599*	0.4141*	0.2467*	0.1383*	0.6166*
CW					0.2352*	0.2772*	0.1947*	0.1905*	0.5088*	0.2302*	0.3563*	0.8933*
HSW						0.1264*	0.1173	0.0622	-0.1462	-0.0516	0.1110*	0.2075*
SW							0.3545*	0.0409	-0.0118	0.4089*	0.0114	0.0067
SD								0.2210*	0.1960*	0.4471*	0.2919*	0.0866
TDM									0.1722*	0.062	0.3495*	0.1267*
Chl.C										0.0923	0.3754*	0.4505*
NLP											0.3069*	0.0640
PH												0.3512*

** = Significance at 5% level; * = significance at 1% level; NLP = leaves per plant; PH = plant height; SD = stem diameter; Chl. C = chlorophyll contents; NCP = Cobs per plant; NGRPC = grain rows per cob; CL = Cob length; CD = Cob diameter; CW = Cob weight; SW = Stover weight; HSW = 100-seed weight; GYP = grain yield per plant; TDM = total dry matter.

(2013), Grzesiak et al. (2007), Ali et al. (2011) and Ali et al. (2012a, b).

It was convinced from Tables 2 and 3 that significant positive genotypic and phenotypic correlation coefficients of chlorophyll contents were found with leaves per plant, cob weight, stem diameter, grain yield per plant, total dry matter, plant height, cobs per plant, grain rows per cob and cob diameter while leaves per plant were significantly correlated at genotypic and phenotypic levels with cob weight, stem diameter, chlorophyll contents, grain yield per plant, total dry matter, plant height, grain rows per cob and cob diameter. Higher and significant correlation of chlorophyll contents with leaves per plant, plant height and total dry matter indicated that photosynthetic rate was higher that caused for the accumulation of organic compounds in the plant body and hence helped in the improvement of grain yield (Jension et al., 1981; Mehdi and Ahsan, 2000a; Afarinesh et al., 2005; Grzesiak et al., 2007; Moulin et al., 2009; Ali et al., 2011; Ali et al., 2012a; Ali et al., 2013). Plant height was positively and significantly correlated at genotypic and phenotypic levels with all traits while non-significant at phenotypic level with 100-seed weight and stover weight. Higher 100-seed weight indicated that overall grain yield per plant increased. Total dry matter was positively and significantly correlated at genotypic and phenotypic levels with all traits but non-significant with 100-seed weight, cobs per plant and stover weight. The significant correlation of total dry matter with grain yield, grain rows per cob and chlorophyll contents suggested that the crop plant vigor is higher that may be helpful to improve grain yield. Results were found similar as reported by Jension et al. (1981); Mehdi and Ahsan (2000a); Mehdi and Ahsan (2000b); Afarinesh et al. (2005), Ali et al. (2013), Grzesiak et al. (2007), Moulin et al. (2009); Ali and Ahsan (2011), Ali et al. (2011) and Ali et al. (2014a, b, c). It was persuaded that stem diameter was positively and significantly correlated at genotypic and phenotypic levels with leaves per plant while with cob weight, chlorophyll contents, grain yield per plant, total dry matter, stover weight, plant height, cob weight, leaves per plant and cob diameter while stover weight was positively and significantly correlated with cob weight, cob length, 100-seed weight, grain yield per plant, total dry matter, cob diameter and plant height. The genotypes with higher stem diameter indicated that photosynthetic rate is higher that caused in the increase of accumulation of organic compounds, leaves per plant, total dry matter and hence the crop yield and productivity. Selection of genotypes on the basis of stem diameter may be helpful to improve maize grain yield. The cob diameter and cob weight also contributed great role in the grain yield per plant. Greater diameter, greater will be the grain rows per cob and grain yield per plant. Findings were similar as reported by Mehdi and

Table 3. Phenotypic correlations of various morphological and physiological and grain yielding traits of maize.

Traits	NGRPC	CL	CD	CW	HSW	SW	SD	TDM	Chl.C	NLP	PH	GYP
NCP	0.2189**	0.3251**	0.1935*	0.3532**	-0.1192	0.1062	0.1448	0.1170	0.2669**	0.1716*	0.2905**	0.3133**
NGRPC		0.0328	0.5816**	0.4706**	-0.2128**	-0.0247	0.1016	0.1703*	0.3135**	0.1413	0.2093**	0.4239**
CL			0.1975*	0.4494**	0.1806*	0.2961*	0.1808*	0.1364	0.1655*	0.1745*	0.3011**	0.3060**
CD				0.7441**	0.0629	0.3190**	0.1663*	0.1566*	0.4034**	0.2412**	0.1356	0.6044**
CW					0.2316**	0.2771**	0.1941*	0.1905*	0.5053**	0.2270**	0.3562**	0.8932**
HSW						0.1241	0.1150	0.0610	-0.1408	-0.0513	0.1089	0.2044**
SW							0.3536**	0.0408	-0.0120	0.4031**	0.0113	0.0067
SD								0.2205**	0.1956*	0.4374**	0.2911**	0.0864
TDM									0.1706*	0.0619	0.3494**	0.1267
Chl.C										0.0926	0.3731**	0.4473**
NLP											0.3026**	0.0632
PH												0.3511**

** = Significance at 5% level; * = significance at 1% level; NLP = leaves per plant; PH = plant height; SD = stem diameter; Chl. C = chlorophyll contents; NCP = Cobs per plant; NGRPC = grain rows per cob; CL = Cob length; CD = Cob diameter; CW = Cob weight; SW = Stover weight; HSW = 100-seed weight; GYP = grain yield per plant; TDM = total dry matter.

Ahsan (2000a); Afarinesh et al. (2005); Wang et al. (2007); Ali et al. (2013) and Ali et al. (2012a, b). 100-seed weight was positively and significantly correlated with cob length, cob weight and grain yield per plant. Cob weight and cob diameter were positively and significantly correlated with each other and also with stem diameter, cob length, 100-seed weight, dry matter yield, leaves per plant, chlorophyll contents, grain rows per cob and cobs per plant. Higher 100-seed weight indicated that the individual grain size was higher. 100-seed weight directly effect grain yield per plant and selection on the basis of 100-seed weight may be helpful to improve crop plant yield and production. Cob length was positively and significant correlated with all traits at genotypic and phenotypic levels expect grain rows per cob while cobs per plant was significantly correlated with grain rows per cob, cob length, cob diameter, cob weight, stem diameter, chlorophyll contents, plant height, grain yield per plant and total dry matter. The genotypes with higher cob length indicated that the grain rows per cob may be higher, due to which the grains per cob will also be increased. Due to increase in grain rows and grains per ear row the overall grain yield may be improved and selection of genotypes on the basis of cob length, grain rows per cob, grains per ear row, 100-seed weight and grain yield per plant may be helpful to improve crop yield and production (Mehdi and Ahsan, 1999, 2000a; Afarinesh et al., 2005; Ali et al., 2013; Grzesiak et al., 2007; Ali and Ahsan, 2011; Ali et al., 2014b, c; Ali et al., 2012a, b).

Conclusions

It was concluded from present study that higher heritability and genetic advance was found for grain yield per plant and its contributing traits leaves per plant, cob length, cob weight, stover weight and total dry matter. Positive and significant genotypic correlation was found for grain yield per plant with stem diameter, cob diameter, cob length, cob weight, 100-seed weight, dry matter yield, leaves per plant, chlorophyll contents, grain rows per cob and cobs per plant. Hence selection of higher grain yielding maize genotypes may be useful on the basis of these traits.

Conflict of Interest

The authors have not declared any conflict of interest.

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REFERENCES

Afarinesh A, Farshadfar E, Choukan R (2005). Genetic analysis of drought tolerance in maize (*Zea mays* L.) using diallel method. Seed Plant 20(4):457- 473.

Ali Q, Ahsan M (2011a). Estimation of variability and correlation analysis for quantitative traits in chickpea (*Cicer arietinum* L.). Int. J. Agro Vet. Med. Sci. 5(2):194-200.

Ali Q, Ahsan M, Ali F, Aslam M, Khan NH, Munzoor M, Mustafa HSB, Muhammad S (2013). Heritability, heterosis and heterobeltiosis studies for morphological traits of maize (*Zea mays* L.) seedlings. Adv. Life Sci. 1(1):52-63.

Ali Q, Ahsan M, Ali F, Muhammad S, Manzoor M, Khan NH, Basra SMA, Mustafa HSB (2013b). Genetic advance, heritability, correlation, heterosis and heterobeltiosis for morphological traits of maize (*Zea mays* L). Albanian J. Agric. Sci. 12(4):689-698.

Ali Q, Ahsan M, Khan NH, Ali F, Elahi M, Elahi F (2012b). Genetic analysis for various quantitative traits of chickpea (*Cicer arietinum* L.). Int. J. Agro Vet. Med. Sci. 6(1):51-57.

Ali Q, Ahsan M, Mustafa HSB, Hasan EU (2013a). Genetic variability and correlation among morphological traits of maize (*Zea mays* L) seedling. Albanian J. Agric. Sci. 12 (3):405-410.

Ali Q, Ahsan M, Tahir MHN, Basra SMA (2012a). Genetic evaluation of maize (*Zea mays* L.) accessions for growth related seedling traits. Int. J. Agro Vet. Med. Sci. 6(3):164-172.

Ali Q, Ali A, Ahsan M, Ali S, Khan NH, Muhammad S, Abbas HG, Nasir IA, Husnain T (2014c). Line × Tester analysis for morpho-physiological traits of *Zea mays* L. seedlings. Adv. life Sci. 1(4):242-253.

Ali Q, Ali A, Awan MF, Tariq M, Ali S, Samiullah TR, Azam S, Din S, Ahmad M, Sharif NM, Muhammad S, Khan NH, Ahsan M, Nasir IA, Hussain T (2014b). Combining ability analysis for various physiological, grain yield and quality traits of *Zea mays* L. Life Sci. J. 11(8s):540-551.

Ali Q, Ali A, Tariq M, Abbas MA, Sarwar B, Ahmad M, Awaan MF, Ahmad S, Nazar ZA, Akram F, Shahzad A, Samiullah TR, Nasir IA, Husnain T (2014a). Gene action for various grain and fodder quality traits in *Zea mays*." J. Food Nutrit. Res. 2(10):704-717.

Ali Q, Elahi M, Ahsan M, Tahir MHN, Basra SMA (2011). Genetic evaluation of maize (*Zea mays* L.) genotypes at seedling stage under moisture stress. Int. J. Agro Vet. Med. Sci. 5(2):184-193.

Anonymous (2011-2012). Economic Survey of Pakistan. Govt. of Pakistan, Finance and Economic Affairs Division, Islamabad.

Bureau of Chemistry, U.S. (2010). Wiley, Harvey Washington. Composition of maize (Indian corn), including the grain, meal, stalks, pith, fodder, and cobs.__University of California Libraries, nrlf_ucb:GLAD-151223559.

Burton GW (1951). Quantitative inheritance in pearl millet (*Pennisetum glaucum* L.). Agron. J. 43:409-417.

Chaudhry AR (1983). Maize in Pakistan. Punjab Agriculture Research Coordination Board, University of Agriculture Faisalabad.

Falconer DS (1989). Introduction to quantitative genetics. 3rd Ed. Logman Scientific and Technical, Logman House, Burnt Mill, Harlow, Essex, England.

Grzesiak MT, Rzepka A, Hura T, Hura K, Skoczowski A (2007). Changes in response to drought stress of triticale and maize genotypes differing in drought tolerance. Photosynthesis 45:280-287.

Jenison JR, Shank DB, Penny LH (1981). Root characteristics of 44 maize inbred evaluated in four environments. Crop Sci. 21:233-236.

Kwon SH, Torrie JH (1964). Heritability and interrelationship of two soybean (*Glycine max* L.) populations. Crop Sci. 4:196-198.

Mehdi SS, Ahsan M (1999). Evaluation of S_1 maize (*Zea mays* L.) Families for green fodder yield. Pakistan J. Biol. Sci. 2:1069-1070.

Mehdi SS, Ahsan M (2000a). Genetic coefficient of variation, relative expected genetic advance and inter-relationships in maize (*Zea mays* L.) for green fodder purposes at seedling stage. Pakistan J. Biol. Sci. 11:1890-1891.

Mehdi SS, M Ahsan (2000b). Coefficient of variation, inter-relationship and heritability: estimated for some seedling trails in Maize in recurrent selection cycle. Pakistan J. Biol. Sci. 3:181-182.

Moulin S, Baret F, Bruguier N, Bataille C (2009). Assessing the vertical distribution of leaf Chlorophyll content in a maize crop. INRA - Unite Climat, Soil Environ. pp. 7803-7929.

Steel RGD, Torrie JH, Dicky DA (1997). Principles and procedures of Statistics. A Biometrical Approach 3rd Ed. McGraw Hill Book Co. Inc. New York, pp. 400-428.

Wang BQ, Li ZH, Duan LS, Zhai ZX (2007). Effect of coronatine on photosynthesis parameters and endogenous hormone contents in maize (*Zea mays* L.) seedling under drought stress. Plant Physiolog. Commun.43:269-272.

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Effect of halopriming treatment on seed germination and seedling emergence of Judas tree (*Cercis siliquastrum* L., Caesalpiniaceae) from Zanjan, Iran

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The effect of seed halopriming with potassium nitrate on germination and emergence traits of *Cercis siliquastrum* L seeds, in two experiments were evaluated, one in the laboratory and the other in a greenhouse. The studies were a completely randomized design and 4 replications. The treatments included non-priming and halopriming with potassium nitrate at one of four concentrations (0, 100, 250, 500 and 750 mM) for 48 h, for all treatments the seeds were boiled in water per 24 h. The results showed that seed halopriming with KNO_3 at the 750 mM concentration in the laboratory significantly increased several characteristics of germination. Emergence characteristics also were increased by pretreatment of seeds with potassium nitrate when planting in greenhouse. The highest emergence percentage (57%), speed of germination (1.54 seeds per day) emergence energy (44.8%) and lowest mean germination time (13.9 days) were showed in 100 mM treatment. The best halopriming level for seedling characteristics was detected in 100 mM KNO_3. However, haloprimed achenes resulted in maximum final emergence and shoot length, root length, collar diameter, shoot dry weight, root dry weight, number of leaves, leaf area and seedling quality index treated with primed techniques were increased and compared with non-prime treatment.

Key words: Afforestation, *Cercis siliquastrum*, germination, Potassium nitrate, regeneration, seedling quality index.

INTRODUCTION

Increasing global consumption of natural resources has caused ecological degradation in some area. The annual area has decreased with about 5.2 million hectares of forestlands worldwide over the past ten years (FAO, 2010). Therefore, restoration projects are very important in forest management for increasing forestlands, especially in arid and semi-arid areas. Last year, the amount of forest degradation in Iran reached 2 million hectares according to FAO (Kouhgardi et al., 2012). Iran is a country with an arid and semi-arid climate and restoration of deforested and desertified areas have been done with native tree species such as (*Haloxylon persicum*), (*Quercus persica*), (*Pistasia atlantica*) and *(Olea europaea*) (Jazirei, 2001). Current interest is

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increasing for using Judas tree (*Cercis siliquastrum* L.), a celsipeanacea species with ornamental-conservational importance, for restoration (Jazirei, 2001). Judas trees are also used for protection against soil losses caused by wind and water erosion (Gebre and Karam, 2004; Zahreddine et al., 2007). This deciduous species may also be used in reforestation of disturbed lands to improve the landscape. Judas trees occur most often on the borders of broadleaved or coniferous forests in valleys of streams and rivers in maquis communities (Boratynski et al., 1992).

Like many other woody plants, the artificial regeneration of Judas trees is most commonly by seed propagation, as this method is a cost-effective method (Pipinis et al., 2011). Cuttings are rarely used, but some varieties are propagated by grafting (Ana-Felicia, 1998). The seed of this species, as in many Leguminosae, has a hard seed coat that is impermeable to water, which causes a physical dormancy. Judas trees require a long growing season, have good drought tolerance and are sensitive to frost (Sabina and Cornelia, 2012). They are distributed in Thermo-Mediterranean zones at elevations up to 0-800 m, and on soils with pH above 7.5 (Sternberg, 2012). In Iran Judas tree grows in Giulan, Hamedan, Lorestan and Fars as wild plant. Tree growth is high in soils with gypsum and limestone. It is also resistant to drought and needs direct sunlight for growth (Sabety, 1994).

Nursery production of high quality seedlings is important in forestry for successful regeneration. *Cercis* seeds generally require pre-germination treatment to overcome dormancy, because of their impermeable seed coat and embryo dormancy (Hamilton and Carpenter, 1976; Geneve, 1991; Tipton, 1992; Jones and Geneve, 1995; Rascio et al., 1998). There are different methods for breaking seed dormancy such as thermo-stratification, acid exposure, scarification and chemical treatment (Kermode, 2011). Much research has been done on breaking the dormancy of Judas tree seeds. Geneve (1991) and Dirr and Heuser (1987) tried to break the dormancy of Judas tree seed by cold stratification to allow imbibitions of the hard seed coat, but this method requires a long exposure period to cold temperatures to improve germination efficiency. Judas seeds treated with sulfuric acid and follow stratification had high germination rates, but there were subsequent negative physiological side effects on seedling growth rate (Frett and Dirr, 1979; Liu et al., 1981). In addition, the method required a long time to complete the germinability.

Recently, the priming method has been presented for breaking seed dormancy and improving seedling growth of crops and trees (Bradford, 1986). During osmotic priming (halopriming), ions in a potassium nitrate and sodium chloride solution accumulate within the seeds, increasing water absorption by reducing water potential (Parera and Cantliffe, 1994). Potassium nitrate as one of the main compounds in halopriming increases the concentrations of potassium and nitrogen in seeds (Alevarado and Bradford, 1988; Bellti et al., 1993). Advantages of halopriming include high germination efficiency (Taylor et al., 1998), more rapid germination (Bhan and Sharma, 2011), enhanced growth rates (Geo et al., 2012), and more uniformity of germination that collectively result in higher seedlings quality (Basra et al., 2005).

There are increased opportunities for the application of such seed treatment method in forestry, especially in the restoration of arid and semi-arid lands. But there are many problems with Judas tree seed germination and seedling production that limit its use in restoration. The purpose of this research is to evaluate the effectiveness of treating Judas tree seed with boiling water and halopriming technique followed by soaking in a potassium nitrate solution to break the double dormancy and promote germination simultaneously. We also followed the seedlings in greenhouse conditions obtained from non-priming with primed seeds in order to determine how seedling characteristics are affected by priming treatments conducted by different KNO_3 concentrations.

MATERIALS AND METHODS

Seed characterization or traits

The seeds of Judas tree were received from the Central Seed Center of Caspian (Amol) for this research. The seeds were obtained from Zanjan (Iran), seed of physic-chemical characteristics such as weight, purity, humidity and viability was determined. Also, habitat characteristics of the seeds produced are given in Table 1.

Seed priming

The experiments were performed in College of Natural Resource, Tarbiat Modares University, Iran. The treatments included non-priming and halopriming with potassium nitrate at four concentrations (0, 100, 250, 500 and 750 mM) for 48 h and 4 replications. For all treatments, the seeds were boiled in water per 24 h. After boiling in water (sowing in water with 100°C), they were left up to 24 h until the water temperature reached ambient air temperature. For priming with Potassium nitrate, two hundred seeds were selected randomly for each concentration and were placed in a 10-cm diameter plastic Petri dish on a filterpaper (Whatman filter paper No. 1). The Petri dishes were covered by aluminum foil in order to prevent waste solution and were placed in a germinator (with a temperature of 20°C and in constant darkness). A period of 2 days was allocated to their growth. In order to wash off the solutions from the surface of the seeds, after the 2 days, seeds were rinsed in distilled deionized water for 2 min. Seeds were dried slowly at room temperature for 48 h to reach the initial moisture (control) (Demir and Mavi, 2004). Unprimed seeds (only sowed in boiling water) and control seeds (no seed handling) were used in research.

Seed germination in the laboratory

For seed germination analysis, four replicates of 25 seeds of non-primed, primed (potassium nitrate treatment) and control

Table 1. seeds features from Judas tree (*Cercis siliquastrum* L., Caesalpiniaceae) seeds originated from Zanjan (Iran) and commercialized by forest seed center from Central Seed Center of Caspian (Amol)

Description the area and laboratory characteristic										
Specie	**Origin**	**Latitude**	**Longitude**	**Altitude (M)**	**Climate**	**Viability (%)**	**Humidity**	**Numbers of seed per kg**	**One thousand grain weight (g)**	**Purity (%)**
C. siliquastrum	Zanjan	36.66° N	48.48° E	1663	Semiarid ultra cold	85	4.4	36630	27.7	97

Table 2. Seed germination measurements utilized to characterize seeds from Judas tree (*Cercis siliquastrum* L., Caesalpiniaceae) and their mathematical expressions.

Germination indices	**Mathematical expressions**
Germinability	FG, FE = n/N*100 (n- The total number of seeds germinated in the period N- The number of seeds planted)
Speed of germination, emergence (Maguire, 1962)	SG, SE = N1/1 + n2/2 + ... + nx/x = N (N1 = Nx are the no. of seed germinated on day 1 to day x, 1 = X are the no. of days)
Germination energy, emergence energy (Czabator, 1962)	GE, EE = Mng/N *100 (Mng- Accumulative maximum percentage of germinated seeds N- The number of seeds planted)
Mean germination time, Mean emergence time (Ellis and Roberts, 1981)	MGT, MET = $\sum D_n / \sum n$ (where n is the number of seeds which were germinated on day D)

treatments were placed on filter papers in Petri dishes and were kept moist daily (20 ml). The Petri dishes were transferred to a germinator where the temperature was maintained at 20°C, under the 16 h light and 8 h of dark, light intensity of 1000 Lux and 60% humidity (ISTA, 1985). The papers were replaced every 3 days to prevent fungal growth. Seed germination was recorded daily in a certain time. A seed was considered as germinated when its radicle emerged by about 2 mm in length (Mohammadi, 2009).

Seedling emergence in the greenhouse

Pots with 35 cm diameter contain silt loamy soil with 0.3 dS m electrical conductivity was prepared. In each pot, twenty-five seeds were planted 2 cm in depth and irrigated when soil moisture was slightly below field capacity. There were four replications for each KNO_3 concentration. The experiment was conducted in a greenhouse where daily air temperatures averaged was 20 ± 10°C (ranging from 7 to 15°C at night), and natural light that varied from 6000-10000 lux during the day. Counts of germinating seeds were made daily, starting on the first day of stem emergence. The progress of seedling emergence was measured at 24 h intervals for 45 days. After 45 days of start of the experiment, final germination percentage and final emergence percentage (FGP, FEP), germination speed (GS), mean germination time and emergence time (MGT, MET), germination and emergence energy (GE, EE) were calculated according to the equations (Table 2).

Measurement of growth characteristics in greenhouse

Seedlings obtained from greenhouse condition were planted in pots with dimensions 15 × 20 cm and were irrigated to field capacity once every 2 days for 4 months in greenhouse conditions listed above. Plants were randomly selected per replication in each treatment, a total of 8 plants for each treatment, to determine seedling growth characteristics such as the length of the shoot and root (to the nearest mm). Root collar diameter was measured with a caliper. Shoot and root dry weight, and leaf fresh weight were measured with a microbalance to

Table 3. Comparison of the mean (± standard devaluation in parentheses) of seed germination and seedling emergence of Judas tree (*Cercis siliquastrum* L., Caesalpiniaceae) obtained of seeds from Laboratory and Greenhouse and primed with KNO_3 and non-primed seed.

Germination environment	Treatment	G, E (%)	GS, ES (seed per day)	GE, EE (%)	MGT, EGT (day)
Laboratory	Non-primed	18 (2.3)^{d}	0.26 (0.04)^{e}	2.6 (1.2)^{c}	19.7 (1.6)^{a}
	100 mM KNo3	39 (2)^{c}	0.61 (0.06)^{d}	12.12 (2.6)^{bc}	19.2 (1.4)^{a}
	250 mM KNo3	61 (6)^{b}	1.16 (0.07)^{c}	26.9 (14.4)^{ab}	16.4 (1.6)^{b}
	500 mM KNo3	70 (7.6)^{a}	1.41 (0.17)^{b}	33.8 (18.3)^{a}	15.9 (0.7)^{b}
	750 mM KNo3	72 (6.5)^{a}	1.61 (0.05)^{a}	35.2 (8.4)^{a}	13.6 (0.58)^{c}
	F value	72.61**	143.146**	6.48**	17.91**
Greenhouse	Non-primed	25 (3.8)^{d}	0.24 (0.03)^{c}	4.5 (1.5)^{c}	26.3 (2.1)^{a}
	100 mM KNo3	82 (9.2)^{a}	1.8 (0.2)^{a}	49.3 (11.8)^{a}	12.4 (1)^{c}
	250 mM KNo3	66 (6.9)^{b}	1 (0.1)^{b}	26.7 (7.5)^{b}	17.7 (1.1)^{b}
	500 mM KNo3	53 (3.8)^{c}	0.89 (0.06)^{b}	17.2 (8.8)^{b}	17.3 (1.7)^{b}
	750 mM KNo3	59 (3.8)^{c}	0.97 (0.5)^{b}	18 (7.5)^{b}	18.8 (4.9)^{b}
	F value	47.8**	67.8**	16.46**	25.05**

** P < 0.01; Means with different letters superscripts in a column to each environment indicate significant difference among seed treatment means according to Duncan's multiple range test (P < 0.01). G, E: Germinability, Emergency, GS, ES: Speed of germination, emergence, GE: Germination energy, emergence energy, MGT, MET: Mean germination time, Mean emergence time.

a precision of 0.001 gr. Leaf surface was measured with a CI202 Area meter, CID, Inc. For each plant, expanded leaf area of randomly selected leaves was measured and leaf number was counted by treatment. Leaf area was computed using the formula for specific leaf area (specific leaf area = leaf area (cm²) / leaf dry weight (gr)) according to Arias et al. (2007). Seedling quality (QI) was calculated for each seedling using the formula: QI = TDW / ((SL / SD) + (SDW / RDW)), where TDW is total dry weight, SL is shoot length, SD root collar diameter, SDW shoot dry weight and RDW is root dry weight (Mckay et al., 1999).

Statistical analysis

Experiments were set up in a completely randomized design. Data normality was explored by kolmogorov-smirnov Test. The mean and one-way ANOVA were calculated using SPSS (version 18) software. The mean separations were carried out using Duncan's multiple range tests (Duncan, 1955) and significance was determined at $p \leq 0.05$.

RESULTS

Germination in the laboratory

Control seeds (no seed handling) did not show germination in two substrates, Therefore, they were not involved in computing. The results indicated that halopriming treatments significantly (*P < 0.01*) affected all the measured parameters (Table 3). The largest improvement was achieved when seeds were primed with 750 mM KNO_3. Haloprimed seeds had significantly higher final germination percentage (64%), germination speed (1.34 seed per day), germination energy (32.6%) and mean germination time (6.1 day) compared to un-primed seeds.

Germination in greenhouse

Similar results to the laboratory experiment were observed when seeds were treated and the seedling emergence was evaluated in the greenhouse for 45 days in similar environmental conditions. The KNO_3 treatment had a significant effect on all the response variables (Table 3, *P* < 0.01). Maximum improvement was recorded when seeds were primed with 100 mM KNO_3. Seeds primed had a higher emergence percentage (57%) than those in the unprimed. The highest emergence speed was observed for seeds primed in the 100 mM KNO_3. For unprimed seeds, emergence energy was recorded at about 4.5% compared to about 49.3% for seeds primed with 100 mM KNO_3 (Table 3). Seeds primed with 100 mM KNO_3 had significantly shorter germination times than any other treatment.

Mean cumulative germination in the laboratory

Mean cumulative germination of *Cercis* seeds in the germinator showed that seeds primed in any of the KNO_3 concentrations had faster germination compared to seeds in the control (Figure 1). Seeds primed with 750 mM KNO_3 had the fastest germination start time among tree seeds, and the highest speed of germination. Germination began in non primed treatment later 7 days compared to primed treatment of 750 mM.

Mean cumulative emergence in greenhouse

Similarly, mean cumulative emergence of *C. siliquastrum* seeds grown in the greenhouse germinated faster for

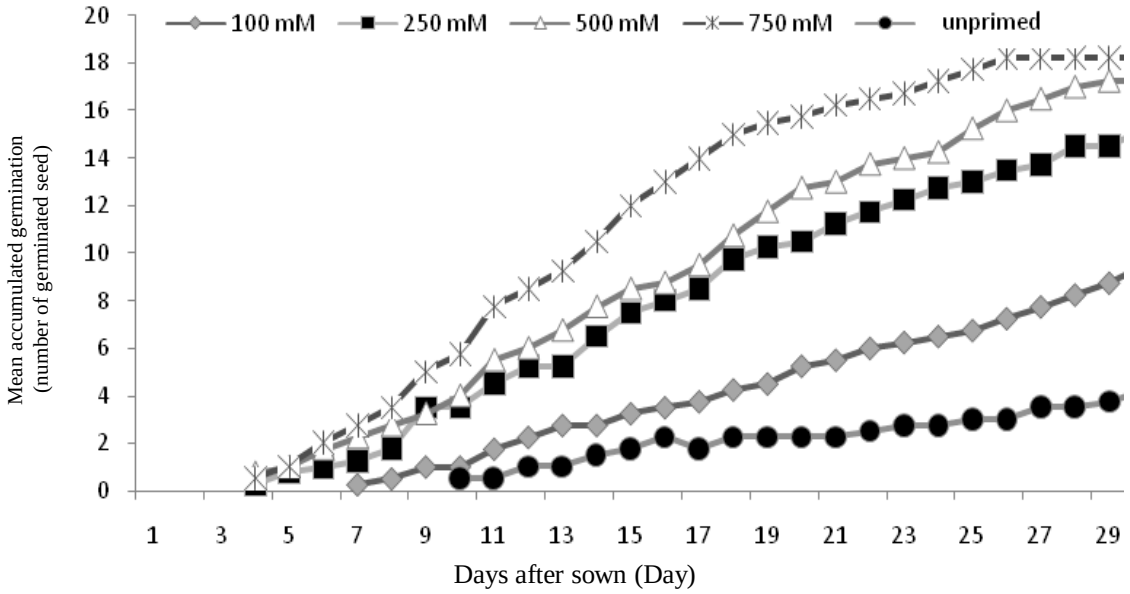

Figure 1. Cumulative mean of seed germinationin the laboratory for *C. Siliquastrum* L. seed obtained from from Zanjan (Iran) in 2011.

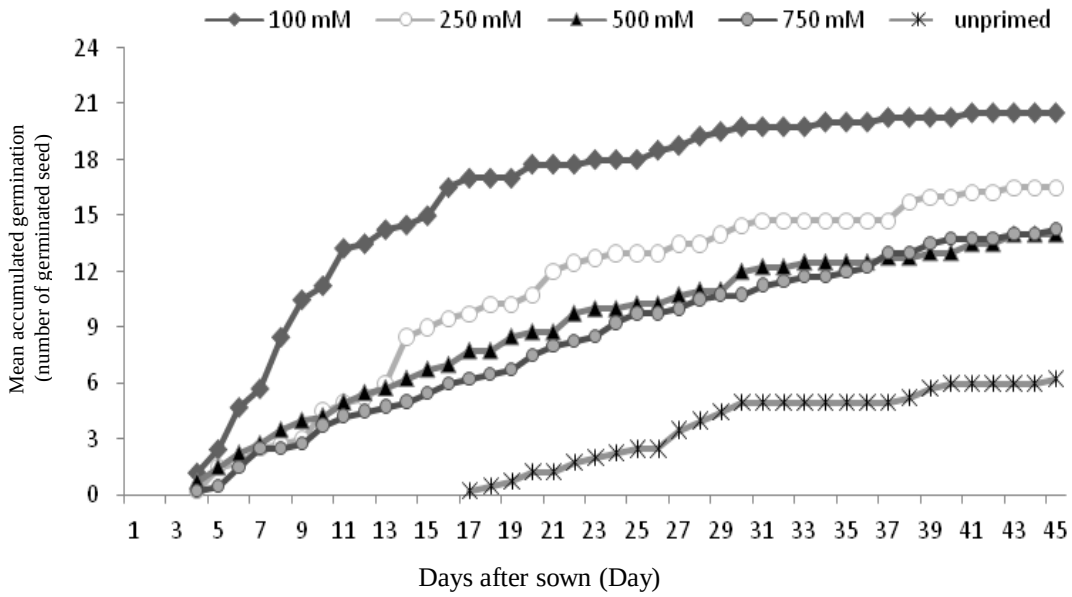

Figure 2. Effects of various concentrations of KNO_3 under seedling emergence of Judas tree (*Cercis siliquastrum* L., Caesalpiniaceae) seed obtained from from Zanjan (Iran) in 2012.

primed seeds compared to those in the control. Seeds primed in each of the KNO_3 solutions had the fastest emergence. Seed in the 100 mM KNO_3 treatment had the fastest emergence start time in any of the treatment, also it completed the emergence period sooner than seed in any other treatments. Seeds in the boiling water of treatment were the most delayed in initiating emergence in 17 days (Figure 2).

Growth characteristics in greenhouse

Halopriming with KNO_3 affected the growth characteristics compared to non-primed seeds, significantly. Seed priming increased shoot, root length and collar diameter seedlings as compared to unprimed seedling. Also 100 mM concentrate indicated the highest lenght of shoot, root and highest collar diameter compared to unprimed

Table 4. Comparison of the seedling growth characteristics of Judas tree (*Cercis siliquastrum* L., Caesalpiniaceae) obtained of seeds from Greenhouse and primed with KNO_3 and non-primed seed.

Treatments	100 mM	250 mM	500 mM	750 mM	Non-primed	F
Shoot length (mm)	24.7(5.9)^{a}	19.1(3.6)^{b}	17.3(3.5)^{bc}	14.6(3.2)^{cd}	11.7(1.7)^{d}	12.88**
Root length (mm)	27.7(6.2)^{a}	20.8(6.0)^{b}	20.7(5.7)^{b}	19.2(4.9)^{b}	12.0(0.9)^{c}	9.43**
Collar diameter (mm)	1.7(0.5)^{a}	1.2(0.3)^{b}	1.1(0.3)^{bc}	1.2(0.4)^{bc}	0.84(0.2)^{c}	5.64**
Shoot fresh weight (g)	1.84(0.5)^{a}	0.78(0.5)^{ab}	0.66(0.4)^{bc}	0.79(0.4)^{ab}	0.31(0.1)^{c}	4.28**
Root fresh weight (g)	1.21(0.5)^{a}	0.58(0.3)^{b}	0.44(0.1)^{b}	0.54(0.2)^{b}	0.29(0.1)^{b}	11.14**
Root dry weight (g)	0.32(0.1)^{a}	0.21(0.1)^{ab}	0.15(0.1)^{bc}	0.26(0.1)^{ab}	0.07(0.04)^{c}	4.98**
Shoot dry weight (g)	0.51(0.03)^{a}	0.16(0.05)^{b}	0.23(0.03)^{b}	0.16(0.06)^{b}	0.07(0.03)^{c}	13.16**
No. of leaves	9.1(1.2)^{a}	6.2(1.9)^{b}	5.1(1.3)^{bc}	5.6(1)^{b}	3.8(0.8)^{c}	19.83**
Leaf area (cm^2)	6.06(2)^{a}	5.11(1.1)^{ab}	3.8(1.4)^{b}	4.3(1)^{b}	3.8(0.7)^{b}	3.90**
Specific leaf area (cm^2/g)	117.7(36.4)^{b}	127.8(53.7)^{b}	148.4(58.7)^{b}	128.2(35.2)^{b}	417.3(98.88)^{a}	35.45**
Seedling quality index	0.0061(0.003)^{a}	0.0026(0.001)^{bc}	0.0029(0.002)^{b}	0.0039(0.002)^{bc}	0.0011(0.0007)^{c}	5.33**

** $P < 0.01$; Means with different letters superscripts in a line indicate significant difference among seed treatment means according to Duncan's multiple range test ($P < 0.01$).

treatment, 13, 15.7 cm and 0.86 mm, respectively. Halopriming increased fresh weight shoot and root of seedling ($P < 0.01$) compared to unprimed seedling, and 100 mM treatment had the highest fresh weight shoot and root compared to other concentrations and non-primed treatments, 1.53 and 0.92 g, respectively. Similar trend was observed in weight of dry shoot and root in seedlings. Particularly, concentrate of 100 mM was better than other concentrates and unprimed treatment (Table 4). Haloprimig increased the number of leafs and leaf area for seedlings, significantly ($P < 0.01$).

However, the seedlings primed by 100 mM KNO_3 had highest number of leaf and leaf area for seedlings, compared to unprimed seedlings, 5.3 and 2.2 cm^2, respectively.

Although, unprimed treatment had the highest specific leaf area, the Seedlings primed by 100 mM concentration had highest seedling quality index among other concentrate and unprimed treatment (Table 4).

DISCUSSION

Germination characteristics of holoprimed seed in germinator

The required time for emergence of seedlings has an important role in survival and for competitiveness with other plants; and delays in seedling germination can affect total biomass and sapling growth, especially under competitive conditions (Ross and Harper, 1972). A dormant seed is one that is unable to germinate in a specified period of time under a combination of environmental factors that are normally suitable for the germination of the non-dormant seed (Baskin and Baskin, 1998). Dormancy is a mechanism to prevent germination during unsuitable ecological conditions, when the probability of seedling survival is low (Bewley and Black, 1994). Affordable methods that can improve seed germination and increase the chance of seedling success in establishment and dominance in regeneration are valuable. Priming treatments have much promise to improve seed germination and emergence of hard to regenerate species such as the Judas tree (Heydecker and Coolbear, 1977); these two traits, germination and rapid early growth, are important parameters that determine seedling competitiveness and ability to dominate in regeneration (Alizadeh and Jafari, 2006).

In this study, we hypothesized that enhancing germination and early growth of Judas trees can be achieved with the seed halopriming technique. We observed that Halopriming with KNO_3 caused increased seed germination percentage, speed, and energy, while decreasing mean germination time. In the laboratory study, the highest rate of germination occurred when seed was treated with a 750 mM KNO_3 solution, and the highest germination in the greenhouse was in the 100 mM KNO_3 treatment. These results are consistent with other studies (Afzal et al., 2009; Khan et al., 2009; Bhan and Sharma, 2011; Guo et al., 2012).

Increased germination percentage may be due to the effect of KNO_3 on biochemical changes involving hydrolysis and increased synthesis of enzyme activity that increases cell wall elasticity, thus promoting germination.

Another biochemical mechanism of seed priming is the increased activity of andobetabanaz enzymes that act to weaken cell walls, permitting rapid emergence of the rootlet (McDonald, 1999). Increased germination speed in seed treated by halopriming may be related to the production and of metabolites important to germination (Lee and Kim, 2000; Basra et al., 2005), or it may be associated with the synthesis of DNA, RNA and proteins during priming (Bray et al., 1989). For seed in the laboratory experiment, germination energy of the seed that was haloprimed was significantly larger than it was for seed treated with boiling water only. This may be due to the effect of boiling water on dormancy breaking of the seed coat and removing endosperm dormancy and decomposition of Frulic acid layer surrounding the endosperm (Christine et al., 1992). Halopriming effect on seed germination energy showed significant improvement in comparison with control in both experiments, however, the increase in seed germination energy was higher in the greenhouse study perhaps due to the amount of nitrogen in the soil pots compared to the filter paper medium in the laboratory study (Sivritepe et al., 2003). It may also be related to differences in nutritional elements such as NO_2^- in the soil, the uptake of nutrients, and the rate of seedling growth during the germination period (Wang et al., 2003).

In this study, we found that increases in the germination percentage, germination speed and germination energy of haloprimed seed can short the seed germination period, thus decreasing mean germination time. Decreased mean germination time in Judas tree seed may be also due to water absorption that promotes earlier germination. Similar results were reported by Afzal et al. (2009) who investigated the effect of halopriming treatments on seed germination of marigold. Also, others have reported that the mean of seed germination time was improved in halopriming treatments with KNO_3 (Afzal et al., 2009; Guo et al., 2012). Results of mean cumulative seed germination of Judas tree showed that using the halopriming technique quickened the initiation of germination and reduced the overall germination period compared to non-haloprimed seed. More rapid onset and completion of germination may be due to the priming effect on seed condition, Seed priming improves the performance of heterogeneous seeds and uniformity of germination (Olouch and Welbaum, 1996).

Most previous research done on breaking dormancy in Judas tree seed had emphasized treatments of chemical scarification with sulfuric acid for 20 to 30 min and cold stratification for a period of 90 to 120 days (Piotto et al., 2004; Pipinis et al., 2011; Zenkirkiran et al., 2010).

We found that seed dormancy could be broken using the halopriming technique with potassium nitrate salt so that in a 30 days period in laboratory conditions or 45 days in the greenhouse germination was complete, thus shortening the time required compared to the other chemical and cold stratification methods.

Using a solution of 750 mM KNO_3 to haloprime Judas tree seed in the laboratory increased average germination by 54%, germination speed by 1.35 and germination energy by 32.6% over germination performance in the unprimed.

Seed germination and seedlings characteristics in greenhouse

In the greenhouse study, use of the halopriming technique with a solution of 100 mM KNO_3 increased the average germination percentage by 57%, germination speed by 1.56, germination energy by 44.8%, and decreased the average time of germination by nearly 14 days compared to seed in the control treatment (without halopriming). Our results indicated that by halopriming seed of the Judas tree that germination increased significantly, and also, the length of the main and secondary roots were increased, which may increase root fresh and dry biomass of emerging seedlings. Halopriming also reduced the mean duration of germination by 6.1 days. Halopriming with KNO_3 may affect root nutrition and enhance growth. Increases in leaf number and area in seedlings from haloprimed seed can improve the absorbtion of nitrogen compared to seedlings from unprimed seed (Rouhi et al., 2012).

The overall effect of potassium nitrate is to stimulate the growth and development of roots by improved nutrition and higher absorption capacity, which enhances the growth and establishment of Judas tree seedlings. Hadinezhad et al. (2013) studied the effect of potassium nitrate on seedling emergence and improvement in growth of *Quercus castaneifolia*. They observed properties of KNO_3 that could improve germination and seedling growth when seeds were haloprimed compared with no-primed seed.

Other studies have reported that halopriming with boiling water and KNO_3 increased the speed of germination and growth (Riggio-Bevilacqua et al., 1985; Rascio et al., 1998; Smiris et al., 2006; Afanasiev, 1944; Frett and Dirr, 1979; Liu et al., 1981; Zencirkiran, 2010; Gebre and Karam, 2004). The authors found positive effects of stratification with boiling water and halopriming with KNO_3 on Judas tree seed germination and seedling development. Finally, it can be concluded that halopriming with KNO_3 salt solution can improve and invigorate the germination of Judas tree seed, thus increasing growth potential of seedlings and improving seedling competitiveness in a shorter time compared with non-haloprimed seed.

Conflict of Interest

The authors have not declared any conflict of interests.

REFERENCES

Afanasiev M (1944). A study of dormancy and germination of seeds of *Cercis canadensis*. J. Agric. Res. 69(10):405-420.

Afzal I, Ashraf S, Qasim M, Basra SMA, Shahid M (2009). Does halopriming improve germination and seedling vigour in marigold (*Tagetes* spp.). Seed Sci. Technol. 37(2):436-445.

Alevarado AD, Bradford KJ (1988). Priming and storage of tomato (*Lycopersicon esculentum*) seeds, effect of storage temperature on germination rate and viability. Seed Sci. Technol. 16:601-612.

Arias D, Calvo-Alvarado J, Dohrenbusch A (2007). Calibration of LAI-2000 to estimate leaf area index (LAI) and assessment of its relationship with stand productivity in six native and introduced tree species in Costa Rica. For. Ecol. Manage. 247(1-3):185-193.

Baskin CC, Baskin JM (1998). Seeds: ecology, biogeography, and evolution of dormancy and germination. Academic press. San Diego, P. 666.

Basra SMA, Afzal I, Anwar S, Shafique M, Haq A, Majeed K (2005). Effect of different seed invigoration techniques on wheat (*Triticuma estivum* L.) seeds sown under saline and non-saline conditions. J. Seed Technol. 28:36–45.

Bergmark CL, Jackson WA, Volk RJ, Blum U (1992). Differential Inhibition by Ferulic Acid of Nitrate and Ammonium Uptake in *Zea mays* L. Plant Physiol. 98(2):639–645.

Bewley JD, Black M (1994). Seeds. Physiology of Development and Germination. Second Edition. New York, Plenum Press P. 445.

Bhan S, Sharma NC (2011). Effect of seed stratification and chemical treatments an seed germination and subsequent seedling growth of wild apricot *(Prunus armeniaca* L.). Res. J. Agric. Sci. Technol. 2.1:13-16.

Boratynski A, Browicz K, Zielinski J (1992). Chorology of Trees and Shrubs in Greece. Polish Academy of Sciences. Institute of Dendrology, Sorus, Poznan-Kornik. P. 286.

Bradford KJ (1986). Manipulation of seed water relations via osmotic priming to improve germination under stress conditions. Hortic. Sci. 21:1105–1112.

Bray CM, Davison PA, Ashraf M, Taylor RM (1989). Biochemical changes during osmopriming of leek seeds. Ann. Bot-London 36:185–193.

Demir I, Mavi K (2004). The effect of priming on seedling emergence of differentially matured watermelon seeds. Scient. Hortic. 102(4):467-473.

Dirr MA, Heuser Jr CW (1987). The reference manual of woody plant propagation: From seed to tissue culture. Varsity Press, P. 239.

Duncan DB (1955). Multiple range and multiple F tests. Biometrics 11(1):1-42.

Frett JL, Dirr MA (1979). Scarification and stratification requirements for seed of *Cercis canadensis* L. (redbud), *Cladrastis lutea* (Michx. F) C. Koch (yellowwood) and *Gymnocladus dioicus* (L.) C. Koch (Kentucky Coffee Tree). Plant Propag. 25(2):4-6.

Gebre GH, Karam NS (2004). Germination of *Cercis siliquastrum* seeds in response to gibberellic acid and stratification. Seed Sci. Technol. 32(1):255-260.

Geneve RL (1991). Seed dormancy in eastern redbud *(Cercis canadensis*). J. A. Soc. Hortic. Sci. 116(1):85-88.

Guo S, Wang Y, Wang W (2012). Effects of priming treatments on germination and biochemical characteristics of *Pinus bungeana* seeds. For. Stud. China 14(3):200–204.

Hadinezhad P, Payamenur V, Mohamadi J, Ghaderifar F (2013). The effect of priming on seed germination and seedling growth in *Quercus castaneifolia*. Seed Sci. Technol. 41(1):121-124.

Hamilton DF, Carpenter PL (1976). Regulation of seed dormancy in *Elaeagnus angustifolia* by endogenous growth substances. Can. J. Bot. 54(10):1068-1073.

Heydecker W, Coolbear P (1977). Seed treatments for improved performance-survey and attempted prognosis. Seed Sci. Technol. 5(35):3-425.

ISTA (1985). International Rules for Seed Testing Rules. Seed Sci. Technol. 13:299-255.

Jazirei MH (2001). Aforestation in dryland. Tehran University Publication 447 p.

Jones RO, Geneve RL (1995). Seedcoat structure related to germination in eastern redbud (*Cercis canadensis* L.). J. A. Soc. Hortic. Sci. 120(1):123-127.

Kouhgardi E, Akbarzadeh M, Shahrokhi S (2012). How plantations can affect sustainable forest management in Iran. Int. Proc. Chem. Biol. Environ. Eng. 41:4-8.

Lee SS, Kim SONG (2000). Total sugars, α-amylase activity, and germination after priming of normal and aged rice seeds. Korean J. Crop Sci. 45:108–111.

Liu NY, Khatamian H, Freta TA (1981). Seed coat structure of three woody legume species after chemical and physical treatments to increase seed germination. J. A. Soc. Hortic. Sci. 106 (5):691- 694.

McDonald MB (1999). Seed deterioration: physiology, repair and assessment. Seed Sci. Technol. 27(1):177-237.

McKay HM, Jinks RL, McEvoy C (1999). The effect of desiccation and rough-handling on the survival and early growth of ash, beech, birch and oak seedlings. Ann. For. Sci. 56:391–402.

Mohammadi GR (2009). The influence of NaCl priming on seed germination and seedling growth of canola (*Brassica napus* L.) under salinity conditions. Am. Eur. J. Agric. Environ. Sci. 5(5):696-700.

Olouch MO, Welbaum GE (1996). Effect of post-harvest washing and post-storage priming on viability and vigor of 6-year old muskmelon (*Cucumis melo* L.) seeds from eight stages of development. Seed Sci. Technol. 24:195–209.

Parera CA, Cantliffe DJ (2010). Presowing seed priming. Hortic. Rev. 16:109-141.

Piotto D, Viquez E, Montagnini F, Kanninen M (2004). Pure and mixed forest plantations with native species of the dry tropics of Costa Rica: a comparison of growth and productivity. For. Ecol. Manage. 190:359-372.

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Full Length Research Paper

Production and utilization of *Musca domestica* maggots in the diet of *Oreochromis niloticus* (Linnaeus, 1758) fingerlings

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Musca domestica **maggots were produced from poultry dung for five weeks, and its meal utilized as a replacement for fishmeal in the diet of the fingerlings of Nile tilapia, *Oreochromis niloticus*. The maggots were cultured in four different enclosures: aluminium mobile maggotry, aluminium, plastic and wooden boxes. They were harvested at the end of the culture period, processed by oven-drying, and grinding into powdery form as maggot meal. The produced maggot meal was used to replace fishmeal in eight compounded isonitrogenous diets at levels of 0% (Control diet), 20, 30, 40, 50, 60, 70 and 80%. The diets were fed to *O. niloticus* fingerlings to determine the effects of maggot meal in comparison with fishmeal on the growth, nutrient utilization and survival of the fingerlings. The feeding experiment was carried out in 40 L plastic tank in triplicates, with 20 Nile tilapia fingerlings per tank. The fingerlings were fed 5% of their body weight on a daily ration for 10 weeks. Aluminium culture box was best for maggot production with the highest weight, and the most cost-effective. Highest mean weight gain, relative growth rate and specific growth rate was in fingerlings fed 50% maggot meal diet, and lowest in the control diet. Food conversion ratio was lowest in fish fed 60% maggot meal diet, and highest in fish fed the control diet. The protein efficiency ratio was highest in fish fed 60% maggot meal diet, and lowest in fish fed the control diet. Survival was higher, 100% in fish fed maggot meal-based diets, and lower, 95% in fish fed the control diet. These results indicate that replacement of fishmeal with maggot meal at 50 to 60% inclusion level is suitable for optimal growth performance, nutrient utilization and survival in *O. niloticus* fingerlings.**

Key words: Maggot meal, feeding trial, cost-effectiveness, nutrient utilization, growth rate.

INTRODUCTION

Aquaculture has the same target as agriculture, namely, to increase the production of food above the level that would be produced naturally. This brings about competition in the use of fish as food for direct human consumption and in animal husbandry for feed production. Shortages of major feedstuff has been on the increase in recent times in Nigeria (FDF, 2008), and with poultry and farm animals industry expanding at the rate of 10% annually, the aquaculture industry is finding it more difficult to source for critical feed ingredients (Gao and

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Lee, 2012; Nzeka, 2013).

The future growth of the aquaculture industry depends upon the availability of suitable and economical feeds. The cost of feeding is a major factor affecting the development of aquaculture in Nigeria (FAO, 2007). Fagbenro et al. (2003) reported that the use of commercial pellets and supplementary fish feeds accounts for about 60 and 40% respectively of the cost of fish farming venture in Nigeria, because fishmeal, a major protein source for many commercial feeds is expensive and scarce. In 2015, the cost of imported fishmeal in Nigeria ranged from US$2800–3050 per tonne, while the cost of locally produced fishmeal was US$1270–1550 per tonne. At present, around 10% of global fish production goes to fishmeal (either whole fish or fish remains resulting from processing) and is used mainly in aquaculture (FAO, 2012). Recent high demand and consequent high prices for fishmeal, together with increasing production pressure on aquaculture, has led to the research on non-conventional animal proteins for aquaculture and livestock (which could eventually supplement or replace fishmeal).Meanwhile, aquaculture is growing and fishmeal production is declining rapidly as a source of feed because of decreased supplies of caught fish (FAO, 2012). The search for alternative and sustainable proteins is an issue of major importance that needs viable solutions in the short term, making insects an increasingly attractive feed option.

Musca domestica maggots have potentially supplemented fishmeal in the diet of fish including *Oreochromis niloticus* and mud catfish fingerlings (Ugwumba and Abumoye, 1998; Ugwumba et al., 2001; Ajani et al., 2004; Sogbesanet al., 2005; Ogunji et al., 2006, 2008). Culture of maggots is used in converting wastes (of low economic value) e.g. animal dung into valuable animal protein (Calvert, 1976; Ugwumba et al., 2001; Omoyinmi et al., 2005). The major problem with maggot production is harvesting/collection of the maggots from dung, hence, the need for Aluminium mobile maggotry which makes harvesting/collection easier. This study was carried out to investigate the suitability of different culture enclosure for the production of maggots and to determine the effect of replacement of fishmeal with maggot meal in the diet of *O. niloticus*, which is popularly cultured in Nigeria.

MATERIALS AND METHODS

Production of maggots

Maggots were produced from poultry dung (which was collected from Oluwalonse Farm, Lanniba Village, Ajibode, Ibadan, Oyo State from March to May, 2013) using four culture enclosures namely: Aluminium Mobile Maggotry, aluminium, wooden and plastic boxes.

Aluminiummobile maggotry

The mobile maggotry (1.2×0.8×0.5 m) was made with aluminum sheets and consisted of three chambers: the top, middle and bottom chambers. The top chamber of the maggotry was the culture chamber and it had a lid at the top and at the front for easy introduction of the culture substrate. The lids were left open for access to *M. domestica* adult for egg-laying on the exposed dung. The base of the culture chamber was screened with 3mm wire mesh net to allow dropping of the maggots. Mosquito netting of 1.8mm mesh size was placed on the wire mesh to overcome the problem of dung dropping with the maggots. The middle chamber was the cleaning chamber for maggots emerging from the culture chamber. The base of this chamber was also screened with 3mm wire mesh for cleaning of maggots from the remains of the culture substrate. The bottom chamber was the collection chamber where the maggots were collected.

Aluminium, plastic and wooden culture boxes

The Aluminium boxes (0.2×0.33×0.5m), plastic boxes (0.5×0.35×0.22m), and the wooden boxes (0.6×0.5×0.3m) were perforated at the sides to allow adequate aeration and the perforations were screened on the inside with mosquito mesh net (1.8mm mesh size) to prevent escape of maggots. The upper part of each box was open while the bottom which was also perforated to allow dropping of maggots and was placed on an aluminium tray to serve as collection platform for the dropping maggots. The inner sides of the collection trays were painted black as maggots are known to be negatively photo-tactic (Ugwumba et al., 2001). All the culture boxes were placed on a constructed long wooden table.

Experimental set-up for maggot production

The culture enclosures were set-up in quadruplicates for the experiment. A shed was constructed over the enclosures to avoid direct effect of rainfall and sunlight. Freshly collected dung was tied in air tight sacks for two days in order to kill any maggots before loading into the culture enclosures. Twenty kilogrammes of dung was placed in the culture chamber of each maggotry, wooden, plastic and aluminium boxes and exposed to flies for egg-laying. The dungs were moistened with half a litre of water every day to prevent them from drying up. The set-up was checked every day for collection of maggots. The dung was collected weekly for a period of five weeks, from March to May 2013. The production was done in the Animal House of the Department of Zoology, University of Ibadan, Ibadan, Nigeria.

Harvesting and processing of maggots

Maggots were harvested daily once they emerge from the dung. Harvesting of maggots from the mobile maggotry was done by collecting the maggots from the outlet of the maggotry, while in the boxes, it was done by lifting each box and collecting the maggots from the collection tray that was placed beneath each box. Harvested maggots were rinsed with water to remove any dung on them and then weighed. The maggots were then blanched with hot water and re-weighed. The maggots were oven-dried at 60°C for 24 h, cooled, processed into powdery form as maggot meal using pestle and mortar. The maggot meal was packed in an air-tight plastic container and stored at 4°C till when needed.

Feeding trial with *O. niloticus*

Experimental diet

Eight dry isonitrogenous feeds were formulated based on the

Table 1. Percentage composition (% dry weight) of experimental diets.

Ingredients	Experimental diets							
	Diet A	Diet B	Diet C	Diet D	Diet E	Diet F	Diet G	Diet H
Fishmeal (g)	28.71	25.20	22.86	20.53	17.97	15.14	11.99	8.47
Maggot meal (g)	-	6.30	9.79	13.69	17.97	22.71	27.98	33.87
Corn meal (g)	61.29	58.50	57.34	55.78	54.06	52.16	50.03	47.66
*Vitamin & Mineral Premix (g)	0.05	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Soybean oil (g)	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Cassava starch binder (g)	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0
Total (g)	100	100	100	100	100	100	100	100
Calculated crude protein (%)	30	30	30	30	30	30	30	30
Inclusion levels of maggot meal (%)	0	20	30	40	50	60	70	80

*Vitamins and Minerals Premix: 2.5kg of premix contained: Vitamin A- 12,500,000.00 I.U, Vitamin D_3- 2,500,000.00 I.U, Vitamin E – 40,000.00 mg, Vitamin K3 - 2,000.00 mg, Vitamin B1 - 3,000.00 mg, Vitamin B2 - 5,500.00 mg, Niacin - 55,000.00 mg, Calcium pantothenate - 11,500.00 mg, Vitamin B6 - 5,000.00 mg, Vitamin B12 - 25.00 mg, Folic acid - 1,000 mg, Choline chloride - 500,000.00 mg, Biotin - 80.0 mg, Manganese - 120,000.00 mg, Iron - 100,000.00 mg, Zinc - 80,000.00 mg, Copper- 8,500.00 mg, Iodine - 1,500.00 mg, Cobalt - 300.00 mg, Selenium - 120.00 mg, Anti-oxidant - 120,000.00 mg.

protein content of the major feed ingredients namely fishmeal, maggot meal and corn meal using the Pearson Square Method described by Pearson (1976). Fishmeal was replaced in the diets with increasing levels of inclusion of maggot meal at 0% (Diet A, Control Diet), 20% (Diet B), 30% (Diet C), 40% (Diet D), 50% (Diet E), 60% (Diet F), 70% (Diet G), 80% (Diet H). Table 1 shows the percentage composition of experimental diets.

Fishmeal, corn meal, mineral and vitamin premix were purchased from Kesmac Feeds, Orogun, Ibadan, Nigeria; while starch (binder) and soybean oil were purchased from Bodija Market, Ibadan, Nigeria. Appropriate quantities of ingredients in each diet were weighed and mixed thoroughly in a bowl before adding gelatinized starch. The feeds were sun-dried for five days, after which, they were packed in air-tight plastic bags and stored at 4°C in the laboratory. Proximate analyses of maggot meal, fishmeal, corn meal and the compounded diets were carried out using the methods of AOAC (2012).

Experimental procedure

Fingerlings of *Oreochromis niloticus* with mean weight of 0.95±0.03g, and mean length of 3.63±0.160cm were acclimatized in the laboratory for one week, during which they were fed with fishmeal. They were then starved for 24 h before the commencement of the experiment, after which, the standard length and weight of each fingerling was measured and randomly assigned to plastic culture tanks at a stocking density of 20 fingerlings per tank, giving a total of 60 fingerlings per experimental diet. Total and average lengths and weights of fish for each tank were calculated and recorded as initial lengths and weights. The length and weight of five randomly selected fingerlings from each tank were measured weekly to access growth rate and to calculate feed rations from estimated total fingerling weight in each tank. The fish were fed diet corresponding to 5% of their body weight daily for ten weeks, from June to September 2013; half of the ration was fed at 09.00 h and the other half at 18.00 h. Feeding was done manually at a particular point in each tank and visual observations of the fingerlings were made during this process. The tanks were monitored daily for mortality; dead fish were removed, and counted. At the end of the experiment, final lengths and weights of all *O. niloticus* fingerlings left in each tank were measured, and total and average final lengths and weights were calculated.

Economic evaluations of diets formulated

The economic evaluations of the diets formulated were calculated using the formulae reported in Sogbesan et al. (2006).

Estimated investment cost analysis= Cost of feed ($) + Cost of fingerlings stocked ($)

$$\text{Profit Index} = \frac{\text{Value of fish (\$)}}{\text{Cost of feed (\$)}}$$

$$\text{Incident of cost} = \frac{\text{Cost of feed (\$)}}{\text{Mean weight gain of fish produced (g)}}$$

Net profit = Total cost of fish cropped ($) - Total Expenditure ($)

$$\text{Cost: Benefit ratio (C: Br)} = \frac{\text{Total cost of fish cropped (\$)}}{\text{Total Expenditure (\$)}}$$

Monitoring of water quality

Temperature was taken daily between 07.00 to 08.00h with Mercury-in-glass thermometer. Dissolved oxygen was determined using the Winkler's Method described by Boyd (1990) and pH with a pH meter (JENWAY 3510) weekly. During the experimental period, water temperature ranged from 26.1 to 26.5°C, dissolved oxygen 5.0 to 5.9 mg/L and pH 8.01 to 8.10.

Evaluation of growth and nutrient utilization of fish

The following growth, nutrient utilization and survival of fish were computed for fingerlings on each diet using the following formulae reported in Monebi and Ugwumba (2013).

Mean weight gain = FMW– IMW

Where FMW = final mean weight (g/fish), IMW = initial mean weight (g/fish)

$$\text{Relative growth rate (RGR) \%} = \frac{W_f - W_i}{W_i} \times 100$$

Where W_i = initial weight of fish (g), W_f = final weight of fish (g).

$$\text{Specific Growth Rate (SGR) \%} = \frac{\log_e W_f - \log_e W_i}{t} \times 100$$

Where Log_e = the natural logarithm, t = duration of experiment in days.

$$\text{Food conversion ratio (FCR) \%} = \frac{\text{Total food supplied to fish (g)}}{\text{Total weight gain by fish (g)}}$$

Protein Intake = Food supplied (g) × % crude protein of feed.

$$\text{Protein efficiency ratio (PER) \%} = \frac{\text{Mean weight gain by fish (g)}}{\text{Mean protein intake (g)}}$$

$$\text{Protein productive value (PPV) \%} = \frac{B - B_0}{PI} \times 100$$

Where B= Final body protein (at the end of experiment), B_0 = Initial body protein (at the beginning of experiment), and PI= Protein Intake.

$$\text{Survival (S)} = \frac{N_f}{N_i} \times 100$$

Where N_i = Number of fish at the beginning of the experiment, and N_f = Number of cultured fish at the end of the experiment.

Economic evaluation of maggot production

Economic evaluation of maggot production was calculated using the formula reported in Sogbesan et al. (2006) as:

$$\text{Cost of production} = \frac{\text{Cost of constructing culture enclosure (\$)}}{\text{Quantity of maggot produced (g)}}$$

Statistical analysis

All data collected were subjected to descriptive statistics, student's t-test and one-way analysis of variance (ANOVA).

RESULTS

Production of maggots

There was a gradual increase in the weight of maggots followed by a drop in the third week after which production is increased in the third and fifth weeks for maggots cultured in aluminium and wooden boxes. Production from plastic box decrease steadily from the first to the fourth week, followed by an increase in the fifth week.

Production from the aluminium mobile maggotry was relatively the same throughout the culture period. (Figure 1).

Maggots produced fom aluminium box had the highest total quantity of 1.603 kg maggot/5 weeks while the lowest value, 0.230 kg maggot/5 week was recorded in aluminium mobile maggotry (Table 2). There was significant difference ($p < 0.05$) between the mean weight of maggots produced in all the four culture enclosures. Also, aluminium box was found to be the most cost-effective, 0.002$/kg maggot, while the least cost-effective, 0.081$/kg was recorded in aluminium mobile maggotry (Table 2). There was significant difference ($p < 0.05$) in the cost-effectiveness of the four culture enclosures. The result of the production from proximate composition of maggots produced showed that crude protein content was 45.5%, crude lipid, 9.0%; crude fibre, 3.0% and ash content, 12.0%.

Feeding trial with *O. niloticus*

The crude protein content of the eight experimental diets ranged from 37.60 to 37.70% and were not significantly different ($p>0.05$) (Table 3). The weekly changes in weight of the fingerlings fed the different diets are illustrated in Figure 2. There was progressive increase in the weight gain of fish fed all the experimental diets. Results on the growth and nutrient utilization of fingerlings are shown in Table 4. The mean weight gain, relative and specific growth rates were highest, 9.29 g/fish, 967.7 and 1.47%) in fish fed 50% maggot meal diet and lowest, 4.05g/fish, 430.85% and 1.04% respectively in fish fed control diet. Differences in growth rates between the diets were significantly different ($p<0.05$).

The food conversion ratio was highest (3.01) in fish fed the control diet while the lowest (2.37) was in fish fed 60% maggot meal diet and the differences were significant ($p<0.05$). The highest protein intake (8.38g) was obtained in fish fed 50% maggot meal diet while the lowest value (4.59g) was in fish fed the control diet and the differences were significant ($p<0.05$). Protein efficiency ratio was highest (1.12) in fish fed 60% maggot meal diet and lowest (0.88)in fish fed the control diet and the differences were significant ($p<0.05$). There was no mortality, that is, 100% survival in the maggot-based diets unlike the control, where survival was 95%. The difference was significant ($p<0.05$).

The carcass composition before and after the experiment is shown in Table 5. There was significant difference between the crude protein, lipid, fibre and ash

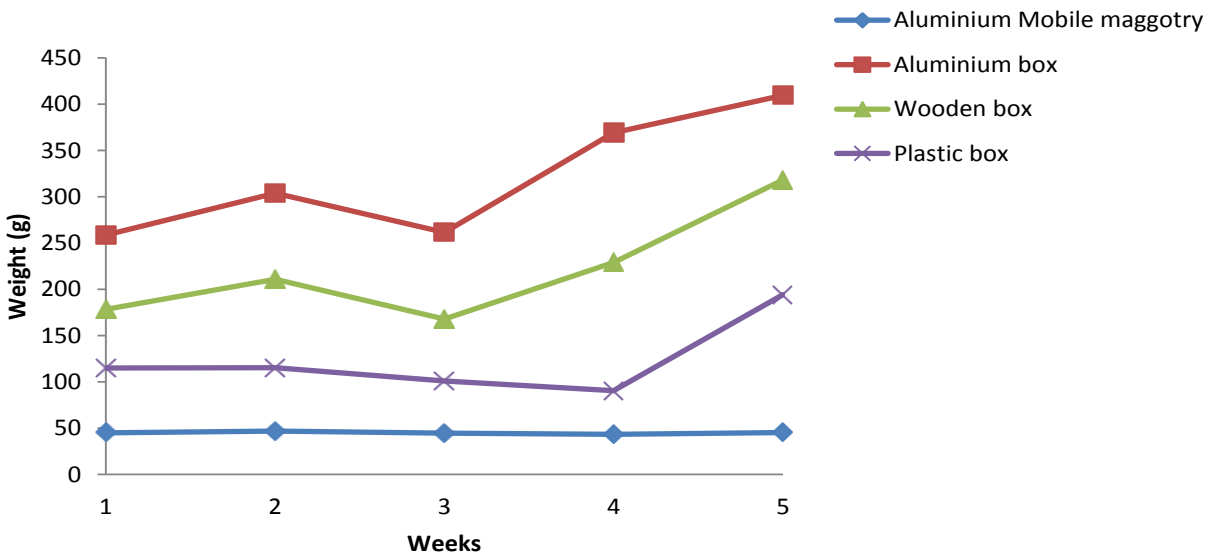

Figure 1. Weekly production of *M. domestica* maggot in different enclosures.

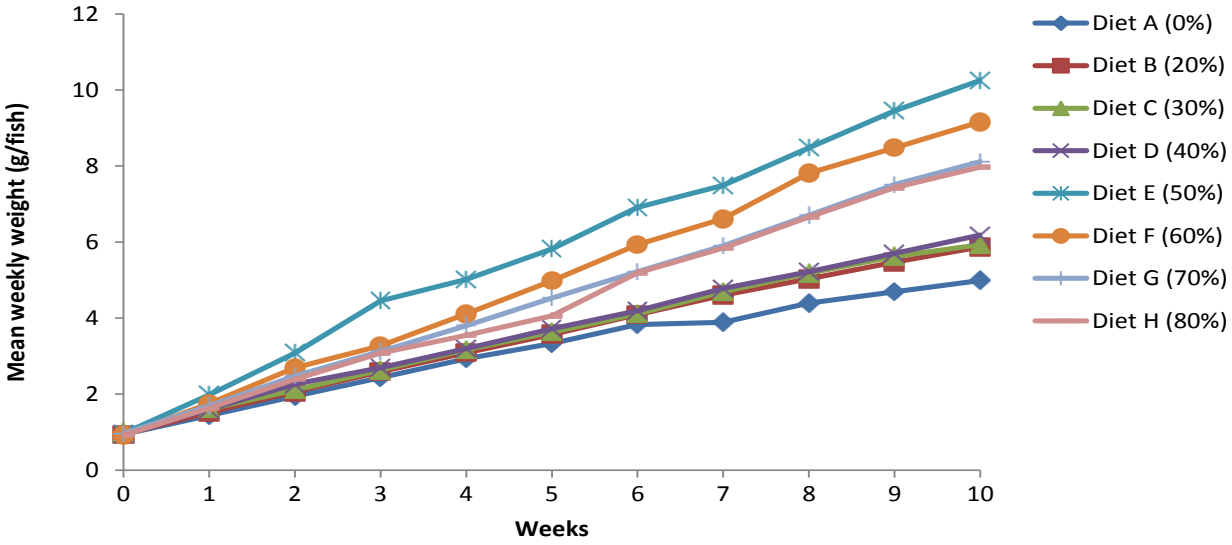

Figure 2. Weekly weight changes of *O. niloticus* fingerlings fed the various experimental diets.

contents of the initial and final carcass composition of *O. niloticus.* At the end of the experiment, the highest percentage crude protein (76.75%) was obtained in fish fed 50% maggot meal diet, while the lowest (69.30%) was in fish fed the control diet and the differences were significant ($p < 0.05$).

Economic evaluations of experimental diets

Results on the economic evaluation of experimental diets are shown in Table 6. At the end of the experiment, the best net profit of $12.97 and cost: benefit ratio of 2.90 was obtained in fish fed 50% maggot meal diet while the lowest net profit value of $1.89 and cost: benefit ratio of 1.48 was in fish fed the control diet.

DISCUSSION

The water parameters monitored during the study were within the suitable range for tropical fish indicating that the environmental conditions of the fish during the experimental period were adequate. Recommended temperature range for optimum growth of tilapias is 22 to 29°C (Sarig, 1969; Mires, 1995) while Magid and Babiker

Table 2. Cost effectiveness of maggot production from the different enclosures.

Enclosure	Cost of enclosure ($)	Quantity of maggot produced (kg maggot/ 5 week)	Incidence cost ($/kg maggot)
Aluminium mobile maggotry	18.67	0.230	0.081
Aluminium box	3.55	1.603	0.002
Plastic box	2.54	0.620	0.004
Wooden box	5.08	1.104	0.005

Table 3. Proximate composition of experimental diets (% dry weight).

Nutrients	Diet A (Control, 0%MM)	Diet B (20% MM)	Diet C (30% MM)	Diet D (40% MM)	Diet E (50% MM)	Diet F (60% MM)	Diet G (70% MM)	Diet H (80% MM)
Crude protein	37.70^{a}	37.68^{a}	37.66^{a}	37.64^{a}	37.65^{a}	37.63^{a}	37.62^{a}	37.60^{a}
Crude lipid	8.00^{b}	7.00^{a}	7.00^{a}	7.00^{a}	8.00^{b}	7.00^{a}	7.00^{a}	7.00^{a}
Crude fibre	4.00^{b}	3.00^{a}	4.00^{b}	4.00^{b}	4.00^{b}	3.00^{a}	4.00^{b}	3.00^{a}
Ash	5.00^{a}	12.00^{d}	12.00^{d}	8.00^{c}	12.00^{d}	14.00^{e}	8.00^{c}	7.00^{b}
Dry matter	90.25^{h}	90.16^{g}	89.46^{d}	89.12^{b}	89.38^{c}	88.74^{a}	89.62^{f}	89.55^{e}

MM = Maggot Meal. Values on the same row with different superscript are significantly different ($p < 0.05$).

Table 4. Growth and Nutrient Utilization of *O. niloticus* fed maggot meal diets for 10 weeks.

Parameters	Diet A (Control, 0% MM)	Diet B (20% MM)	Diet C (30% MM)	Diet D (40% MM))	Diet E (50% MM)	Diet F (60% MM)	Diet G (70% MM)	Diet H (80% MM)
Duration (Days)	70	70	70	70	70	70	70	70
No of fish stocked	60	60	60	60	60	60	60	60
No of fish left	57	60	60	60	60	60	60	60
IMW (g/fish)	0.94^{ab}	0.94^{ab}	1.0^{c}	0.94^{ab}	0.96^{b}	0.92^{a}	0.96^{b}	0.92^{a}
FMW (g/fish)	4.99^{a}	5.87^{b}	5.93^{c}	6.18^{d}	10.25^{h}	9.16^{g}	8.11^{f}	7.97^{e}
MWG (g/fish)	4.05^{a}	4.93^{b}	4.93^{b}	5.24^{c}	9.29^{g}	8.24^{f}	7.15^{e}	7.05^{d}
RGR (%)	430.85^{a}	524.47^{c}	493^{b}	557.45^{d}	967.7^{h}	895.65^{g}	744.79^{e}	766.30^{f}
SGR (%)	1.04^{a}	1.14^{c}	1.1^{b}	1.17^{c}	1.47^{f}	1.42^{e}	1.33^{d}	1.34^{d}
FCR	3.01^{f}	2.74^{d}	2.83^{e}	2.71^{d}	2.40^{ab}	2.37^{a}	2.47^{c}	2.42^{b}
PI	4.59^{a}	5.09^{b}	5.25^{bc}	5.38^{c}	8.38^{g}	7.35^{f}	6.64^{e}	6.41^{d}
PER	0.88^{a}	0.97^{b}	0.94^{ab}	0.97^{b}	1.11^{c}	1.12^{c}	1.08^{bc}	1.10^{c}
PPV	46^{a}	75^{b}	86^{c}	115^{e}	114^{e}	116^{e}	108^{d}	106^{d}
Survival (%)	95.0^{a}	100.0^{b}	100.0^{b}	100.0^{b}	100.0^{b}	100.0^{b}	100.0^{b}	100.0^{b}

Values with different superscripts on the same row are significantly different ($p < 0.05$). Key: MM = Maggot meal; IMW = Initial Mean Weight; FMW = Final Mean Weight; MWG = Mean Weight Gain; RGR = Relative Growth Rate; SGR = Specific Growth Rate; FCR = Food Conversion Ratio; PI = Protein Intake; PER = Protein Efficiency Ratio; PPV = Protein Productive Value.

(1975) and also Ross (2000) reported dissolved oxygen values >3 mg/L as the standard range for optimum growth of tilapias. Boyd and Lichtkoppler (1979) reported dissolved oxygen concentration of 5.0 mg/L and above as desirable for survival of fish. Ross (2000) recommended pH range of 7-9 as the optimum for the growth of tilapias.

Maggot production

The four enclosures investigated were suitable for the production of maggots. However, aluminium culture box was the best enclosure for the production of maggots and also the most cost-effective. The higher maggot

Table 5. Carcass composition of *O. niloticus* fingerlings fed experimental diets at the beginning and end of feeding trial (% dry weight).

Nutrients	Initial carcass composition	Experimental diets							
		Final carcass composition							
		Diet A (Control, 0% MM)	Diet B (20% MM)	Diet C (30% MM)	Diet D (40% MM)	Diet E (50% MM)	Diet F (60% MM)	Diet G (70% MM)	Diet H (80% MM)
Crude protein	67.20[a]	69.30[b]	71.00[c]	71.74[d]	73.35[e]	76.75[h]	75.70[g]	74.35[g]	74.00[f]
Crude lipid	7.00[a]	7.50[b]	8.00[d]	7.50[b]	7.50[b]	7.70[c]	7.50[b]	8.00[d]	7.50[b]
Crude fibre	2.00[b]	1.50[a]	1.50[a]	1.50[a]	1.50[a]	1.50[a]	1.50[a]	1.50[a]	1.50[a]
Ash	22.00[e]	21.00[d]	18.00[b]	19.00[c]	21.00[d]	18.00[b]	17.50[a]	19.00[c]	18.00[b]

MM= Maggot Meal. Values on the same row with different superscripts are significantly different ($p<0.05$).

Table 6. Economic analysis of experimental diets.

Diets	Parameter determined				
	Estimated investment cost analysis ($)	Profit index ($)	Incident of cost	Net profit ($)	Cost: Benefit ratio (C:Br)
Diet A (Control, 0% MM)	3.90	0.04	0.69	1.89	1.48
Diet B (20% MM)	4.45	0.02	0.93	2.25	1.51
Diet C (30% MM)	4.75	0.02	1.15	1.95	1.41
Diet D (40% MM)	5.09	0.02	1.28	4.04	1.79
Diet E (50% MM)	6.82	0.03	1.33	12.97	2.90
Diet F (60% MM)	6.86	0.02	1.52	11.42	2.67
Diet G (70% MM)	6.99	0.02	1.81	9.76	2.40
Diet H (80% MM)	7.40	0.02	2.03	8.75	2.18

production in aluminium, plastic and wooden boxes compared to aluminium mobile maggotry in this present study could be due to the use of collection trays introduced for harvesting of maggots from the culture boxes, thereby making harvesting easier.

The crude protein of maggots recorded in this study (45.5%) is similar with that reported by Gado et al. (1982) (45.0%), Atteh and Olegbenla (1993) (45.0%), close to those reported by Ugwumba et al. (2001) (41.3%)and Okah and Onwujiariri (2012) (44.44%) but at variance with those of Calvert et al. (1971) (63%), Hwangbo et al. (2009) (63.99%), Awoniyi et al. (2003) (55.1%), Sogbesan et al. (2006) (55.4%). Also, the crude fibre recorded in this study (3.0%) is also slightly similar with that reported by Omoyinmiand Olaoye (2012) (2.41%) but clearly at variance with those of Ugwumba et al. (2001) (9.5%), Sogbesan et al. (2006) (1.56%), and Aniebo et al. (2009) (7.5%). Variations in nutritional components of maggot meal have been reported to be mostly due to differences in age at harvest (Inoaka et al., 1999; Newton et al., 2004; Aniebo et al., 2008; Aniebo and Owen, 2010), method of drying (Fasakin et al., 2003; Aniebo and Owen, 2010) and larval feed substrate (Newton et al., 1977). The age of the maggots in the present study was not known. Poultry dung used for the production of the maggots in the present study has been reported to be the best substrate for maggot production (Omoyinmi et al., 2005; Anene et al., 2013).

Feeding trial with *O. niloticus*

Progressive weight gain of *O. niloticus* recorded in all the dietary treatments throughout the duration of the experiment is an indication that the fish responded positively to all the diets in terms of growth, and that the protein content of the experimental diet was likely adequate for growth of the fish. The results obtained from this present study show that fingerlings fed 50% maggot meal inclusion diet has the best growth performance, an indication that they were able to properly convert their food into body growth than those on all the other diets. This result is in accordance with that recorded by Omoruwou and Edema (2011), and Ajani et al. (2004), who recorded the highest weight gain in 50% maggot meal inclusion in the diet of 'Heteroclarias' and *O. niloticus* fingerlings respectively.

Food conversion ratio was lowest in fish fed 60%

maggot meal diet indicating that they were most efficient in converting their food to body growth.Protein efficiency ratio and protein productive value were highest in fish fed 60% maggot meal diet, an indication of good protein digestibility and bioavailability for optimum body protein increase and growth (Pellett, 1989). Survival was high for all diets during the experiment, and was significantly different ($p<0.05$) between fingerlings fed the control diet and all the other maggot meal based diets, 100% survival in the maggot-dependent diets, further indicates the suitability of maggot meal diets for *O. niloticus* fingerlings.

The proximate composition of the fish carcass from all the diets showed an increase in the values of crude protein at the end of the experiment. This may be attributed to the ability of *O. niloticus* to convert and utilize the crude protein in their diets for body growth.The economic evaluation of feeding *O. niloticus* fingerlings on experimental diets shows that 50% maggot meal diet recorded the highest net gain and cost: benefit ratio. The positive net gain and cost: benefit ratio recorded in all the diets indicate that *O. niloticus* can be economically reared on all the diets. However, the replacement of fishmeal with maggot meal from 40-80% in the diets of *O. niloticus* showed better cost: benefit ratio with optimum at 50% maggot meal inclusion. The cost of production and the benefits positively favoured all treatments since the values computed are > 1.0 which shows an increase in the fish value above the amount invested. This is not withstanding, more monetary profits awaits a farmer when 50% of maggot meal is used to replace fishmeal in the diet of *O. niloticus*. This agrees with the findings of Sogbesan et al. (2006) who reported that cost of production and benefits positively favoured (> 1.0) all treatments when maggot meal was partially substituted at 0, 25, 50, 75 and 100% in the diet of 'Heteroclarias' fingerlings.Aluminium culture box is the best and most cost-effective enclosure for the production of maggots. The present study showed that though maggot meal can completely replace fishmeal in the diet of *O. niloticus* fingerlings, replacement of fishmeal with maggot meal is best at 50% for optimum growth and 60% for optimum nutrient utilization. Therefore, replacement of fishmeal with maggot meal at 50 to 60% inclusion level is recommended.

Conflict of Interest

The authors have not declared any conflict of interest.

REFERENCES

Ajani EK, Nwanna LC, Musa BO (2004). Replacement of fishmeal with maggot meal in the diets of Nile Tilapia, *Oreochromis niloticus*. World Aquaculture- Baton Rouge 35(1):52-55.

Anene A, Afam-Anene OC, Ike K, Ekekwe NH (2013). Preliminary investigations on quantity of maggots produced from four different sources of livestock wastes. J. Res. Biol. 3(6):1060-1065.

Aniebo AO, Erondu ES, Owen OJ (2008). Proximate composition of housefly larvae (*Musca domestica*) meal generated from mixture of cattle blood and wheat bran. Livest. Res. Rural Dev. 20(12):1-5.

Aniebo AO, Erondu ES, Owen OJ (2009).Replacement of fish meal with maggot meal in African catfish (*Clarias gariepinus*) diets. Revista UDO Agricola 9(3):666-671.

Aniebo AO, Owen OJ (2010). Effects of age and method of drying on the proximate composition of housefly larvae(*Musca domestica,* Linnaeus) meal (HFLM). Pak. J. Nutr. 9(5):485-487.

AOAC (Association of Official Analytical Chemists) International (2012). Official Methods of Analysis of AOAC International, 19th Edition. Gaithersburg, Maryland, USA, AOAC International. P. 1230.

Atteh JO, Olegbenla FD (1993). Replacement of fishmeal with maggots in broiler diet: Effects on performance and nutrient retention. Nig. J. Anim. Prod. 20:44-49.

Awoniyi TAM, Aletor VA, Aina JM (2003). Performance of broiler chickens fed on maggot meal in place of fishmeal. Int. J. Poult. Sci. 2:271-274.

Boyd CE (1990). Water quality in ponds for Aquaculture. Alabama Agriculture Experiment Station, Auburn University. Alabama. P. 462.

Boyd CE, Lichtkoppler F (1979). Water Quality Management in Fish Ponds. Research and Development Series No. 22 International Centre for Aquaculture (J.C.A.A) Experimental Station Auburn University, Alabama. pp. 45-47.

Calvert CC (1976). Systems for indirect recycling by using animal and municipal wastes as a substrate for protein production. In: New feed resources proceedings of technical consultation held in Rome, 22–24 November 1976. Part IV, Chapter 18.

Calvert CC, Martins RD, Eby HJ (1971). Biodegraded hen manure and adult houseflies: Their nutritional value to the growing chick. In: Livestock Waste Management and Pollution Abatement. Proc. Internat, Symp. Livestock waste, Columbus, Ohio. pp. 319–320.

Fagbenro OA, Adeparusi EO, Fapohunda OO (2003). Feedstuff and dietary substitution for farmed fish in Nigeria. In: Proceedings of the Joint Fisheries Society of Nigeria/National Institute for Freshwater Fisheries Research/FAO- National Special Programme for Food Security National Workshop on Fish feed development and Feeding Practices in Aquaculture (Eyo AA Ed). National Institute for Freshwater Fisheries Research, New- Bussa. pp. 60– 72.

FAO (2007). State of World Fisheries and Aquaculture. Rome. P. 240.

FAO **(**2012).State of the world fisheries. Rome. P. 209.

FDF (2008). Federal Department of Fisheries, Fisheries Statistics of Nigeria Projected human population; fish demand and supply in Nigeria, 2000 – 2015. P. 56.

Fasakin EA, BalogunAM, Ajayi OO (2003). Evaluation of full-fat and defatted maggot meals in the feeding of clariid catfish, *Clarias gariepinus* fingerlings. Aquaculture Res. 34(9):733-738.

Gado MS, El-Aggory SM, Elgawaad AA, Mahmoud AK (1982). The possibility of applying insect protein in broiler rations. Nutr. Abstracts Rev. 53:76.

Gao Y, Lee JY (2012). Compensatory responses of Nile tilapia *Oreochromis niloticus* under different feed deprivation regimes. Fish. Aquat. Sci. 15(4):305-311.

Hwangbo J, Hong EC, Jang A, Kang HK, Oh JS, Kim BW, Park BS (2009).Utilization of house fly-maggots, a feed supplement in the production of broilerchickens. J. Environ. Biol. 30(4):609–614.

Inoaka T, Okubo G, Yokota M, Takemasa M (1999). Nutritive value of house fly larvae and pupae fed on chicken faeces as food source for poultry. J. Poultry Sci. 3(3):174-180.

Magid A, Babiker MM (1975). Oxygen consumption and respiratory behaviour of three Nile fishes. Hydrobiologia 46:359–367.

Mires D (1995). The tilapias.In: Production of Aquatic Animals: Fishes (eds. Nash CE and Novotony AJ) Elsevier, New York. pp. 133–152.

Monebi CO, Ugwumba AAA (2013). Utilization of the Earthworm (*Eudrilus eugeniae*) in the diet of Heteroclarias Fingerlings. Int. J. Fish. Aquacult. 5(2):19-25.

Newton GL, Booram CV, Barker RW, Hale OM (1977).Dried *Hermetia illucens* larvae meal as a supplement for swine. J. Animal Sci. 44(3):395-400.

Newton L, Sheppard C, Watson D, Burtle G, Dove R (2004).Using the black soldier fly, Hermetia illucens, as a value-added tool for the management of swine manure. University of Georgia, College

of Agriculture & Environmental Science. Department of Animal & Dairy Science Annual Report. P. 17.
Nzeka UM (2013).Nigeria Food Processing Ingredients Market.A report from Global Agricultural Information Network (GAIN).United States Department of Agriculture (USDA) Foreign Agricultural Service. P. 34.
Ogunji JO, Kloas W, Wirth M, Schulz C, Rennert B (2006). Housefly maggot meal: An emerging substitute of fishmeal in Tilapia Diets, Conference on International Agricultural Research for Development. DeuscherTropentag, Stuggart-Hoheheim, Oct 11-13, 2006. pp. 1-7.
Ogunji JO, Toor RS, Schulz C, Kloas W (2008). Growth performance, nutrient utilization of Nile Tilapia, *Oreochromis niloticus* fed Housefly Maggot Meal (Magmeal) Diets. Turk. J. Fish. Aquatic Sci. 8:141-147.
Okah U, Onwujiariri EB (2012). Performance of finisher broiler chickens fed maggot meal as a replacement for fish meal. J. Agric. Technol. 8(2):471-477.
Omoruwou PE, Edema CU (2011). Growth response of *Heteroclarias* hybrid fingerlings fed on maggot based diet. Nig. J. Agric. Food Environ. 7(1):58-62.
Omoyinmi GAK, Olaoye OJ (2012). Growth Performance of Nile Tilapia-*Oreochromis niloticus* fed diets containing different sources of animal protein. Libyan Agric. Res. Center J. Int. 3(1):18-23.
Omoyinmi GAK, Fagade SO, Adebisi AA (2005).Assessment of different organic substrates in the laboratory culture of some live food organisms. Zoologist 3:105-111.
Pearson D (1976). The chemical analysis of foods. 7th Edition, Churchill Livingstone, London.
Pellett P (1989). Report of a joint FAO/WHO expert consultation on protein quality evaluation, Bethesda, M.D., U.S.A, 4-8 December, 1989. FAO and Food Nutrition Paper 51:7.
Ross LG (2000). Environmental physiology and energetics. In: Beveridge MCM, McAndrew BJ (Eds.) Tilapias: Biology and Exploitation, Kluwer Academic Publishers, Dordrecht, The Netherlands. Fish Fish. Series 25:89–128,
Sarig S (1969). Winter storage of Tilapia. FAO Fish Culture Bulletin 2:8–9.
Sogbesan OA, Ajuonu ND, Ugwumba AAA, Madu CT (2005). Cost benefits and growth performances of catfish hybrid fed maggot meal diets. J. Sci. Ind. Res. 3(2):51-56.
Sogbesan AO, Ajuonu N, Musa BO, Adewole AM (2006). Harvesting techniques and evaluation of maggot meal as animal protein source for 'Heterobranchus' in outdoor concrete tanks. World J. Agric. Sci. 2(4):394-402.
Ugwumba AAA, Abumoye OO (1998). Growth response of Clarias gariepinus fingerlings fed live maggot from poultry droppings. In: Sustainable Utilization of Aquatic/Wetland Resources. Selected papers from 9th Annual Conference of the Nigerian Association for Aquatic Sciences, Abeokuta, Nigeria, 30thNov.-2nd Dec. 1995, pp. 60-68.
Ugwumba AAA, Ugwumba AO, Okunola AO (2001). Utilization of live maggot as supplementary feed on the growth of *Clarias gariepinus* (Burchell) fingerlings. Niger. J. Sci. 35(1):1-7.

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Full Length Research Paper

Nitrogen levels effect on wheat nitrogen use efficiency and yield under field conditions

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A two year (2009-10 and 2010-11) field experiment was conducted on twelve wheat genotypes supplied with four levels of nitrogen (recommended dose of nitrogen; RDN, RDN-50%, RDN-25% and RDN+25%) to identify genotypes with maximum nitrogen use efficiency. Physiological parameters namely plant height, tiller number, spikelet number, grain yield, thousand grain weight and biomass increased with increases in nitrogen dose. During both years, yield and nitrogen use efficiency were higher in PBW 621 and PBW 636 while BW 9022 showed maximum thousand grain weight over other genotypes. Among all genotypes, HD 2967 was the tallest and tiller number and spikelet number were maximum in BW 9183.

Key words: Nitrogen, yield, nitrogen use efficiency, wheat, genotypes.

INTRODUCTION

Nitrogen (N) is an essential element for both crop development and biomass. The absorption of N by plants plays an important role in their growth. However, excessive use of N is economically costly (Ju et al., 2009) as well as environmentally damaging with excess N lost by leaching into groundwater and runoff into surface water (Galloway et al., 2008; Gruber and Galloway, 2008; Conley et al., 2009). Both to avoid pollution by nitrates and to maintain economic balance, there is need to identify genotypes that can efficiently use N. Efficient use of N by wheat is needed to sustain or increase yield and quality, while reducing the negative impacts of fertilizer on the environment (Hirel et al., 2007; Foulkes et al., 2009).

Assimilation of inorganic N into organic form has a marked influence on plant productivity and crop yield. Grain yield is the main target of crop production. Adequate N nutrition is required for full development of tillers and leaves. Rahman et al. (2011) reported that N application has a tremendous effect on tiller formation and survival of tillers. Application of N at later stages of maize (Amanullah et al., 2009) increased plant height, kernel number and high biomass at maturity that results in high yield (Amanullah et al. 2009; Hokmalipour et al., 2010). Nitrogen use efficiency (NUE) is defined as the grain yield per unit of available N in the soil (Moll et al., 1982). NUE varies with the growth stage of the plant (Woolfolk et al., 2002). NUE as reflected in grain yield of winter wheat has also been shown to change with time and rate of application (Woolfolk et al., 2002). Grain yield and N content of cereal crops increase significantly with applied N (Viller and Guillaumes, 2010).

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Efficient N utilization is crucial for economic wheat production and protection of ground and surface water (Vukovic et al., 2008). The genetic variation in both acquisition and internal-use efficiencies indicates that there is potential for increases in efficiency of nitrogen use through plant selection, particularly in low nitrogen environments (Giller et al., 2004). There is a need to increase NUE of cereal crops by selecting new hybrids or cultivars from the available ancient and modern germplasm collection. These objectives can be met through identification of varieties that have better NUE while at least maintaining or optimally increasing crop productivity. Field-based studies have shown differences in the NUE of barley genotypes by Abeledo et al. (2008) and Anbessa et al (2009). The study was conducted under field conditions over two years with the objective to assess the effect of different doses of N on wheat yield and yield related traits and to identify genotype that possesses high yield and NUE.

MATERIALS AND METHODS

Two year (2009-10 and 2010-11) experiment was conducted on the fields of Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana (30.91°N, 75.85°E and 252 m asl), India. to study the response of twelve wheat genotypes namely PBW 621, PBW 636, PBW 590, DBW 17, HD 2967, PBW 509, BW 9178, BW 9183, BW 8989, BW 9022, PBW 343 and PBW 550 to four doses of N fertilizer. The crop was sown in plots consisting of 4 rows of 1 m each in 22.5 cm spaced rows and with 40 cm between the plots. The experimental design was a split plot plots with three replicates. The crop was raised under normal planting time, that is, 28th October, 2009 and 29th October, 2010 in the field under 4 different N levels (in the form of urea) including the presently recommended N dose (RDN) (120 Kg N/ha), two suboptimal N doses [RDN-50% (60 Kg N/ha) and RDN-25% (90 Kg N/ha)] and supra-optimal N dose [RDN+25% (150 Kg N/ha)]. NPK and Diammonium Phosphate were applied as basal dose and urea was applied in different doses as described earlier. N dose was added in two split applications: First at the time of sowing and second at 1st irrigation (almost month after sowing). In the results, mean values of both years are discussed.

Grain yield was determined by recording the yield after shelling. The grain yield of each plot was recorded and expressed as kg/m^2. Biomass was determined by taking the weight of above ground dried plants along with the ears. The biomass was expressed as kg/plant. Thousand grain weight was calculated by randomly selecting sample from the pool of harvested seeds from each plot. NUE was calculated by the formula given by Moll et al. (1982):

$$NUE = \frac{\text{Grain yield}}{\text{Nitrogen fertilizer application}}$$

Statistical analysis

All the values were mean of three replicates. Data obtained was subjected to split plot design at 5% level of critical difference using CPCS1 software developed by department of Statistics, Punjab Agricutural University, Ludhiana.

RESULTS AND DISCUSSION

Yield and yield attributes

Mean values of both years showed an increase in grain yield and its attributes supplied with higher doses of N. Results showed that yield was in the range of 0.306 to 0.563 Kg/m^2 (Table 1). The effect of genotypes- N levels interaction was significant and for all genotypes, yield was significantly affected by N levels. As reported earlier, grain yield is significantly influenced by N application (Singh et al., 2000; Sial et al., 2005). As compared to recommended dose (RDN), under low N levels viz. RDN-50% and RDN-25%, the reduction in yield was 41 and 9%, respectively. Increased grain yield with increase in N application could be ascribed to increased biomass production with N fertilization.

Ample nutrient supply results in enhancement in growth and production of more reproductive structures per plant (that is, tiller number – Table 2) thereby increasing overall yield of the crop as reported by Lawlor (2002) and Valerol et al. (2005). Genotype PBW 621 significantly produced greatest grain yield at RDN-50% (0.385 Kg/m^2) and RDN-25% (0.605 Kg/m^2), RDN (0.625 Kg/m^2) while at RDN+25%, two genotypes PBW 621 and PBW 636 gave the highest yields (0.635 Kg/m^2). Genotype BW 8989 gave very low yield (0.306 Kg/sqm).

There was 6 and 2% decrease in thousand grain weight with the application of RDN-50% and RDN - 25%, respectively as compared to optimum N dose (Table 1). The low N supply decreases grain weight due to less supply of the grain with carbohydrates and amino compounds during the lag phase when the number of storage cells and starch granules are being formed as reported by Paponov et al. (2005). Thousand grain weight was in the range of 31.51 to 37.21 g and the maximum thousand grain weight of 35.64 g (RDN-50%), 36.64 g (RDN-25%), 37.83 g (RDN) and 38.72 g (RDN+25%) was recorded for the genotype BW 9022 as depicted in Table 1. The interaction of genotypes and N levels showed significant differences in thousand grain weight. As observed in our study, reports in the literature also indicate that thousand grain weight increases with increasing dose of N in wheat (Gouis et al., 2000; Guarda et al., 2004) and corn (Hokamlipour et al., 2010).

As compared to optimum N dose, the average decrease in biomass at RDN-50% and RDN-25% was 25 and 8%, respectively. The highest biomass was recorded in the treatment RDN+25% (Table 1). Similar to our observations, biomass increased significantly with increasing N level as reported in corn and sweet sorghum by Almodares et al. (2009). Biomass varied in the range of 1.32 to 1.84 Kg/plant (Table 1). In genotypes, PBW 621 and PBW 343 at RDN-50% (1.29 Kg/plant) and in PBW 636 at RDN-25%, RDN and RDN+25%, the largest biomasses were recorded. Results indicated that decrease in biomass was proportional with the decrease

Table 1. Effect of different doses of nitrogen on yield (Kg/sqm), thousand grain weight (g) and biomass (Kg/plant) during years and 2009-10 and 2010-11.

N Doses / Genotypes	Yield (Kg/m^2)					Thousand grain weight (g)					Biomass (Kg/plant)				
	RDN-50%	RDN-25%	RDN	RDN+25%	Mean	RDN-50%	RDN-25%	RDN	RDN+25%	Mean	RDN-50%	RDN-25%	RDN	RDN+25%	Mean
PBW 621	0.385	0.605	0.625	0.635	0.563	32.64	33.31	34.00	34.78	33.68	1.27	1.44	1.70	1.99	1.60
PBW 636	0.350	0.530	0.545	0.635	0.515	32.87	33.45	34.36	34.64	33.83	1.29	1.79	1.96	2.34	1.84
PBW 590	0.320	0.435	0.455	0.470	0.420	33.29	34.53	35.73	36.92	35.11	1.17	1.44	1.50	1.64	1.44
DBW 17	0.280	0.415	0.470	0.485	0.413	31.72	32.92	34.26	36.34	33.81	1.14	1.40	1.54	1.89	1.49
HD 2967	0.295	0.410	0.450	0.480	0.409	33.50	35.95	36.85	37.89	36.05	1.08	1.44	1.48	1.97	1.49
PBW 509	0.240	0.300	0.480	0.510	0.383	34.03	35.28	35.50	36.73	35.38	1.27	1.43	1.54	1.67	1.47
BW 9178	0.195	0.375	0.390	0.485	0.361	32.73	33.66	34.31	37.06	34.44	1.17	1.54	1.68	1.87	1.56
BW 9183	0.215	0.330	0.360	0.410	0.329	33.50	34.47	34.64	36.27	34.72	1.17	1.73	1.80	1.99	1.67
BW 8989	0.200	0.310	0.335	0.380	0.306	34.87	35.47	35.67	36.59	35.65	1.02	1.20	1.30	1.77	1.32
BW 9022	0.190	0.360	0.375	0.405	0.333	35.64	36.64	37.83	38.72	37.21	1.25	1.39	1.68	1.90	1.55
PBW 343	0.205	0.325	0.355	0.440	0.331	29.34	31.50	32.39	32.81	31.51	1.29	1.37	1.55	1.77	1.49
PBW 550	0.215	0.395	0.415	0.465	0.373	32.78	33.92	35.66	35.78	34.53	1.22	1.57	1.64	1.92	1.58
Mean	0.258	0.399	0.438	0.483	0.395	33.08	34.26	35.10	36.21	34.66	1.20	1.48	1.61	1.89	1.54

CD (5%): A- 0.012, B- 0.020, AB- 0.041; A- 0.984, B- 1.704, AB- 1.964; A- 0.118, B- 0.204, AB- 0.738; A- N doses, B-Genotypes, AB-In.

Table 2. Effect of different doses of nitrogen tiller number (per m row length) and spikelet number during years 2009-10 and 2010-2011.

N Doses / Genotypes	Tiller number (per m row length)					Spikelet number				
	RDN-50%	RDN-25%	RDN	RDN+ 25%	Mean	RDN-50%	RDN-25%	RDN	RDN+ 25%	Mean
PBW 621	81	84	89	95	87	17	19	19	20	19
PBW 636	80	84	99	102	91	18	18	20	21	19
PBW 590	61	75	84	86	76	15	18	17	19	17
DBW 17	69	82	78	95	81	18	18	18	19	18
HD 2967	62	80	88	91	80	18	18	19	20	19
PBW 509	68	81	83	94	81	17	18	18	18	17
BW 9178	72	87	96	98	88	16	18	18	18	17
BW 9183	69	87	93	99	87	18	19	19	19	19
BW 8989	66	66	79	95	76	18	19	19	19	19
BW 9022	69	82	85	95	82	18	18	19	19	18
PBW 343	72	76	85	87	80	17	17	18	18	18
PBW 550	71	76	79	87	78	16	18	18	19	17
Mean	70	80	87	94	82	17	18	19	19	18

CD (5%): A- 5.100, B- 8.828, AB- 13.224; A- 0.555, B- 0.962, AB- 1.567; A- N doses, B-Genotypes, AB-Interaction.

Table 3. Effect of different nitrogen doses on variation in nitrogen use efficiency (NUE) (Kg Kg^{-1}) during years 2009-10 and 2010-11.

N Doses / Genotypes	NUE (Kg Kg^{-1})				
	RDN-50%	RDN-25%	RDN	RDN+ 25%	Mean
PBW 621	64.2	67.3	50.9	42.0	56.1
PBW 636	58.4	58.9	45.4	42.4	51.3
PBW 590	53.4	48.4	37.9	31.4	42.7
DBW 17	46.7	46.1	39.2	32.3	41.1
HD 2967	49.2	45.6	37.5	32.0	41.1
PBW 509	40.0	33.4	40.0	34.0	36.8
BW 9178	32.5	41.7	32.5	32.4	34.8
BW 9183	35.9	36.7	30.0	27.4	32.5
BW 8989	34.2	36.1	29.6	29.4	32.3
BW 9022	31.7	40.0	31.3	27.0	32.5
PBW 343	33.4	34.5	28.0	25.4	30.3
PBW 550	35.9	43.9	34.6	31.0	36.3
Mean	39.0	40.2	33.6	29.7	35.6

CD (5%): A- 1.527, B- 2.646, AB- 5.291; A- N doses, B-Genotypes, AB-Interaction.

in sub optimal dose of N. Thousand grain weight and biomass were significantly affected by different N levels but no significant difference was found between control (RDN) and RDN-25% (Table 1).

At RDN+25%, tiller number was maximum while minimum tiller number was recorded in treatment RDN-50% (Table 2). The increase in number of fertile tillers with the increasing levels of N can be attributed to the reduction in mortality of tillers and enabling the production of more tillers from the main stem (Warraich et al., 2002). Tiller number was found to lie in the range of 76 to 91 (Table 2) and highest number was recorded in PBW 621 at RDN-50% (81) and in BW 9178 and BW 9183 at RDN-25% (87), respectively. There was a marginal decrease in spikelet number at RDN-50% and RDN-25%, respectively. PBW 621, PBW 636, DBW 17, HD 2967, BW 9183, BW 8989 and BW 9022 showed maximum spikelet number at lower N doses (Table 2). Spikelet number was affected significantly by different N levels and significant difference was found between treatment RDN-50% and RDN-25% as compared to control.

Nitrogen use efficiency (NUE)

Nitrogen use efficiency is based on yield performance, that is, on grain yield per N input. During both years (2009-10 and 2010-11) of study, genotypic variation for NUE was observed, however, it decreased with increasing dose of N. A decrease in NUE with increasing fertilizer rates is due to less increase in grain yield in comparison to N supply as observed by Zhao et al. (2006) in sorghum. Beatty et al. (2010) reported that the NUE of barley genotypes grown in field depends on the level of N supplied.

Nitrogen use efficiency varied from 30.3 to 56.1 Kg Kg^{-1} as shown in Table 3. Highest NUE was recorded in PBW 621 at both RDN-50% (64.2 Kg Kg^{-1}) and RDN-25% (67.3 Kg Kg^{-1}). Several studies on maize have shown genetic variability for NUE both at low and high N fertilization levels (Presterl et al., 2002; Gallais and Coque, 2005). Between N treatments, maximum NUE was recorded in RDN-25% which was significantly higher than that of control (RDN) but it was statistically close to RDN-50%. Main focus of our study was not to deprive plants for nutrients but to find a N dose up to which N fertilization can be reduced with high yield and NUE. This study confirmed that recently released cultivar by PAU viz. PBW 621 is high yielding and is also efficient in metabolizing N.

Correlation analysis between different physiological traits and NUE indicated that NUE was positively and significantly correlated with grain yield at all the four levels of applied N for all genotypes. However, the relationship of other physiological traits, that is, thousand grain weight, biomass, plant height, tiller number and spikelet number was non-significant. Positive and significant correlation was observed between biomass and tiller number (Table 4).

Mean values for studied parameters of twelve genotypes during first year (2009-10) and second year (2010-11) were grouped into clusters on the basis of all studied physiological parameters. A cluster tree was generated using NTSYS software (Rohlf, 1998). Combined cluster analysis of both years showed that 12 genotypes were divided into three clusters (I, II and III) (Figure 1). In the cluster I, PBW 621 and PBW 636 were clustered together. In Cluster II, PBW 590 and HD 2967 while in cluster III, DBW 17, PBW 550, PBW 509, BW

Table 4. Correlation analysis between different yield attributes in wheat genotypes.

Parameter	Yield	Thousand grain weight	Biomass	Plant height	Tiller number	Spikelet number
NUE	0.982	-0.163	0.447	0.201	0.418	0.301
Yield		-0.243	0.499	0.273	0.468	0.248
Thousand grain weight			-0.230	-0.308	-0.232	-0.013
Biomass				0.341	0.840	0.257
Plant height					0.443	0.373
Tiller number						0.325

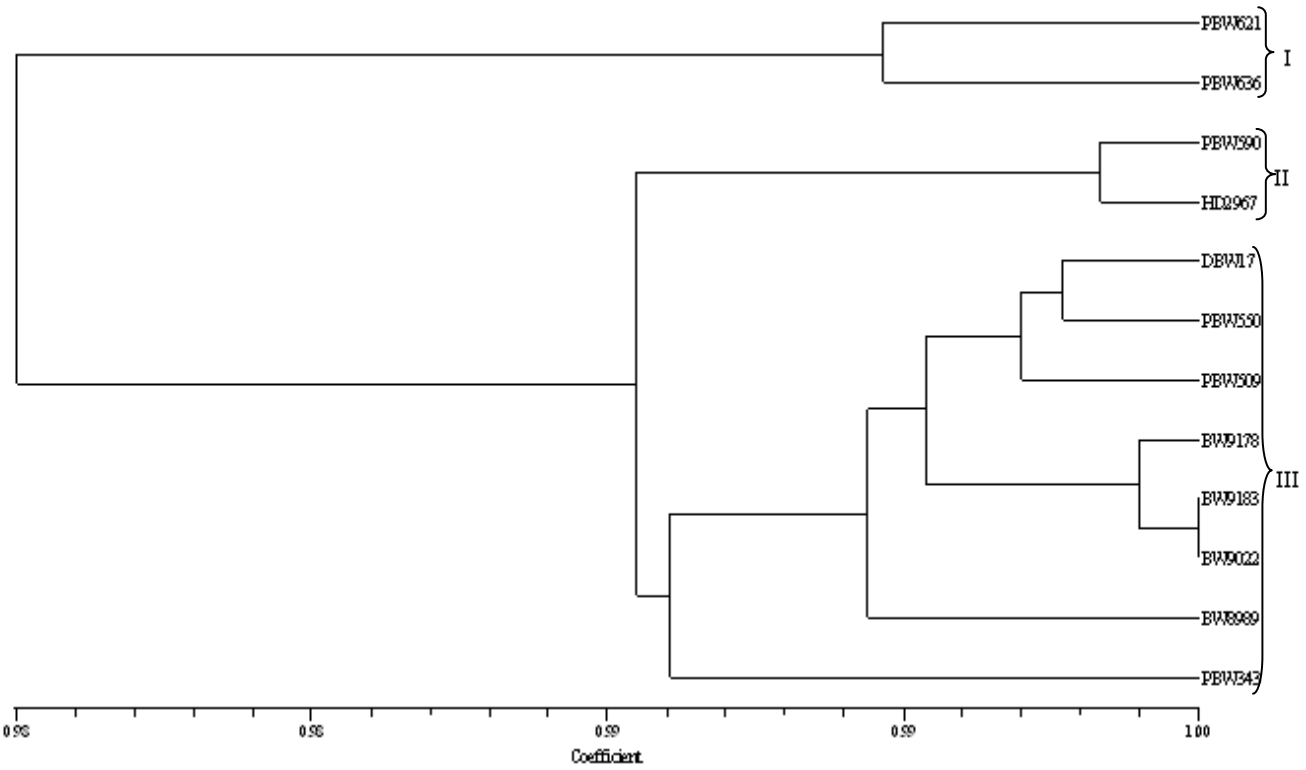

Figure 1. Cluster of twelve wheat genotypes by UPGMA clustering method for the years 2009-10 and 2010-2011.

9178, BW 9183, BW 8989, BW 9022 and PBW 343 were grouped together. PBW 621 and PBW 343 were at the opposite sides of the tree plot showing that they vary with respect to N use as also reflected from the data. Genotypes clustered together showed similar physiological behavior for N use. From cluster analysis of both years, it has been observed that PBW 621 and PBW 636 share similarity with respect to N use.

Conclusion

In conclusion, results of present study showed that PBW 621 and PBW 636 gave highest NUE and grain yield at sub optimal doses of N while widely grown cultivar PBW 343 and advanced breeding lines BW 8989 and BW 9022 showed a lower efficiency for N use. 120 kg/ha is the recommended N dose. However, considering environmental and economic issues, it has been observed that a decrease in N dose up to RDN-25% is tolerated by N efficient genotypes without marked loss in yield whereas further decrease from RDN-25% causes N starvation. It would be particularly interesting to further investigate the differential N responsiveness of contrasting genotypes in terms of complex regulatory network involved in NUE. These N efficient genotypes may be used as donor stocks in wheat breeding programs.

Conflict of Interest

The authors have not declared any conflict of interest.

REFERENCES

Abeledo LG, Savin R, Slafer GA (2008). Wheat productivity in the Mediterranean Ebro Valley: analyzing the gap between attainable and potential yield with a simulation model. Eur. J. Agron. 28:541-50.

Almodares A, Jafarinia M, Hadi MR (2009). The effects of nitrogen fertilizer on chemical compositions in corn and sweet sorghum. J. Agric. Environ. Sci. 6:441-446.

Amanullah, Marwat KB, Shah P, Maula N, Arifullah S (2009). Nitrogen levels and its time of application influence leaf area, height and biomass of maize planted at low and high density. Pak. J. Bot. 41:761-768.

Anbessa Y, Juskiw P, Good A, Nyachiro J, Helm J (2009). Genetic variability in nitrogen use efficiency of Spring Barley. Crop Sci. 49:1259-69.

Beatty PH, Anbessa Y, Juskiw P, Carroll RT, Wang J, Good AG (2010). Nitrogen use efficiencies of spring barley grown under varying nitrogen conditions in the field and growth chamber. Ann Bot. 105:1171-1182.

Conley DJ, Paerl HW, Howarth RW, Boesch DF, Seitzinger SP, Havens KE, Lancelot C, Likens GE (2009). Controlling Eutrophication: Nitrogen and Phosphorus. Science 323:1014-1015.

Foulkes MJ, Hawkesford MJ, Barraclough PB, Holdsworth MJ, Kerr S, Kightley S, Shewry PR (2009). Identifying traits to improve the nitrogen economy of wheat: Recent advances and future prospects. Field Crops Res. 114:329-342.

Gallais A, Coque M (2005). Genetic variation and selection for nitrogen use efficiency in maize, a synthesis. Maydica 50:531-547.

Galloway JN, Townsend AR, Erisman JW, Bekunda M, Cai Z, Freney JR, Martinelli LA, Seitzinger SP, Sutton MA (2008). Transformation of the Nitrogen Cycle: Recent Trends, Questions, and Potential Solutions. Science 320:889-892.

Giller KE, Chalk P, Dobermann A, Hammond L, Heffer P, Ladha JK, Nyamudeza P, Maene L, Ssali H, Freney J (2004). Emerging technologies to increase the efficiency of use of fertilizer nitrogen. In: Mosier AR, Syers JK, Freney JR, editors. Agriculture and the nitrogen cycle: assessing the impacts of fertilizer use on food production and the environment. Washington, DC: Island Press. pp. 35-51.

Gouis LJ, Beghin D, Heumez E, Pluchard P (2000). Genetic differences for nitrogen uptake and nitrogen utilization efficiencies in winter wheat. Eur. J. Agron. 12:163-173.

Gruber N, Galloway JN (2008). An Earth-system perspective of the global nitrogen cycle. Nature 451:293-296.

Guarda G, Padovan S, Delogu G (2004). Grain yield, nitrogen-use effi ciency and baking quality of old and modern Italian bread-wheat cultivars grown at diff erent nitrogen levels. Eur. J. Agron. 21:181-192.

Hirel B, Gouis JL, Ney B, Gallais A (2007). The challenge of improving nitrogen use efficiency in crop plants: towards a more central role for genetic variability and quantitative genetics within integrated approaches. J. Exp. Bot. 58:2369-2387.

Hokmalipour S, Seyedsharifi R, Jamaati-e-Somarin SH, Hassanzadeh M, Shiri-e-Janagard M, Zabihi-e-Mahmoodabad R (2010). Evaluation of plant density and nitrogen fertilizer on yield, yield components and growth of maize. World Appl. Sci. J. 8:1157-1162.

Ju XT, Xing GX, Chen XP (2009). Reducing environmental risk by improving N management in intensive chinese agricultural systems. Proc. Natl. Acad. Sci. USA 106:3041-3046.

Lawlor DW (2002). Carbon and nitrogen assimilation in relation to yield: mechanisms are the key to understanding production systems. J. Exp. Bot. 53:773-787.

Moll RH, Kamprath EJ, Jackson WA (1982). Analysis and interpretation of factors which contribute to efficiency of nitrogen utilization. Agron. J. 74:562-64.

Paponov IA, Sambo P, Erley G, Presterl T, Geiger HH, Engels C (2005). Grain yield and kernel weight of two maize genotypes differing in nitrogen use efficiency at various levels of nitrogen and carbohydrate availability during flowering and grain filling. Plant Soil 272:111-123.

Presterl T, Groh S, Landbeck M, Seitz G, Schmidt W, Geiger HH (2002). Nitrogen uptake and utilization efficiency of european maize hybrids developed under conditions of low and high nitrogen input. Plant Breed. 121:480-486.

Rahman MA, Sarker MAZ, Amin MF, Jahan AHS, Akhter MM (2011). Yield response and nitrogen use efficiency of wheat under different doses and split application of nitrogen fertilizer. Bangladesh J. Agric. Res. 36:231-240.

Rohlf FJ (1998). NTSYSpc: Numerical Taxonomy and Multivariate Analysis System. Version 2.02. Exeter Software, Setauket, New York.

Sial MA, Arain MA, Naqvi SKMH, Dahot U, Nizamani NA (2005). Yield and quality parameters of wheat genotypes as affected by sowing dates and high temperature stress. Pak. J. Bot. 37:575-584.

Singh MK, Thakur R, Verma UN, Upasani RR, Pal SK (2000). Effect of planting time and nitrogen on production potential of Basmati rice cultivars in Bhiar Plateau. Indian J. Agron. 45:300-303.

Valerol DJJA, Maturano M, Ramirez AA, Martin-Benito JMT, Alvarez JFO (2005). Growth and nitrogen use efficiency of irrigated maize in a semiarid region as affected by nitrogen fertilization. Span. J. Agric. Res. 3:134-144.

Viller JM, Guillaumes E (2010). Use of nitrification inhibitor DMPP to improve nitrogen recovery in irrigated wheat on a calcareous soil. Span. J. Agric. Res. 8:1218-1230.

Vukovic I, Mesic M, Zgorelec Z, Jurisic A (2008). Nitrogen use efficiency in winter wheat. Cereal Res. Commun. 36:1199-1202.

Warraich EA, Ahmad N, Basra SMA, Afzal I (2002). Effect of nitrogen on source–sink relationship in wheat. Int. J. Agric. Biol. 4:300-302.

Woolfolk CW, Raun WR, Johnson GV, Thomason WE, Mullen RW, Wynn KJ, Freeman KW (2002). Influence of late-season foliar nitrogen applications on yield and grain Nitrogen in winter wheat. Agron. J. 94:429-434.

Zhao RF, Chen XP, Zhang FS, Zhang H, Schroder J, Romheld V (2006). Fertilization and nitrogen balance in a wheat-maize rotation system in North China. Agron. J. 98:935-945.

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Full Length Research Paper

Rice farming in saline lowland of Sahel: Combination of anti-salt dam, salt-tolerant varieties, fertilizers to improve yields

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Salinity stress, drought and the lack of water supply are major constraints limiting rice productivity in rainfed lowland of the Sine Saloum region. To alleviate these constraints, several actions have been undertaken including construction of anti-salt dykes and using of salt tolerant varieties. The objectives of this study were: (a) analyze the hydraulic operation of these lowlands and salinity rate during wet season. (b) To test, the response of new salt-tolerant varieties, with different fertilizers and to identify the best suitable. Trials were conducted in two sites. The level of groundwater and salinity were measured during two wet seasons in two sites. At least 100 mm of rainfall are required to decrease salinity (EC) below 3 dS / m on a leveled land, before sowing. Before sowing, the management of water flow at the anti-salt dam must take into account not only the leaching of salt, but also the groundwater recharge. It is this groundwater that will allow rice to reach maturity, at the end of rains by mitigating late season drought. In both sites, *D14* and *IR70870-B-P-2-2* were the most biomass productive varieties. Among the eleven rice varieties tested, five have performed well with the average grain yields of 4 t.ha^{-1}.

Key words: Rainfall, groundwater, sea water, new salt-tolerant varieties, lowland.

INTRODUCTION

Rice is the most important food crop of the developing world and the staple food in the Sahelian nations of West Africa. Rice consumption in West Africa still increases, due to accelerating urban population growth and increasing levels of consumption per-capita (Seck et al., 2012). Senegal is one of the biggest rice importers in West Africa, (800,000 t per year) about 60% of local consumption (FAO-stat, 2012). Nevertheless, the part of imported rice can be reduced, by increasing local production, when considering the potential of various

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agro-ecological conditions in which rice can be produced (Lancon and Erenstein., 2002). In term of surface area, the rainfed lowland system is the most important reaching 60% of total rice-farming in Senegal (SAED, 2012). However, biophysical characteristics, rainfall hazard and water provision infrastructures determine the availability of water throughout the year (Kilian et al., 1999). Rice yields are generally low (Yoshida and Benta, 1983). Also, salt-stress, water deficiency and lack of best fertilizer management destabilize further more this fragile situation (Fukai et al., 1998; Diop et al., 2002). In Sine Saloum region, western Senegal, lowlands cover more than 500,000 ha, representing important resources for rural and national economy (Camara et al., 2007). Rice farming remains one of the main agricultural activities in this area (Mbodj, 2008). However, salinity stress caused by intrusion of sea water in these lowlands is the major constraint limiting rice-farming (Mbodj, 2001), (Camara et al., 2007). As consequence of land degradation, farmers are obliged to exploit upland areas, while the potential yield of the upland is lower than that of lowland (Yoshida and Benta, 1983), (DingKhun and Sow, 1997). In addition, water use efficiency on-farm is low, due to meantime and late season droughts, high evapotranspiration and failure of water control infrastructures (Tomar and Toole, 1980; Lilley and Fukai, 1994).

Rice crop suffers alternately from water excess and deficits, thus leaching and volatilization of nutrients are increased (Camara et al., 2008; Kaushal et al., 2009). Low water use efficiency illustrates the priority for improved water management in this region. To reduce the vulnerability of rice-farming activities, anti-salt dams have been built, in order to mitigate the salinity rate. Also, the using of salt-tolerant rice varieties gives opportunity to minimize salt effects and to produce higher yields than existing local varieties in this environment (Camara et al., 2008). It is in this context that Africa-Rice in collaboration with National Agricultural Research Station has just set up ten salt tolerant breeding lines for the Sahel Africa. In addition, best agricultural practices (e.g water control, management of fertilizer), contribute to reach that goal (WARDA, 2004). Therefore, the objectives of this paper were: (a) analyze the hydraulic operation of these lowlands and salinity rate during wet season in the presence of anti-salt dam. (b) To test, in these ecologies, the response of new salt-tolerant varieties with different types of fertilizers. (c) And to identify the best salt-tolerant varieties, suitable for saline lowland conditions in the Sahel.

MATERIALS AND METHODS

Experimental sites

Two field experiments were conducted during the wet season (from June to November 2011) in Ndour ndour (14°06'N, 16°18'W) and Nderderling (13°40'N, 16°24'W), located in Sine Saloum region, western Senegal. Sine Saloum region is located in Soudano Sahelian zone. Climate is characterized by a wet season, between 600 and 800 mm rainfall from June to October and a dry season from November to May. Average monthly maximum temperature is 39°C, reached between May-June. Average solar exposure is 9 h per day. Air humidity is high all year long (ANSD, 2009). In Ndour ndour, more than 50% of lowland areas are severely salt affected while Ndinderling site is moderately saline. Evaporation is very important about 2950 mm/year at Ndour ndour. Soil texture varies from sandy to clay sandy at both sites (Mankeur, 1999).

Anti-salt dikes

An anti-salt dam is a structure of water retention made in the valley of Sine Saloun to prevent the invasion of land by sea water and to protect and recover saline soils upstream of the structure. It includes a dike and evacuator regulated by a valve. The way of operation is described hereafter. At the beginning of the wet season, the valve remains closed to allow runoff to accumulate in the basin. Part of the retained water infiltrates and contributes to groundwater recharging. Salt accumulated in the soil dissolves in standing water. The valve is then opened to allow drainage of salt water. The capillary rise from the groundwater through layers of saline soil and its evaporation lead to accumulation of salt on soil surface. The valve is then closed to permit submersion again. Processes of submersion and drainage will continue until the threshold level of salinity (less than 3 dS/m) is obtained. At this time, land preparation is undertaken, followed by sowing or transplanting. At the end of wet season, the valve is closed to help keeping water in the lowland. This allows rice to reach maturity and also prevents capillarity rise of salt water.

Agronomic experiments

Trials were conducted during the wet season 2011 in the farmer fields at Ndinderling and Ndour Ndour sites. A factorial design was used with three replications, four fertilizer treatments as main plot, measuring 54 m^2 (27 m × 2 m) each and eleven rice varieties as sub plot, measuring 4 m^2 (2 m × 2 m) each. Direct sowing was done with three grains per hill, using inter-row distance of 20 cm and spacing of 20 cm within hills. Three fertilizers treatments were used (Table 1). For the first treatment, manure (3,000 kg ha^{-1}) was applied basally. For the second, mineral fertilizers used were urea (46% N), triple super phosphate (20% P), and potassium chloride (50% K). The third treatment was a combination of manure and mineral fertilizers.

Plant materials

The salt-tolerant breeding lines (Table 2) used in these trials were selected during the “Stress Tolerant Rice for Africa and South Asia” (STRASA) project, after a long screening process, in research station (Africa-Rice), and after experimentation in three countries (Gambia, Senegal, Mali) for tolerance to salinity. The screening program started with over 200 lines that were tested at 6 dS/m level of salinity, in the research station of Africa Rice in Ndiaye. After that screening, the salt-tolerant lines were selected and completed with many salt-tolerant lines from International Rice Research Institute (IRRI) which were screened also at 6 dS/m. The second step of this screening process took place in farmer’s fields in Senegal, Gambia and Mali between 2009 and 2010 during wet season.

Sampling, measurements and analyses

Three sub samples of top soil horizons (0 to 20 cm depth) were

Table 1. Description of fertilizer treatments used in the two experimental sites.

Fertilizers treatment (kg ha^{-1})	
MF: Mineral Fertilizer	90N-30P-30K
OF: Manure	3000 kg ha^{-1}
OMF: Manure and Mineral Fertilizer	3000 kg ha (OF)+ 90N-30P-30K (MF)
Control	No fertilizers

Table 2. Origins of the eleven rice varieties used.

Plant material	Origin
D14	IRRI
IR66946-3R-178-1-1	IRRI
WAS161-B-6-1	AfricaRice (Saint Louis)
IR70870-B-P-2-2	IRRI
IR67076-2R-15-3	IRRI
WAS197-B-8-2	AfricaRice (Saint Louis)
IKP	IRRI
SAHEL 236	AfricaRice
IR4630	IRRI
Sahel 201	Africa Rice
IR31785	IRRI

taken in the center of each plot. Sub samples were mixed thoroughly to get a composite sample, air dried and stored at 60°C for one week at laboratory. Analyses included pH_{H2O} on 1:2.5 extract and electrical conductivity (EC) on the 1:5 extract. Soil organic carbon was determined using the wet digestion method (Walkley et al., 1934). Available phosphorus was determined with the Bray 1 test (Bray and Kurtz., 1945). Groundwater's levels were measured using thirty piezometers at both sites. Electrical conductivities of standing water were checked, daily, using a portable conductivity meter during 2010 and 2011 wet season. Contour maps of salinity level at Ndour ndour and Ndinderling farms area were created using the Surfer 8.0 package before setting up the trial. For determination of dry biomass, three plants were harvested (cut flush to the ground) at the end of the vegetative growth stage. These samples are then passed in oven at 60°C for at least 3 days until complete dehydration. Then, the weight is measured with an electronic weight sensor. Grain yields were measured at 4 m^2 (2 m × 2 m). Analysis of variance (ANOVA) has been performed and the mean values have been compared using Student Newman Keuls (SNK) range test. Statistical procedures have been performed using the 9.1 version of SAS software (SAS Institute, 2004). Weather data were collected from stations near the experiment sites and the daily reference evapotranspiration (ETo) was estimated using Penman-Montieth method (Allen et al., 1998). Water use efficiency (WUE) on biomass basis has been calculated with respect to rainfall as follows:

$$WUE = \frac{BIOM}{\sum Rf}$$

BIOM: total above-ground biomass on dry weight basis; Rf: Rainfall during the cropping season.

RESULTS

Soil characteristics

Analyses of top soil horizon (0 to 20 cm) was done (Table 3). Soil in Ndour ndour was acidic with pH (H_2O) and pH (KCl) values of 4.74 and 4.46, respectively. In beginning of cropping season, the EC was already at 4.51 dS.m^{-1}, the P level was 8.33 mg.kg^{-1} (Bray 1) and the organic matter was 3.21%. While in Ndinderling, soil was slightly acidic with pH (H_2O) and pH (KCl) values of 6.19 and 5.11, respectively. EC was 0.54 dS.m^{-1}, P level was 8.59 mg.kg^{-1} (Bray 1) and organic matter was 4.0%. Intrusion of sea water in lowland has induced higher salinity in Ndour ndour compared to Ndinderling. The acidity could be explained by the nature of soils, and chemical reactions between iron transported by water drained from the continent and sea water (Maryse, 1991). However, the soil organic matter was relatively high at both sites, probably due to alluvial and colluvial deposits of organic sediments in the bed of the stream (Perez, 1994).

Evolution of salinity in relation of rainfall

In Figure 1, the salinity in the lowland falls under 3 dS/m after 103.5 mm of rainfall in June 2010 ending. In 2011, after 150 mm of cumulative rainfalls, reached on 30th of July, the salinity was approximately 2 dS/m. This shows that at least 100 mm of rainfall is required for enough leaching of salt in order to reach the threshold of 3 dS/m before sowing. As seen in the contour map (Figures 2 and 3), salinity is not uniform, because leaching also is not uniform due to the fact that terrain is not levelled.

Rain, groundwater, salinity dynamics and their impacts on rice growth

Ndour Ndour: Rice roots are in 90% located in 0 to 30 cm depth. So long as the level of the groundwater is less than 30 cm, it hardly contributes to the rice water supply. In this lowland, the groundwater table, despite of a gradual recharge, was less than 50 cm (Figure 4) during the seed germination and young seedlings growth. However, more than 80% of rains occurred during this period. This allowed having relatively high moisture in the

Table 3. Main characteristics of top soil horizon.

Parameter	Ndour Ndour	Ndinderling
pH (H_2O)	4.74	6.19
pH (KCl)	4.46	5.11
EC ($dS.m^{-1}$)	4.51	0.54
C (%)	1.87	2.34
MO (%)	3.21	4.03
P Bray 1 ($mg.kg^{-1}$)	8.33	8.59

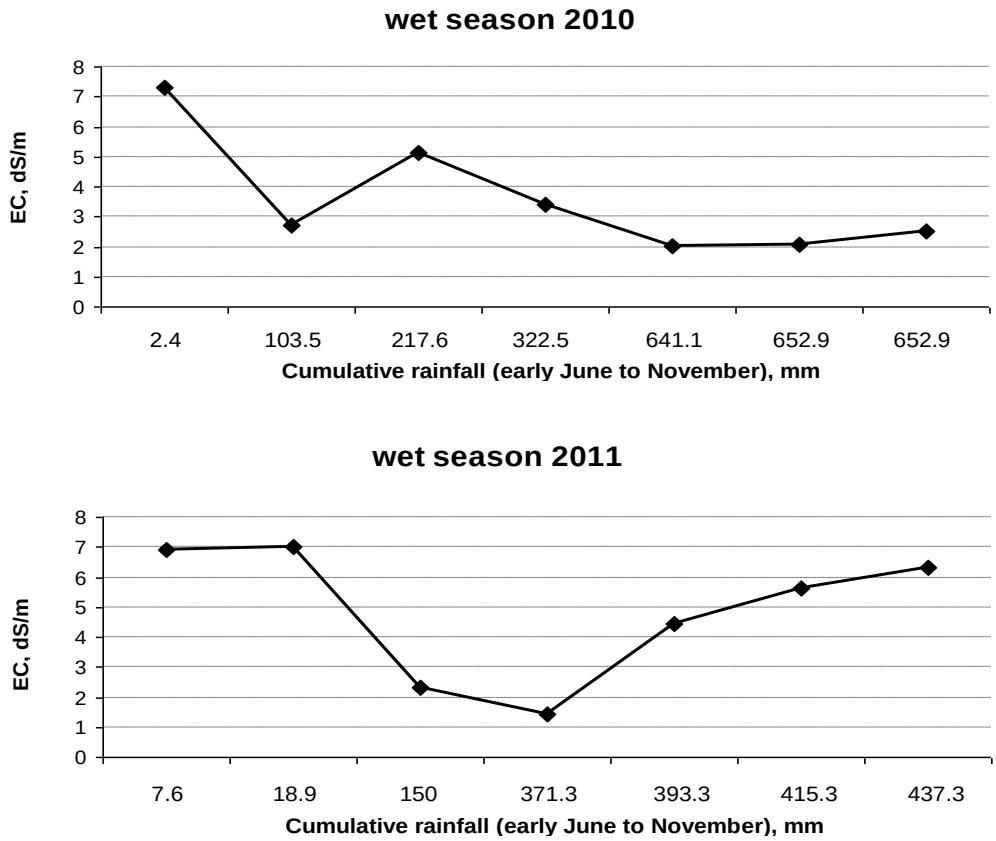

Figure 1. Evolution of mean salinity in the lowland of Ndour Ndour, versus cumulated rainfalls during the wet season 2010 and 2011.

soil. First rains have also allowed leaching of salt thanks to anti-salt dam. Water stress was not very pronounced during the end of vegetative growth at the beginning of the reproductive phase (Figure 4). Rains having stopped in October, this has resulted in a decrease in groundwater table (Figure 4). During the end of the reproductive phase, at maturity, soil moisture was near the permanent wilting point.

As shown in the salinity map of Ndour Ndour (Figure 2), average salinity was lower than 3 dS/m of sowing day. From early June to late August (Figure 1: wet season 2011) lowland has received a significant quantity of rain: 371.3 mm which helped to lower the salt level (Figure 1, wet season 2011).

This decrease is also emphasized by Figure 5. On the opposite, after the end of August, the decline in rainfall has resulted in an increased level of salt. This increase in salinity (Figure 5) is due to the lack of standing water (Figure 4) to prevent salt accumulation coming up from the ground via capillarity and remaining after evaporation.

Ndinderling: Given that the salinity was below the threshold during sowing (Figure 3), the valve stayed closed. That led to keeping water for long time and

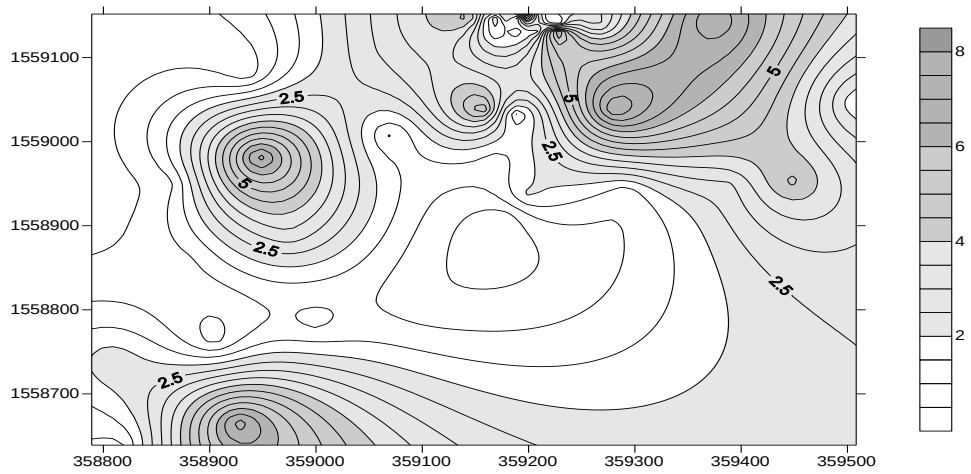

Figure 2. Contour of spatial variability of electrical conductivity (dS.m^{-1}) in the early wet season 2011 at Ndour Ndour before sowing in 28 ha area (x scale: 1.0 inche = 119.865 m, y scale: 1.0 inche = 119.865).

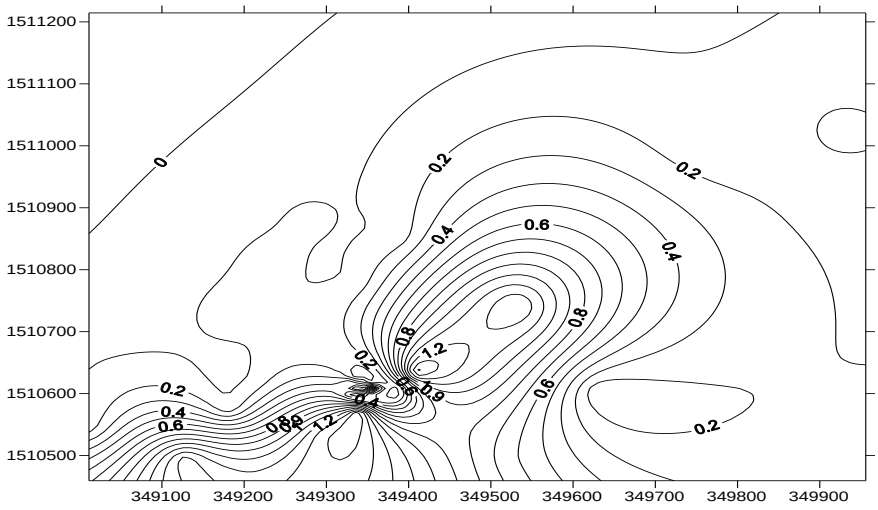

Figure 3. Contour of spatial variability of electrical conductivity (dS/m) in the early wet season 2011 at Ndinderling before sowing in 56 ha area (x scale: 1.0 inche = 157.42 m, y scale: 1.0 inche = 157.42).

permitted its infiltration and deep leaching of salt. Indeed, standing water has achieved the height of 100 cm at surface between September and October. At this stage the main issue is the risk of submersion: At least, one of the leaves should stay out of water for respiration purpose. From October to December, water table decreased from 100 cm to -20 cm (Figure 6). The abundance of rainfall (Figure 6) and the permanent standing water have allowed a continuous leaching of salt and the dilution of the concentration. This has resulted in a low rate of salinity throughout the crop cycle (Figure 7). Crops have used water of the groundwater table after the end of the rains in October. In Ndinderling, rainfall of 758 mm exceeded ETo (554 mm). Finally, there was no salt stress or rainfall deficit during the trial in the lowland of Ndinderling, allowing subsequently, each variety to reach maturity.

Biomass and grain yields

Ndour Ndour: Biomass production was significantly influenced by the type of fertilizer and the different varieties used, but no interaction was observed between the type of fertilizer and variety (Table 4). The average biomass produced was 4.68 tha^{-1} and the control, OF,

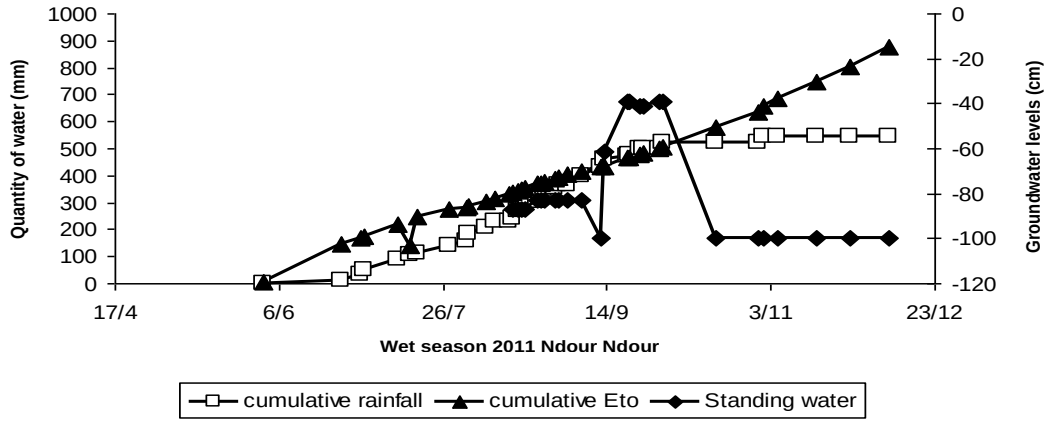

Figure 4. Variation of the rainfall, reference evapotranspiration (ETo) and groundwater table during the wet cropping season from June to December 2011 in Ndour Ndour.

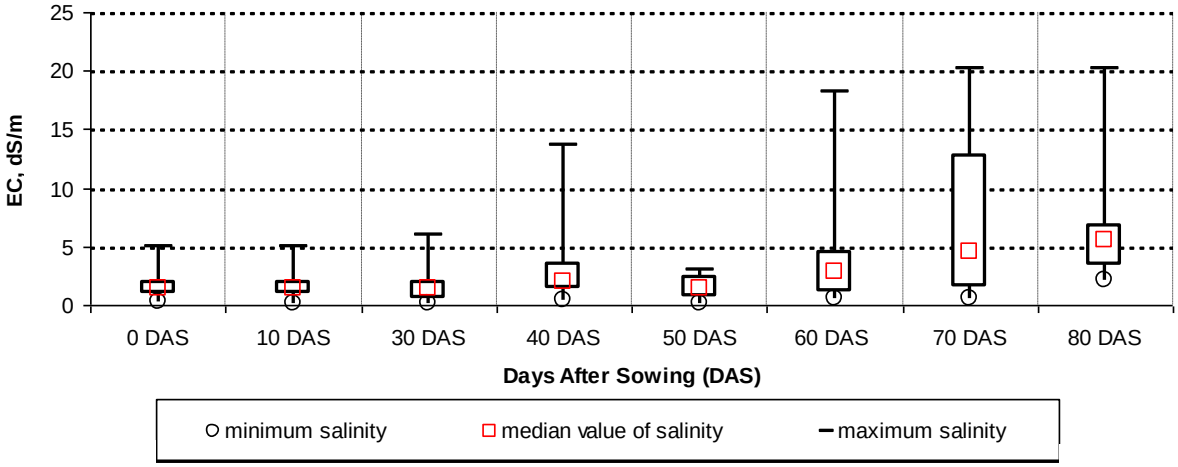

Figure 5. Trend of electrical conductivity (EC) during 80 days after seedling in Ndour Ndour.

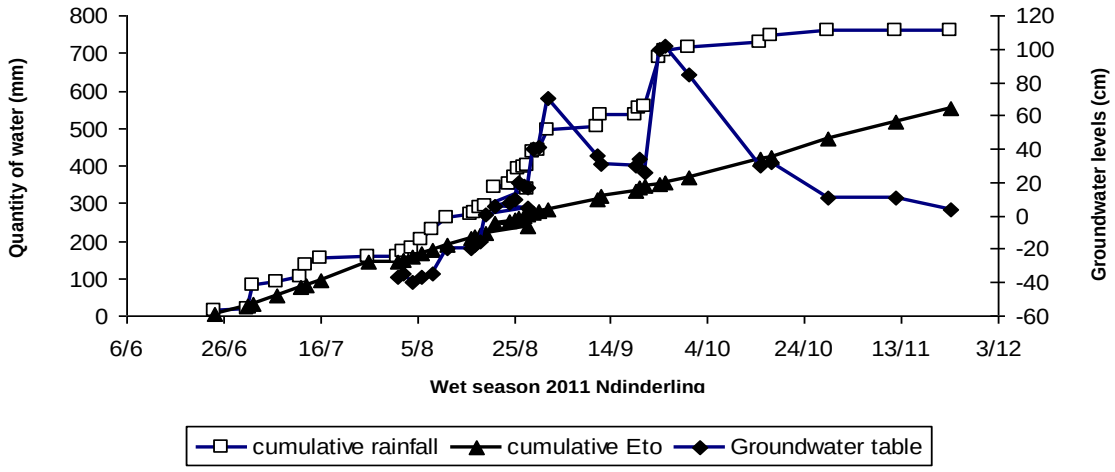

Figure 6. Variation of rainfall, reference evapotranspiration (ETo) and groundwater table during the wet cropping season from June to December 2011 in Ndinderling.

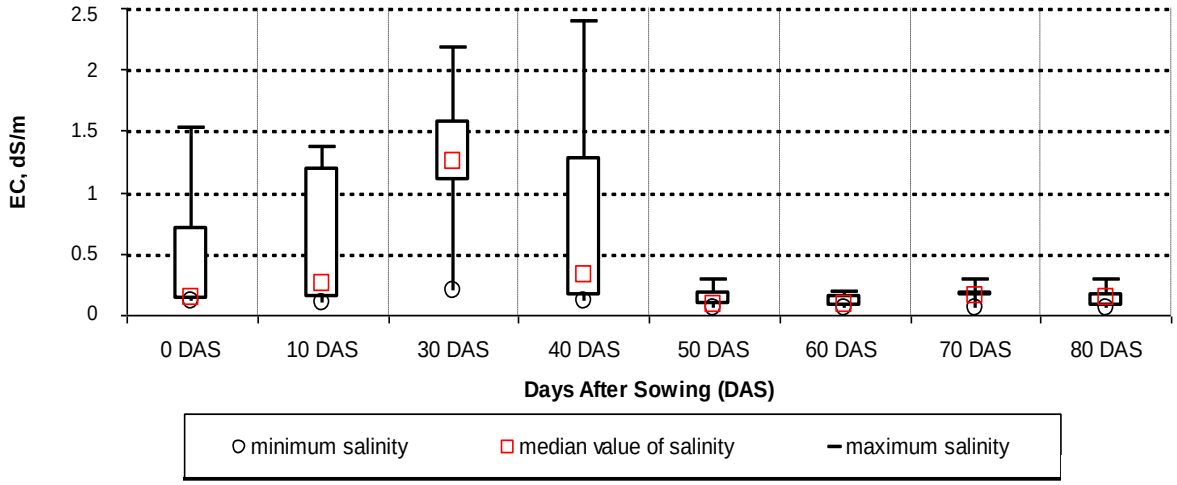

Figure 7. Trend of electrical conductivity (EC) during 80 days after seedling in Ndinderling.

Table 4. Analysis of the variance of the effects of fertilizer treatments, varieties, sites, and interactions within factors on rice biomass production.

Source	DF	Mean square	F Value	Pr > F
Fertilizer	3	202.4307435	25.83	0.0001
Variety	10	20.7133373	2.64	0.0059
Site	1	512.0516156	65.34	0.0001
Treatment*Variety	30	7.165653	0.91	0.5982
Treatment*Site	3	2.2452228	0.29	0.8351
Variety*Site	10	6.4760474	0.83	0.6040
Treatment*Variety*Site	30	8.203849	1.05	0.4136
R-Square	0.683426			
Coeff Var	49.72243			

MF and OMF gave respectively 2.01, 3.7, 6.14, and 6.89 t.ha^{-1}. *D14*, *IR4630* and *IR70870-B-P-2-2* were the most productive varieties, respectively 5.52, 5.37 and 5.19 t.ha^{-1}; the sensitive check *IR31785* produced 3.21 t.ha^{-1}(Table 5).

Ndinderling: Biomass production was significantly influenced by the type of fertilizer and the different varieties used, but no interaction was observed between the type of fertilizer and variety (Table 4). The average biomass was 7.79 t.ha^{-1} and the control, OF, MF, and OMF gave respectively 4.53, 6.94, 8.88, and 10.81 t.ha^{-1}. *Sahel 236*, *D14* and *IR70870-B-P-2-2* varieties were the most productive respectively 9.67, 9.10 and 8.92 t.ha^{-1}. The sensitive check *IR31785* produced 4.26 t.ha^{-1} (Table 5). *D14*, *WAS161-B-6-1*, *Sahel 236*, *Sahel 201* and *IR4630* varieties have performed well; they can produce up to 4 t.ha^{-1} grain yield in a salt water environment (Figure 8) higher than average yield obtained by farmers in this region (1.5 t.ha^{-1}) (Mbodj, 2008). Thus, this can provide better alternatives for farmers.

Water use efficiency (WUE)

Ndour Ndour: WUE was significantly influenced by the type of fertilizer and the variety. Difference between tolerant varieties and the sensitive variety was significant ($P < 0.05$), however no significant difference within tolerant varieties was observed ($P < 0.05$). Average WUE of sensitive variety (IR 31785) was 0.59 kg.m^{-3} and among the tolerant varieties, D14 gave the greater WUE (1.01 kg.m^{-}3). Average WUE was 0.37; 0.68; 1.12 and 1.26 kg.m^{-3} respectively under control, OF, MF and OMF (Table 6). As comparison to control, OF and MF, average WUE under OMF was increased by 71, 46 and 11%, respectively.

Ndinderling: WUE was significantly influenced by the type of fertilizer and the variety. Difference between tolerant varieties and the sensitive variety was significant ($P < 0.05$); however no significant difference within tolerant varieties was observed ($P < 0.05$). Average WUE

Table 5. Effects of fertilizer treatments on above-ground biomass production (t.ha^{-1}).

Rice cultivar	Ndour Ndour					Ndinderling				
	Ctrl	OF	MF	OMF	Mean	Ctrl	OF	MF	OMF	Mean
D14	2.20	4.93	6.63	8.31	5.52^{a}	4.99	7.90	9.58	13.93	9.10^{a}
IR66946-3R-178-1-1	2.74	2.49	6.39	7.47	4.77^{ab}	3.98	6.62	9.31	11.02	7.73^{ab}
WAS161-B-6-1	1.30	3.90	5.66	5.95	4.20^{ab}	5.19	5.13	10.48	9.62	7.60^{ab}
IR70870-B-P-2-2	1.82	4.11	8.90	5.92	5.19^{a}	4.95	9.40	10.13	11.18	8.92^{a}
IR67076-2R-15-3	1.93	3.64	6.71	6.73	4.75^{ab}	5.19	7.10	9.81	10.05	8.04^{ab}
WAS197-B-8-2	2.22	4.33	5.92	6.42	4.72^{ab}	4.55	5.67	7.82	10.09	7.03^{ab}
IKP	1.27	3.52	4.83	7.03	4.16^{ab}	4.47	6.01	6.50	8.85	6.46^{ab}
Sahel 236	3.10	3.00	6.37	7.13	4.90^{ab}	4.78	8.40	11.08	14.40	9.67^{a}
IR4630	1.62	4.52	6.99	8.35	5.37^{a}	3.80	8.48	9.32	12.56	8.54^{ab}
Sahel 201	2.27	4.56	4.72	7.30	4.71^{ab}	4.98	7.44	9.22	11.79	8.36^{ab}
IR31785	1.58	1.73	4.37	5.13	3.21^{b}	3.00	4.23	4.40	5.40	4.26^{b}
Mean	2.01^{b}	3.70^{b}	6.14^{a}	6.89^{a}		4.53^{b}	6.94^{b}	8.88^{ab}	10.81^{a}	

Mean with the same letters are not significantly different by Student Newman Keuls test at the 0.05 level.

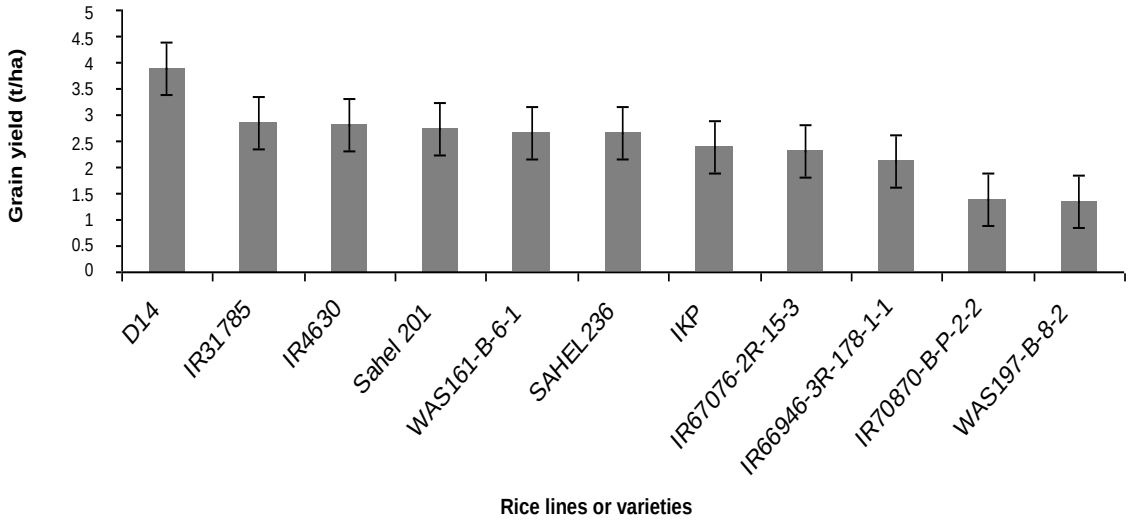

Figure 8. Average grain yields of the eleven rice varieties in moderate saline condition of Ndinderling with statistical range equal 1.3 t/ha.

of sensitive variety (*IR 31785*) was 0.56 kg.m^{-3} and among the tolerant varieties, *Sahel 236* gave the greater WUE (1.28 kg.m^{-3}). Average WUE was 0.60, 0.92, 1.17, and 1.43 kg.m^{-3} under control, OF, MF and OMF (Table 6). As comparison to control, OF and MF, average WUE under OMF was increased by 58, 36 and 18% respectively.

DISCUSSION

In these lowlands degraded by salt, there should be a gap between the beginning of the rainy season and planting date. In fact, the sowing should intervene after the lowlands have received rainfall of at least 100 mm. This is much enough to leach the salt that has been accumulated during the dry season under 3 dS/m which is tolerable for rice. In these lowlands, rice water need must therefore take into account that quantity needed for the leaching of salt. As the map of salinity (Figure 2) shows, the levelling is an essential element for the proper salt leaching. The lowlands must have a low slope (eg0.1%) descending toward the anti-salt dam. Once the valve is closed there will be a uniform height of water throughout the lowland and saline water can be evacuated. Good leaching allows uniform salinity. Salinity higher than 3 dS/m will have no effect on the germination

Table 6. Effects of fertilizer treatments on the water use efficiency (WUE in kg.m^{-3}).

Rice cultivars	Ndour ndour					Ndinderling				
	Ctrl	OF	MF	OMF	Mean	Ctrl	OF	MF	OMF	Mean
D14	0.40	0.90	1.21	1.52	1.01 ^{a}	0.66	1.04	1.26	1.84	1.20^{a}
IR66946-3R-178-1-1	0.50	0.46	1.17	1.37	0.87^{a}	0.53	0.87	1.23	1.45	1.02^{a}
WAS161-B-6-1	0.24	0.71	1.03	1.09	0.77^{a}	0.68	0.68	1.38	1.27	1.00^{a}
IR70870-B-P-2-2	0.33	0.75	1.63	1.08	0.95^{a}	0.65	1.24	1.34	1.48	1.18^{a}
IR67076-2R-15-3	0.35	0.66	1.23	1.23	0.87^{a}	0.68	0.94	1.30	1.33	1.06^{a}
WAS197-B-8-2	0.41	0.79	1.08	1.17	0.86^{a}	0.60	0.75	1.03	1.33	0.93^{a}
IKP	0.23	0.64	0.88	1.29	0.76^{a}	0.59	0.79	0.86	1.17	0.85^{a}
Sahel 236	0.57	0.55	1.16	1.30	0.90^{a}	0.63	1.11	1.46	1.90	1.28^{a}
IR4630	0.30	0.83	1.28	1.53	0.98^{a}	0.50	1.12	1.23	1.66	1.13^{a}
Sahel 201	0.42	0.83	0.86	1.34	0.86^{a}	0.66	0.98	1.22	1.56	1.10^{a}
IR31785	0.29	0.32	0.80	0.94	0.59^{b}	0.40	0.56	0.58	0.71	0.56^{b}
Mean	0.37^{b}	0.68^{b}	1.12 ^{a}	1.26 ^{a}		0.60^{b}	0.92^{ab}	1.17^{ab}	1.43^{a}	

Mean with the same letters are not significantly different by Student Newman Keuls test at the 0.05 level.

Table 7. Average young seedling survival rate (YSSR) in saline soil of Ndour Ndour.

Rice varieties	Average YSSR (%)
D14	82^{a}
WAS161-B-6-1	80^{a}
WAS197-B-8-2	81^{a}
IR66946-3R-178-1-1	74^{a}
IR70870-B-P-2-2	78^{a}
IR67076-2R-15-3	79^{a}
IR4630	83^{a}
IKP	85^{a}
Sahel 236	80^{a}
Sahel 201	85^{a}
IR31785	58^{b}

Values with the same letter are not significantly different by Student Newman Keuls test at the 0.05 level.

rate but will result in death of seedlings. Soil moisture was not used by the seedlings where the salinity exceeds 6 dS.m^{-1} because its increase osmotic potential and the varieties used have been selected at 6 dS/m. Indeed, at -2.34 MPa (osmotic potential = -039EC) (Chinnusamy et al., 2005), which is less than -1.5 MPa (soil water potential at permanent wilting point) (Chinnusamy et al., 2005) led to the death of many young seedlings (Table 7).

Indeed, the young seedlings were very sensitive to salinity (Zeng et al., 2000). At this, salt stress is added to hydric stress at the lowland Ndour Ndour. The effects of drought on yield are most severe when paddies are stressed by water deficit in the pre-flowering stage (Tsubo et al., 2006). The rainfall deficit versus the rice need for water was 18.3%. That does not include the losses of water due to lateral and downward movement (Tsubo et al., 2006). The impact of water deficit on rice is much accentuated in saline soils. As consequences, rice survival rate after seedling was decreased approximately by 20% with tolerant varieties and by 42% with sensitive variety (Table 7), and the number of tiller per plant was decreased by 25%, in Ndour ndour compared to Ndinderling (Table 8). This sensitivity of rice to salinity and water deficit stresses have been emphasized by some authors as (Tsubo et al., 2006; Walia et al., 2005; Tabbal et al., 2002; Ceuppens et al., 1997).

This lower rate of tillering at Ndour Ndour, compared to Ndinderling has affected biomass. Biomass production was greater in Ndinderling compared to Ndour ndour. At both sites, the OMF gave the best above-ground biomass. In fact, this combination increases the capacity of nutrient reserves and makes easy the availability of nutrients to plants (Craswell and Lefroy., 2001; Bado et al., 1997; Bationo and Mokwunye, 1991).

However, because of the relatively high organic matter at both sites, only application of MF gave also greater above-ground biomass compare to OF and the control treatments. Low rainfall, after August was not sufficient to prevent the capillary rise of water from the salt water which led to the high salinity at the end of the season at Ndour Ndour and resulted in the spikelets sterility. Nevertheless, at Ndinderling site, the sensitive check *IR31785* produced 4.26 t.ha^{-1}. *D14*, *WAS161-B-6-1*, *Sahel 236*, *Sahel 201* and *IR4630* varieties have performed well; they can produce up to 4 t.ha^{-1} grain yield in a salt water environment, higher than average yield obtained by farmers in this region (1.5 t.ha^{-1}) (Mbodj, 2008). Thus, this can provide better alternatives for farmers.

Significant yield and WUE increase can be expected by using both organic and mineral fertilizers and more water supplies. Moreover, WUE in Ndour ndour was lower

Table 8. Average number of tillers per plant.

Rice varieties	Ndour ndour	Ndinderling
D14	21[a]	24[a]
WAS161-B-6-1	18[a]	26[a]
WAS197-B-8-2	22[a]	26[a]
IR66946-3R-178-1-1	18[a]	21[a]
IR70870-B-P-2-2	19[a]	24[a]
IR67076-2R-15-3	20[a]	22[a]
IR4630	20[a]	26[a]
IKP	17[a]	22[a]
Sahel 236	20[a]	25[a]
Sahel 201	19[a]	22[a]
IR31785	13[b]	23[a]

Values with the same letter per column are not significantly different by Student Newman Keuls test at the 0.05 level.

than Ndinderling, respectively 0.86 and 1.03 $kg.m^{-3}$ in average.

Conclusion

Sowing at these salt lowlands must occur after a rainfall of at least 100 mm. This amount of water should be integrated into the rice water need. However, levelling the lowlands allows a uniform leaching. After sowing, the management of water flow at the anti-salt dam must take into account, not only leaching of salt, but also groundwater recharge. Indeed, it is this groundwater table that will allow rice to reach maturity at the end of rains in the rainfed lowlands, by mitigating late season drought on the one hand. On the other hand, this infiltration will reduce the salinity of water. Biomass production was greater in Ndinderling compared to Ndour ndour. In both sites, *D14* and *IR70870-B-P-2-2* were the most biomass productive varieties, respectively 5.52, 5.19 $t.ha^{-1}$ in Ndour Ndour; and 9.10 and 8.92 $t.ha^{-1}$ in Ndinderling. At both sites, OMF gave the best above-ground biomass. Because this combination increases the capacity of nutrient reserves and makes easy the availability of nutrients to plants. Among the eleven rice varieties tested, five have performed well: *D14*, *WAS161-B-6-1*, *Sahel 236*, *Sahel 201* and *IR4630*, with greater average grain yields of 4 $t.ha^{-1}$ compared to the 1.5 $t.ha^{-1}$ obtained by farmers. Significant yield and WUE increase can be expected by using both organic and mineral fertilizers and more water supplies. Results of this study provided useful information for integrated management of tolerant rice varieties, water control and fertilizers in the rainfed lowland rice. As perspective to improve this system, using of high yielding tolerant rice varieties associated with organic and mineral fertilizers and additional irrigation source can help to recover the abandoned land and improve rice productivity in this region.

Conflict of Interest

The authors have not declared any conflict of interest.

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REFERENCES

Agence Nationale de la Statistique et de la démographie (2009). Situation Economique et sociale de la Région de Kaolack. Kaolack, Senegal.

Allen RG, Pereira LS, Raes D, Smith M (1998). Crop evapotranspiration. FAO irrigation and drainage P. 56. FAO, Rome.

Bado BV, Sedogo MP, Cescas MP, Lompo F, Bationo A (1997). Effets à long terme des fumures sur le sol et les rendements du maïs au Burkina Faso. Agricultures 6(6):571-575.

Bado BV, De Vries ME, Haefele SM, Wopereis MCS, Ndiaye MK (2008). Critical Limit of Extractable Phosphorous in a Gleysol for Rice Production in the Senegal River Valley of West Africa. Communications in Soil Sci. Plant Anal. 39:202-206.

Bationo A, Mokwunye AU (1991). Role of manures and crop residue in alleviating soil fertility constraints to crop production with special reference to the Sahelian and Sudanian zones of West Africa. Fertil. Res. 29:117-125.

Bray RM, Kurtz LT (1945). Determination of total, organic and available forms of phosphorus in soils. Soil Sci. 59:39-45.

Camara M, Kebe M, Miezan KM (2008). Intensification de la riziculture de bas-fonds dans le Sine-Saloum (Sénégal). Cahiers Agric. N°5, Septembre-Octobre. P. 17.

Camara M, Miezan KM, Kebe M (2007). Caractérisation agronomique, économique et biophysique du continuum bas fond : cas des complexes sahéliens et soudano - sahéliens du Sine-Saloum au SENEGAL. Sci. Nat. 4(1):1-15.

Ceuppens J, Wopereis MCS, Miezan KM (1997). Soil Salinization Processes in Rice Irrigation Schemes in the Senegal River Delta. Soil Sci. Soc. Am. J. 61:1122-1130.

Chinnusamy V, Jagendrorf A, Zhu JZ (2005). Understanding and Improving Salt Tolerance in Plants. Crop Sci. 45:437-448.

Craswell ET, Lefroy RDB (2001). The role and function of organic matter in tropical soils. Nutr. Cycl. Agroecosyst. 61:7-18.

Dingkhun M, Sow A (1997). Potential yields of irrigated rice in the Sahel. In: Miézan, K.M., Wopereis, M.C.S.; Dingkhun et al., eds. Irrigated rice in the Sahel: Prospects for sustainable development. Bouaké (Côte d'Ivoire), WARDA.

Diop NF, Malou R, Da Costa H, Mendy A (2002). Contrainte climatique des nappes d'eau souterraines en zones soudano-sahéliennes : cas de la nappe phréatique du bassin de la Néma dans la région du Sine au centre-ouest du Sénégal. African journal of science and technology (AJST). Sci. Eng. 3(1):44-50.

FAO Statistical Databases (2012) FAO, Rome, Italy. http://faostat.fao.org/ (Accessed January).
Fukai S, Sitisaung P, Chanphengsay M (1998). Increasing production of rainfed lowland rice in drought prone environments: A case study in Thailand and Laos. Plant Prod. Sci. 1:75-82.
Kaushal KG, Bhabani SD, Mohammad S, Pratap BS (2009). Measurement and modelling of soil water regime in a lowland paddy field showing preferential transport. Agric. Water Manage. 96:1705–1714.
Kilian J, Teissier J, Raunet M, Lidon B, Ahmadi N, Traore B, Legoupil JC (1999). Caractérisation agro-écologique des bas fonds : Etude de leur utilisation traditionnelle et des systèmes de production en place. Bouaké, Côte d'Ivoire, ADRAO-CBF, Rapp. Tech. Du comité d'Experts du Consortium Bas-fonds P. 50.
Lancon F, Erenstein O (2002). Potential and prospects for rice production in West Africa. Sub-Regional Workshop on harmonization of policies and co-ordination of programmes on Rice in the ECOWAS sub-region. Accra, Ghana pp. 25-28.
Lilley JM, Fukai S (1994). Effect of timing and severity of water deficit on four diverse rice cultivars.I. Rooting pattern and soil water extraction.Field Crops Res. 37:205-213.
Mankeur F (1999). Rapport de prospection pédologique et de classification des sols dans la région de Fatick autour du Djilor. Kaolack, Sénégal, PAGERNA, Rapp. Technol. P. 20.
Maryse G (1991). Gestion de l'environnement et étude d'impact. Masson, Paris, P. 231.
Mbodj S (2001). Étude d'impact environnemental et méthodologie de construction des ouvrages anti sel ou de retenue dans le contexte PAGERNA. Kaolack, Senegal, PAGERNA, Rapp. Tech, P. 75.
Mbodj S (2008). Une meilleure valorisation des ressources des bas-fonds du Sine Saloum par la gire : expérience du programme de lutte contre la pauvreté en milieu rural dans le Bassin Arachidier.PRODDEL Rapp. Technol. P. 88.
Perez P (1994). Genèse du ruissèlement sur les sols cultivés du Sud Saloum (Sénégal), du diagnostic à l'aménagement de parcelle. Thèse. École Nationale Supérieure Agronomique de Montpellier P. 240.
SAED (2012). La filière riz au Sénégal. Panel de communication. Ministère de l'agriculture et de l'équipement rural P. 20.
SAS Institute Inc (2004) SAS/STAT User's Guide, Version 9.1, Cary, NC: SAS Institute.
Seck PA, Diagne A, Mohanty S, Mohanty S, Wopereis MCS (2012). Crops that feed the world 7: Rice. DOI 10.1007/s12571-012-0168-1.
Tabbal DF, Bouman B, Bhuiyan SI, Sibayan EB, Sattar MA (2002). On-farm strategies for reducing water input in irrigated rice; case studies in the Philippines. Agric. Water Manage. 56:93–112.
Tomar VS, Toole JCO (1980). Water use in lowland rice cultivation in Asia: a review of evapotranspiration. Agric. Water Manage 3:83-106.
Tsubo M, Basnayake, Fukai S, Sihathep V, Siyavong P, Sipaseuth, Chanphengsay M (2006). Top sequential effects on water balance and productivity in rainfed lowland rice ecosystem in Southern Laos. Field Crops Res. 97:209–220.
Walia H, Wilson C, Condamine P, Liu X, Ismail AM, Zeng L, Wanamaker SI, Mandal J, Xu J, Cui X, Close TJ (2005). Comparative transcriptional profiling of two contrasting rice genotypes under salinity stress during the vegetative growth stage. Plant Physiol. 139(2):822-835.
WARDA (2004) Strategic plan 2003-2012: The Africa Rice Center (ADRAO/WARDA). Bouaké, Côte d'Ivoire, Rap. Technol. P. 56.
Yoshida S, Benta WH (1983). Potential productivity of field crops under different environments. IRRI, Los Banos, Philippines.

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Full Length Research Paper

Protective effects of Ca^{2+} against NaCl induced salt stress in two lentil (*Lens culinaris*) cultivars

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Salinity affects ~ 950 million ha of the world's land area. More importantly, this worldwide problem is gradually increasing and limiting productivity. The aim of the present study was to investigate the protective effects of Ca^{2+} against NaCl induced salt stress in *Lens culinaris*. We comparatively analyzed growth, oxidative stress, photosynthetic potential and antioxidant enzyme activities in red and green lentils. Plants were allowed to germinate and then treated with or without NaCl (50, 200 mM) and/or $CaCl_2$ (5, 10 mM) for seven days. NaCl treatment decreased growth, chlorophyll content, carotenoid content and the activities of CAT and Ascorbate peroxidase (APX in both tested plants. Moreover MDA, H_2O_2 and proline levels were increased by NaCl treatment in red and green lentils, indicating that antioxidant system was disrupted by salinity. This study indicated that Ca^{2+} ameliorated the inhibitory effects of NaCl on growth and photosynthesis by regulating the activities of pivotal antioxidant enzymes such as superoxide dismutase (SOD), APX and catalase (CAT) in red and green lentils. Further studies are needed to investigate the underlying molecular mechanisms of Ca^{2+} dependent protective effects under salt stress.

Key words: *Lens culinaris*, calcium, salt stress, oxidative stress, antioxidant enzymes, lipid peroxidation.

INTRODUCTION

Salinity can be defined as the presence of soluble salts with excessive levels in soils or waters. If these salts contain a high proportion of sodium ions, it is called sodicity. Over six percent of the world's land and approximately one-third of agricultural areas are affected by these two increasing environmental problems (Laohavisit et al., 2013; Yadav et al., 2011). Decreasing in crop productivity is one of the most harmful consequences of salinity in particularly arid and semi-arid areas of the world.

Several metabolical and physiologica processes regulating plant growth can be damaged by salinity and sodicity. At first, cellular ionic balance is disrupted. The most common ions associated with salinity are Na^+, Mg^{2+}, Cl^- and HCO_3^-. Because of their dominant toxicity to plants, Na^+ and Cl^- are considered the most important. Increased concentrations of cellular Na^+ and Cl^- inhibit most enzymes and interfere RNA binding (Serrano et al., 1999). It is also shown that excessive uptake of Na^+ disrupts Na^+/K^+ homeostasis and may cause cellular injury and even cell death (Lockhart., 2013). The main phenotypic response of plants to salinity is inhibition

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of growth (Romero et al., 2001; Hilal et al., 1998). The mechanism underlying this response directly depends on absorption of water. When dissolved salt concentration in the soil is higher than inside the plant roots, water tends to move from roots to soil via osmosis, as a result, absorption of water to plant is reduced. Moreover, salt accumulation causes premature senescence in leaves, reduces the photosynthesis rate and directly affects vital processes by generating reactive oxygen species (ROS) such as hydrogen peroxide, nitric oxide, superoxide and hydroxyl radicals. Despite low levels of ROS can mediate salinity tolerance, excessive levels mostly damage to lipids, proteins, and nucleic acids (Xue et al., 2008). Conversely, plant cells have non-enzymatic antioxidants such as proline and antioxidant enzymes such as catalase (CAT) and superoxide dismutase (SOD) which are protect cell from oxidative damage. Therefore, the balance between ROS and antioxidants is crucial for plant survival and is needed to keep on the side of antioxidants (Munns, 2002; Tsuganea et al., 1999).

Unlike Na^+ and Cl^-, it is well known that some ions such as Ca^{+2} increase the adaptation of plant to salt stress by mainly regulating ion transport and exchange mechanisms. Moreover, calcium can also act as a second messenger in stress signaling, stabilizes cell wall structure and restores photosynthesis under NaCl stress. The underlying molecular mechanism of plant cell response to salt stress is regulated by salt overly sensitive (SOS) signaling pathway. Salt stress induces a cytosolic calcium-signal that activates the calcium sensor protein SOS3. SOS3 binds and activates SOS2, which is a member of serine/threonine kinase family. Activated SOS2 regulates the activities of Na^+/H^+ antiporters localized in both plasma membrane and vacuole. This results in Na^+ efflux out of cytosol or vacuole (Hadia and Karimib, 2012).

Lens culinaris is one of the first cultivated annual plants in Middle East and Europe. The wild subspecies, L. culinaris orientalis, is found in particularly Middle East including Turkey. Since this ancient plant has high protein content and is a good source of vitamin B, iron and phosphorus, it is widely consumed as food (Faris et al., 2013). The aim of the present study is to investigate the protective effects of Ca^{2+} against NaCl induced salt stress in red and green lentils.

MATERIALS AND METHODS

Plants Material and Metal Treatment

In this study, the red (yerli) and green (kışlık pul 11) lentil (L culinaris L.cv) cultivars seeds were provided by Field Crops Central Research Institute, Ankara, Turkey. Seeds were surface disinfected with sodium hypochloride (30%) for 10 min and washed with sterile water thoroughly. After imbibition, approximately 15 to 20 seeds were planted onto plastic trays covered with filter paper and cotton containing half-strength Hoagland's solution. They were grown for 10 days in a growth chamber at 23±2°C with 16-h light: 8-h dark photo-cycle at a light intensity of 40 mmol m^{-2} s^{-1}. At the 7th day of growth, salt stress treatment was initiated by applying half-strength Hoagland's solution containing 1.02 g/L KNO_3, 0.492 g/L $Ca(NO_3)_2$, 0.23 g/L KH_2PO_4, 0.49 g/L $MgSO_4.7H_2O$, 1.81 mg/L $MnCl_2.4H_2O$, 0.08 mg/L $CuSO_4.5H_2O$, 0.22 mg/L $ZnSO_4.7H_2O$, 0.09 mg/L $NaMoO_4.H_2O$ and $FeSO_4.7H_2O$ (0.5%) contained 0.6 ml/L (0.4%) tartaric acid to seedlings. Control plants, salt stressed (50, 200 mM NaCl) and NaCl+$CaCl_2$ treated (50 mM NaCl+5 mM Ca, 50 mM NaCl+10 mM Ca, 200 mM NaCl+5 mM Ca, 200 mM NaCl+10 mM Ca) and plants were grown in the growth chamber with the same physical parameters for another 7 days. The shoot and root tissues of 7 days old seedlings were then freshly used in the experiments.

Growth analysis

After 0 and 7 days of metal treatment, 1 g plants for each group were taken at random and divided into separate shoot and root fractions. The fresh weights of shoots and roots were weighed, and root lengths were measured. The samples were then dried in a forced draft oven at 70°C for 24 h, and the dry weights (g/g FW) were determined.

Determination of proline and H_2O_2 content

Proline content was determined according to the modified method of Bates et al. (1973). 0.5 g of shoot and root tissues from control and NaCl, Ca^{2+} treated plants were homogenized 1 ml of 5 % sulfosalicylic acid solution using homogenizer. The homogenate was then centrifuged at 13.000 g for 10 min. 1 ml of the supernatant was then added into a test tube to which 1 ml of glacial acetic acid and 1 ml of freshly prepared acid ninhydrin solution were added (1.25 g ninhydrin dissolved in 30 ml of glacial acetic acid and 20 ml of 6 M orthophosphoric acid). Tubes were incubated in a water bath for 1 h at 95°C and then allowed to cool to room temperature. 2 ml of toluene was added and mixed on a vortex mixture for 20 s in a fume hood. The test tubes were allowed to stand for at least 10 min to allow the separation of toluene and aqueous phase. The toluene phase was carefully pipetted out into a glass test tube and the absorbance was measured at 520 nm in a spectrophotometer. The concentration of proline was calculated from a proline standard curve. The concentration of proline was expressed as µmol/g FW.

The hydrogen peroxide content was determined according to Jana and Choudhuri (1981). Aliguats of fresh shoots and roots were homogenized in 50 mM potassium phosphate, pH 6.5 and centrifuged at 10 000 g for 25 min. The solution was mixed with 1% titanium chloride (in concentrated HCl) and then centrifuged at 10 000 g for 15 min The absorbance of the supernatant was measured at 410 nm and the H_2O_2 content calculated using 0.28 $\mu M^{-1}cm^{-1}$ as extinction coefficient.

MDA content

Lipid peroxidation was evaluated by measuring the amount of MDA amounts according to Heath and Packer (1968). 500 mg plant material was homogenized with 3 ml of 0.5% TBA in 20% TCA (W/V). The homogenized was incubated at 95°C for 30 min and the reaction was stopped in ice. The plant samples were centrifuged at 10 000 g for 15 min and absorbance of the resulting supernatant was recorded at 532 and 600 nm. The non-specific absorbance at 600 nm was subtracted from the 532 nm absorbance.

Chlorophyll and carotenoid contents

Concentration of chlorophylls and carotenoids were determined in

Table 1. Effect of Ca^{2+} enrichment on the fresh shoot weight, fresh root weight, shoot lenght root lenght and electrolyte leakage of 7-day red lentil seedling treated with or without 5.0-10 mol L−1 $CaCl_2$, 50 to 200 mmol L^{-1} NaCl. (a= compared to the control, b= compared to the 50 mM NaCl , c= compared to the 200 mM NaCl).

Red lentil	Shoot length(cm)	Root length(cm)	Shoot weight(g)	Root weight(g)	Electrolyte Leakage (µS/cm)	
					Shoot	Root
Control	19.85	8.78	0.1426	0.0522	48.00	57.53
50 NaCl	18.44 [a]	8.28 [a]	0.1360 [a]	0.0470 [a]	75.14 [a]	83.12 [a]
50 NaCl +5 Ca	18.87 [a]	8.90 [b]	0.1410 [b]	0.0526 [b]	60.98 [ab]	72.11 [ab]
50 NaCl + 10 Ca	19.71 [b]	9.35 [ab]	0.1544 [ab]	0.0639 [ab]	50.76 [ab]	64.45 [ab]
200 NaCl	16.28 [a]	5.05 [a]	0.0698 [a]	0.0417 [a]	120.45 [a]	133.17 [a]
200 NaCl + 5 Ca	17.30 [ac]	5.42 [ac]	0.0801 [ac]	0.0462 [ac]	110.66 [ac]	112.10 [ac]
200 NaCl + 10 Ca	18.78 [ac]	6.81 [ac]	0.1057 [ac]	0.0676 [ac]	98.70 [ac]	107.11 [ac]
RSD	0.36	0.06	0.004	0.003	1.25	3.38

DMSO extract of the young fully expanded leaf by the method of Lichtenthaler and Wellburn (1983). The homogenate was centrifuged at 4000×g for 10 min to remove the residue. The color intensity of clear supernatant was measured at 665, 645 and 470 nm for chlorophyll a, chlorophyll b and carotenoids, respectively. Results have been expressed as mg chlorophyll or carotenoids mg/g fresh weight.

Enzyme extracted and enzyme activities assays

Fresh shoot and root samples weighting about 1 g were homogenized using chilled mortar and pestle in 5 ml of cold 20 mM potassium phosphate buffer (pH 7.0) containing 1.0% insoluble polyvinyl pyrolidone (PVP) in ice bath. The homogenates were centrifuged at 12 000 g for 30 min. The supernatant was stored at 4°C and used for enzyme assays.

Ascorbate peroxidase (EC. 1.11.1.11) activity was measured immediately in fresh extracts and was assayed as describes by Nakano and Asada (1981) using a reaction mixture containing 25 mM potassium phosphate buffer (pH 7.0), 0.1 mM H_2O_2, 0.5 mM ascorbate and 0.1 mM EDTA. The hydrogen peroxide-dependent oxidation of as was followed by a decrease in the absorbance at 290 nm. The activity of APX was calculated using the extinction coefficient of 2.8 mM^{-1} cm^{-1}.

Catalase (EC 1.11.1.6) activity was determined by monitoring the described of H_2O_2 which was carried out by measuring the decrease in absorbance at 240 nm of a reaction mixture containing 25 mM potassium phosphate buffer (pH 7.0), 10 mM H_2O_2 and enzyme extract (Aebi, 1984).

SOD (EC 1.15.1.1) activity was measured spectrophotometrically as described by Beyer and Fridovich (1987). In this assay, 1 unit of SOD is defined as the amount required to inhibit the photo reduction of nitroblue tetrazolium by 50%. The activity of SOD was expresed as Unit/g fresh weight.

Statistical analysis

All experiments were carried out in triplicate and results were expressed as mean ± S.D. Data analysis and graphs were done by Graphpad Prism 6.0 software (La Jolla, CA, USA). Differences were analyzed by using two-way ANOVA at level of significance of $p<0.05$.

RESULTS

Growth analysis

For growth analysis, plants were allowed to germinate and then treated with or without NaCl (50, 200 mM) and/or $CaCl_2$ (5, 10 mM) for seven days in sand culture under glass house conditions. At the end of the day 7, growth of both red and green lentil was significantly reduced by 50 and 200 mM NaCl treatment (Tables 1 and 2).

In 50 and 200 mM NaCl treated red lentil, shoot length was decreased by 7.1 and 17%, while it was decreased by 4.65 and 12% in green lentil, respectively, as compared to untreated control. Combination of 200 mM NaCl and 10 mM Ca^{2+} treated resulted in an increased shoot length in red and green lentils by 15 and 9%, respectively as compared to 200 mM NaCl. Shoot weight was decreased by 62 and 39% in red and green lentils treated with 200 mM NaCl, respectively, as compared to untreated control. Combination of 200 mM NaCl and 10 mM Ca^{2+} treated resulted in increased shoot weight by 42 and 14% in red and green lentils, respectively as compared to 200 mM NaCl.

Chlorophyll (Chl) content

The presence of NaCl decreased total chlorophyll (Chl a + Chl b) and total carotenoid levels in a concentration dependent manner in the shoots of both plants. In red lentil, 200 mM NaCl treatment decreased Chl a, Chl b, and total carotenoid levels by 54, 58 and 61%, respectively, as compared to untreated control. Combination of 10 mM Ca^{2+} and 200 mM NaCl significantly decreased the inhibitory effects of NaCl on Chl a, Chl b and total carotenoid levels by 22, 76 and 34%, respectively, as compared to 200 mM NaCl (Table 3). In green lentil, 200 mM NaCl treatment decreased Chl a,

Table 2. Effect of Ca^{2+} enrichment on the fresh shoot weight, fresh root weight, shoot lenght root lenght and electrolyte leakage of 7- day green lentil seedling treated with or without 5.0 to 10 mol L^{-1} $CaCl_2$, 50-200 mmol L^{-1} NaCl. (a= compared to the control, b= compared to the 50 mM NaCl , c= compared to the 200 mM NaCl).

Green lentil	Shoot length (cm)	Root length (cm)	Shoot weight (g)	Root weight (g)	Electrolyte Leakage (µS/cm)	
					Shoot	Root
Control	21.04	8.24	0.2294	0.1422	73.02	86.26
50 NaCl	20.06 [a]	5.01 [a]	0.2029 [a]	0.0826 [a]	90.13 [a]	102.23 [a]
50 NaCl +5 Ca	24.85 [ab]	7.71 [ab]	0.2414 [ab]	0.1200 [ab]	72.33 [b]	97.12 [ab]
50 NaCl +10 Ca	26.20 [ab]	7.90 [ab]	0.2602 [ab]	0.1321 [ab]	63.16 [ab]	90.10 [ab]
200 NaCl	18.5 [a]	3.40 [a]	0.1393 [a]	0.0706 [a]	130.74 [a]	148.65 [a]
200 NaCl +5 Ca	19.35 [ac]	6.07 [ac]	0.1558 [ac]	0.0810 [ac]	122.23 [ac]	145.00 [a]
200 NaCl +10 Ca	20.21 [ac]	7.85 [ac]	0.1686 [ac]	0.0933 [ac]	111.39 [ac]	127.34 [ac]
RSD	0.32	0.05	0.007	0.004	2.26	4.28

Table 3. Effect of Ca^{2+} enrichment on the photosynthetic pigments of 7- day red lentil seedling treated with or without 5.0 to 10 mol L−1 $CaCl_2$, 50 to 200 mmol L^{-1} NaCl. (a= compared to the control, b= compared to the 50 mM NaCl , c= compared to the 200 mM NaCl).

Red lentil	Chl a (mg/g fresh weight)	Chl b (mg/fresh weight)	Total Chl (mg/g fresh weight)	Chl a/b (mg/g fresh weight)	Total Carotenoid (mg/g fresh weight)
Control	6.31	1.97	8.28	3.20	1.96
50 NaCl	4.52 [a]	1.25 [a]	5.77 [a]	3.61 [a]	1.66 [a]
50 NaCl + 5 Ca	4.70 [ab]	1.60 [ab]	6.33 [ab]	2.95 [ab]	1.74 [ab]
50 NaCl + 10 Ca	5.14 [ab]	1.87 [ab]	7.01 [ab]	2.74 [ab]	1.87 [ab]
200 NaCl	2.90 [a]	0.82 [a]	3.72 [a]	3.53 [a]	0.75 [a]
200 NaCl + 5 Ca	3.14 [ac]	0.94 [ac]	4.08 [ac]	3.34 [ac]	0.88 [ac]
200 NaCl + 10 Ca	3.56 [ac]	1.45 [ac]	5.01 [ac]	2.45 [ac]	1.01 [ac]
RSD	0.08	0.04	0.09	0.04	0.03

Table 4. Effect of Ca2+ enrichment on the photosynthetic pigments of 7- day green lentil seedling treated with or without 5.0 to 10 mol L^{-1} $CaCl_2$, 50 to 200 mmol L^{-1} NaCl. (a= compared to the control, b= compared to the 50 mM NaCl , c= compared to the 200 mM NaCl).

Green lentil	Chl a (mg/g fresh weight)	Chl b (mg/g fresh weight)	Total Chl (mg/g fresh weight)	Chl a/b (mg/g fresh weight)	Total Carotenoid (mg/g fresh weight)
Control	5.56	2.91	8.47	1.91	1.65
50 NaCl	4.65 [a]	1.31 [a]	5.96 [a]	3.54 [a]	1.21 [a]
50 NaCl + 5 Ca	5.67 [b]	2.84 [ab]	8.51 [b]	1.99 [ab]	1.55 [ab]
50 NaCl + 10 Ca	5.83 [ab]	2.98 [b]	8.81 [ab]	1.95 [b]	1.66 [b]
200 NaCl	2.58 [a]	0.76 [a]	3.34 [a]	3.39 [a]	0.86 [a]
200 NaCl + 5 Ca	2.79 [ac]	0.95 [ac]	3.74 [ac]	2.93 [ac]	0.99 [ac]
200 NaCl + 10 Ca	2.95 [ac]	1.13 [ac]	4.08 [ac]	2.61 [ac]	1.11 [ac]
RSD	0.01	0.04	0.08	0.05	0.04

Chl b, and total carotenoid levels by 53, 73 and 47%, respectively, as compared to untreated control. Combination of 10 mM Ca^{2+} and 200 mM NaCl significantly decreased the inhibitory effects of NaCl on Chl a, Chl b and total carotenoid levels by 14, 48 and 29%, respectively, as compared to 200 mM NaCl (Table 4).

MDA, H_2O_2 and proline contents

The presence of NaCl led to an increase in MDA levels in a concentration dependent manner in the shoots of both plants. 200 mM NaCl treatment increased MDA levels by 73 and 66% in the shoots of red and green lentils,

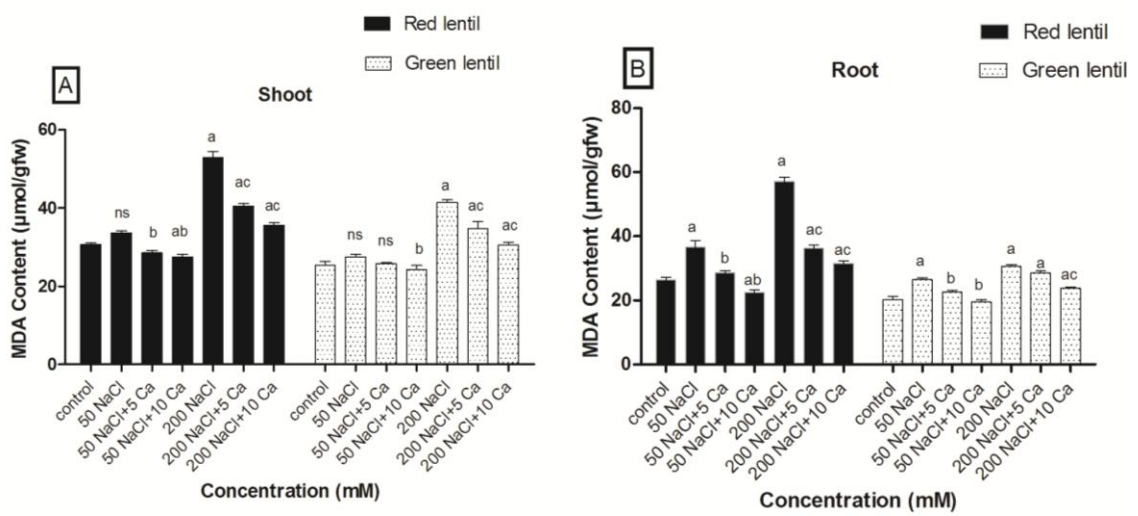

Figure 1. Effect of Ca^{2+} enrichment on the MDA content (mol/gFW) of 7-day old red and green lentil seedlings treated with or without 5,10 mM $CaCl_2$, 50, 200 mM NaCl. (A=Shoot, B=Root) (a= compared to the control, b= compared to the 50 mM NaCl , c= compared to the 200 mM NaCl).

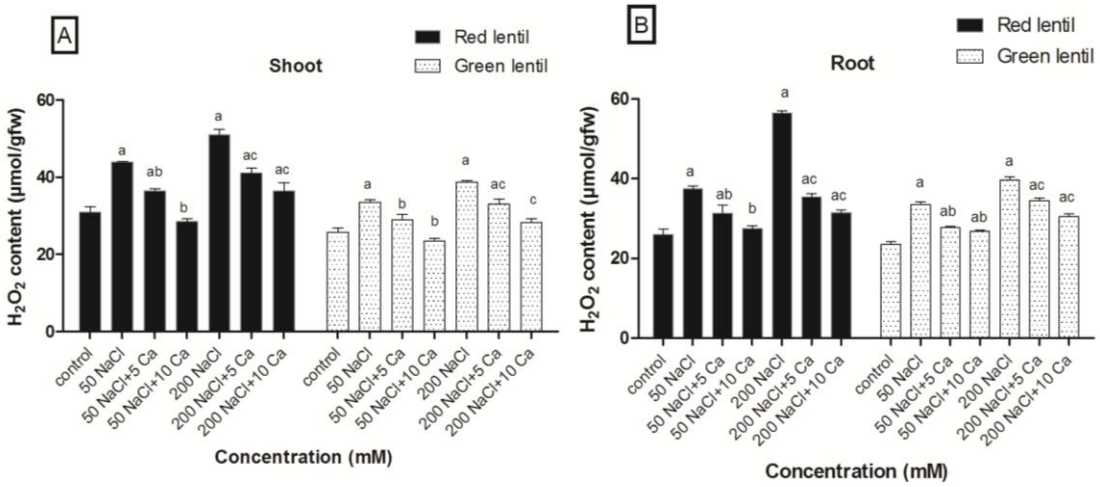

Figure 2. Effect of Ca^{2+} enrichment on the H_2O_2 content (mol/gFW) of 7-day old red and green lentil seedlings treated with or without 5,10 mM $CaCl_2$, 50, 200 mM NaCl. (A=Shoot, B=Root) (a= compared to the control, b= compared to the 50 mM NaCl , c= compared to the 200 mM NaCl).

respectively, as compared to untreated control. Combination of 10 mM Ca^{2+} and 200 mM NaCl solution reduced MDA levels by 33 and 26% in red and green lentils, respectively, as compared to 200 mM NaCl (Figure 1A). Same results were observed in the roots of both plants. Combination of 10 mM Ca^{2+} and 200 mM NaCl decreased MDA content by 44 and 20% in the shoots of red and green lentils, respectively, as compared to 200 mM NaCl (Figure 1B). H_2O_2 content also increased in a concentration dependent manner by NaCl treatment in the shoots of both plants. In the shoots of red lentil treated with 50 and 200 mM NaCl, H_2O_2 content was increased by 41 and 64%, respectively, while it was increased by 29 and 50% in the leaves of green lentil, as compared to untreated controls (Figure 2A). Combination of 10 mM Ca^{2+} and 200 mM NaCl decreased H_2O_2 content by 28 and 27% in the shoots of red and green lentils, as compared to 200 mM NaCl (Figure 2B).

It was observed that proline content in both shoots and roots were significantly increased by NaCl treatment in a concentration dependent manner in red and green lentils

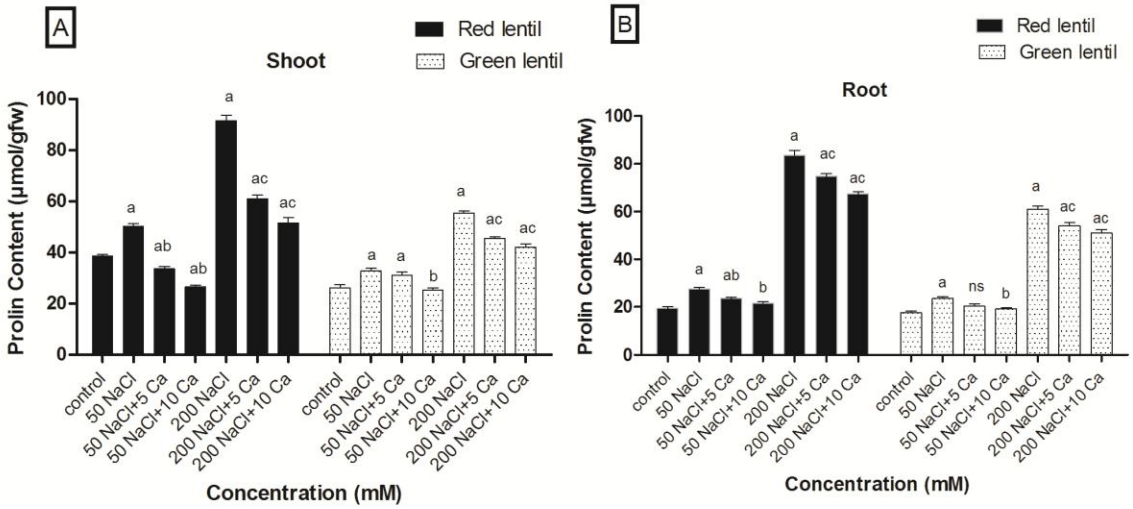

Figure 3. Effect of Ca^{2+} enrichment on the Prolin content (mol/gFW) of 7-day old red and green lentil seedlings treated with or without 5,10 mM $CaCl_2$, 50, 200 mM NaCl. (A=Shoot, B=Root) (a= compared to the control, b= compared to the 50 mM NaCl , c= compared to the 200 mM NaCl).

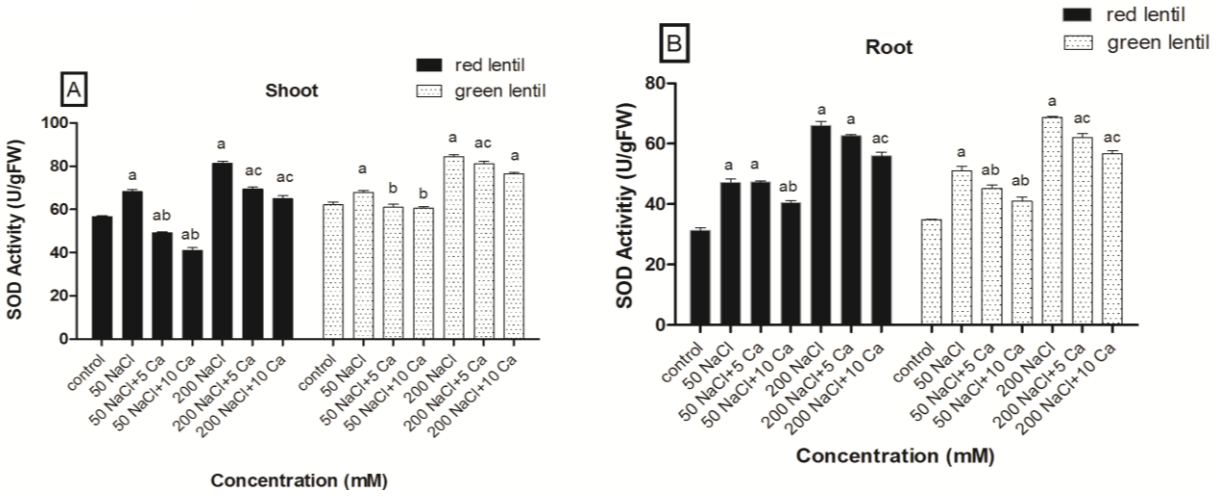

Figure 4. Effect of Ca^{2+} enrichment on the Superoxide Dismutase (SOD) Activity (U/gFW) of 7-day old red and green lentil seedlings treated with or without 5,10 mM $CaCl_2$, 50, 200 mM NaCl. (A=Shoot, B=Root) (a= compared to the control, b= compared to the 50 mM NaCl , c= compared to the 200 mM NaCl).

(Figure 3A). In the shoots of red and green lentils, combination of 10 mM Ca^{2+} and 200 mM NaCl significantly decreased proline content by 43 and 24%, respectively, as compared to 200 mM NaCl while in the roots, proline content was decreased by 19 and 16%, respectively, as compared to 200 mM NaCl (Figure 3B).

Activities of antioxidant enzymes

Activities of SOD, CAT and APX were measured by using spectrophotometric methods.

Superoxide dismutase (SOD) activity

SOD activity was increased by 20 and 9% in the shoots of green and red lentils treated with 50 mM NaCl, respectively. Combination of 50 mM NaCl and 10 mM Ca^{+2} resulted in an increased SOD activity by 39 and 10%, respectively (Figure 4A). 200 mM NaCl treatment increased SOD activity by 43 and 35%, respectively. In the root of both red and green lentils, combination of 200 mM NaCl and 10 mM Ca^{2+} resulted in an increased SOD activity by 15 and 17.5%, respectively, as compared to 200 mM NaCl (Figure 4B).

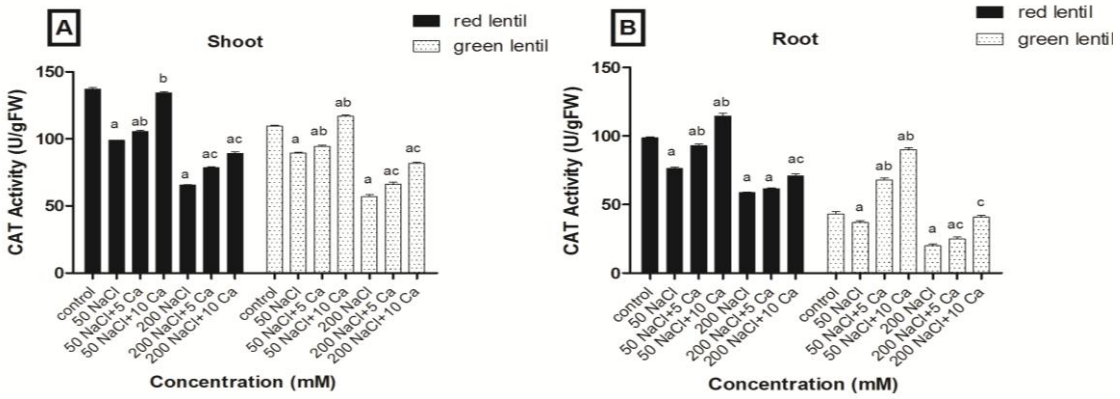

Figure 5. Effect of Ca^{2+} enrichment on the Catalase (CAT) Activity (U/gFW) of 7-day old red and green lentil seedlings treated with or without 5,10 mM $CaCl_2$, 50, 200 mM NaCl. (A=Shoot, B=Root) (a= compared to the control, b= compared to the 50 mM NaCl , c= compared to the 200 mM NaCl).

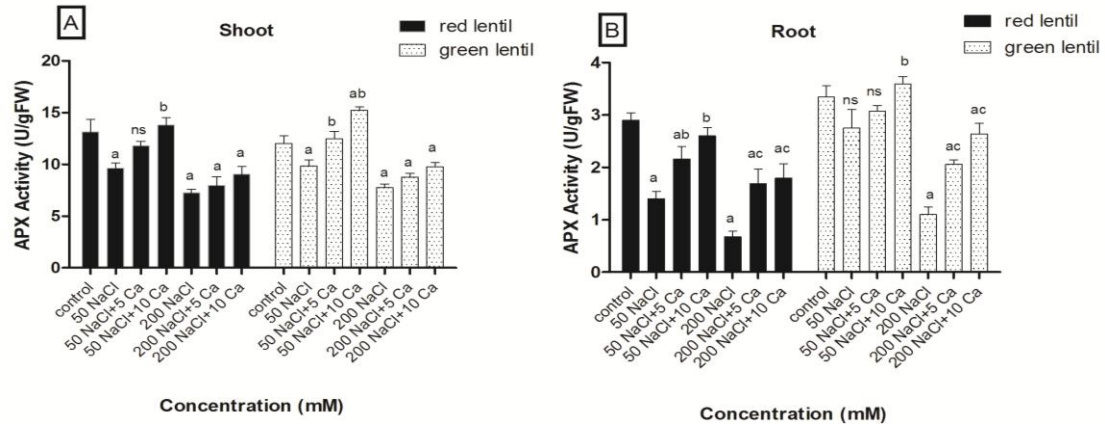

Figure 6. Effect of Ca^{2+} enrichment on the Ascorbat Peroxidase (APX) Activity (U/gFW) of 7-day old red and green lentil seedlings treated with or without 5,10 mM $CaCl_2$, 50, 200 mM NaCl. (A=Shoot, B=Root) (a= compared to the control, b= compared to the 50 mM NaCl , c= compared to the 200 mM NaCl).

Catalase (CAT) activity

In the shoot of red lentil after treatment with 50 and 200 mM NaCl, CAT activity was reduced by 27 and 52%, while in the stem of green lentil, it was reduced by 18 and 47%, respectively, as compared to untreated control. Combination of 200 mM NaCl and 5 mM Ca^{2+} resulted in an increased CAT activity in the shoot of red and green lentils by 19 and 15%, respectively, as compared to 200 mM NaCl (Figure 5A).

Ascorbate peroxidase (APX) activity

It was found that APX activity was reduced in the shoots and roots of both green and red lentils treated with NaCl in a concentration dependent manner (Figure 6A). In the shoot of red lentil treated with 50 mM and 200 mM NaCl, APX activity was reduced by 26 and 44%, while in the shoot of green lentil it was reduced by 18 and 35%, respectively, as compared to untreated control. Moreover, in the root of red lentil treated with 50 mM and 200 mM NaCl, APX activity was reduced by 51 and 76.9%, while in the root of green lentil it was reduced by 17 and 67%, respectively, as compared to untreated control. Combination of 50 mM NaCl and 5 mM Ca^{+2} resulted in an increased APX activity in the root of red and green lentils by 54 and 16%, respectively (Figure 6B).

DISCUSSION

Salinity affects approximately 950 million ha of the world's land area. More importantly, this worldwide problem is

gradually increasing and limiting plant growth and productivity. Currently, saline soil defined as having an electrical conductivity of the saturation extract (ECe) of 4 dS m^{-1} or more, and soils with ECe's exceeding 15 dS m^{-1} are considered strongly saline (Munns, 2002). From bacteria to plants, organisms develop biochemical and molecular mechanisms to adapt saline stress. In plants, many cellular processes including photosynthesis, membrane transport and protein synthesis are mainly affected during development. Salt tolerance can be defined as the ability of plant to complete its life cycle in soil which contains high concentrations of soluble salt (Parida and Das., 2005). It is well known that significant differences are found between salt tolerant plant species (Munns, 2002). Thus, every species and even subspecies should be assessed individually. Lentil species are considered extremely sensitive to salinity (ECe < 2 dS/m) as compared to other legumes such as soybean and broadbean (Sidari et al., 2008; Katerji et al., 2001). In this study, we investigated the protective effects of Ca^{+2} against NaCl induced salt stress in *Lens culinaris*, which is widely consumed as food in Middle East and Europe. We compared red and green lentils in terms of several physiological parameters including growth, chlorophyll content and antioxidant systems which are affected by NaCl stress. Moreover, aside from the effects of NaCl stress, ameliorative effects of Ca^{2+} in both plants were also investigated in this study.

The most common observed phenotypic response of plants to salinity is reduction of growth (Romero et al., 2001; Hilal et al., 1998). We found that NaCl (50 and 200 mM) treatment reduced growth parameters such as length and weight of shoots and roots in both tested plants in a dose dependent manner. Moreover, it was interesting to find that red lentil was more sensitive than green lentil to salt stress, according to our growth analysis. In parallel with our study, Bandeoğlu et al. (2004) reported a dose dependent reduction in the growth of lentil plant treated with 100 and 200 mM NaCl. Kökten et al. (2010) were investigated the effects of salinity on five lentil genotypes and they found that increasing concentrations of NaCl (50 to 200 mg/L) resulted in a significant decrease in length and weight of shoots and roots.

Nutrient enrichment is one of the most useful approaches to minimize the inhibitory effects of salinity (Manaa et al., 2013). N, P, K, Mg and Ca are widely used to reduce Na^{+} and Cl^{-} dependent injuries in plants (Kaya et al., 2003). In the present study, we examined the effects of Ca^{2+} to cope with NaCl dependent salinity in red and green lentils.

Calcium is one of the essential elements for growth and development of several organisms including plants. At first, plants use calcium as a seconder messenger to control numerous cellular processes including cell expansion, elongation, proliferation, circadian rhythms and fertilization. A lot of evidence suggest that calcium plays a crucial role in the adaptation of plants to different kinds of stresses including salt stress. It stabilizes cell wall structure, induces proline synthesis, activates antioxidant enzymes and restores photosynthesis under NaCl stress (Reddy and Reddy., 2004; Yang and Poovaiah., 2002). We showed that Ca^{2+} significantly reduced the growth inhibitory effects of NaCl in red and green lentils. Combination of 10 mM Ca^{+2} and 200 mM NaCl increased length and weight of shoots and roots in both tested plants as compared to 200 mM NaCl. Xue et al. (2008) found that Ca^{2+} enrichment (10 mol L^{-1}) significantly alleviated the inhibitory effect of NaCl on growth of the Jerusalem artichoke. Cha-um et al. (2012) were observed parallel results in *Oryza sativa* by using 1.98 mM $CaCl_2$ transferred to 200 mM NaCl solution. Protective effects of calcium against NaCl stress were also shown in soybean, *Withania somnifera*, linseed and *Rumex sp*. (Arshi et al., 2010; Khan et al., 2010). After the growth analysis, we measured chlorophyll and carotenoid contents to determine the effects of NaCl on the photosynthetic potential of red and green lentils. We found that NaCl treatment significantly decreased Chl a, Chl b, and total carotenoid levels in red and green lentils. Combination of 10 mM Ca^{2+} and 200 mM NaCl reduced the inhibitory effects of NaCl on Chl a, Chl b and total carotenoid levels in red and green lentils. Xue et al. (2008) found that NaCl-treated Jerusalem artichoke showed 12% loss in leaf chlorophyll content after 5 days of treatment. Moreover, they reported that addition of calcium significantly decreased the chlorophyll loss in NaCl-treated plants. Since salt stress is strongly correlated with the generation of reactive oxygen species and Ca^{+2} activates the molecules of antioxidant system, we measured the activities of three antioxidant enzymes (SOD, CAT and APX) and three well known indicators (MDA, proline and H_2O_2) of ROS generation by using spectrophotometric methods. In shoots and roots of both plants, NaCl treatment significantly increased MDA, proline and H_2O_2 levels in a concentration dependent manner. In parallel with the growth results of this study, we observed that red lentil was more sensitive than green lentil to salt stress dependent ROS generation. Combination of 10 mM Ca^{2+} and 200 mM NaCl reduced the effects of NaCl on MDA, proline and H_2O_2 levels in roots and shoots of both tested plants. H_2O_2 is one of the most common ROS which generated in the cell under the normal as well as stressed conditions (Sharma et al., 2012). While it acts as a seconder messenger involved in tolerance to various stresses at low concentrations, it is highly toxic and leads to the programmed cell death at high concentrations (Gill and Tuteja.,2010). Valderrama et al. (2006) and Saha et al. (2010) showed that H_2O_2 content was increased in *Olea europaea* and *Vigna radiata* under the salt stress. We have shown that Ca^{2+} alleviated NaCl dependent excessive H_2O_2 generation in red and green lentils. Malondialdehyde (MDA) is one of the final products of polyunsaturated fatty acid

peroxidation in the cell. Evaluation of MDA is commonly used as a marker of oxidative stress (Sharma et al., 2012). In parallel with this study, Tavallali et al. (2010) and Ashraf et al. (2010) reported that MDA levels were significantly increased in *Pistacia vera* and *Triticum aestivum* under the salt stress. We also showed that Ca^{2+} reduced NaCl dependent increased MDA levels in red and green lentils. Proline, a non-enzymatic antioxidant, is a scavenger of ROS under various stresses including salinity in plants (Gill and Tuteja., 2010). Moreover, accumulation of proline is an indicator of oxidative stress (Hare et al., 1998). We found that increased proline content under NaCl stressed red and green lentils was attenuated by Ca^{2+} treatment.

It was reported that antioxidant enzymes can be activated or inactivated under saline conditions. Xue et al. (2008) also suggested that Ca^{2+} controlled the activities of antioxidant enzymes in stressed plants. We have shown that NaCl treatment increased SOD activity in both tested lentils. However, CAT and APX activities were found to be decreased in both NaCl treated plants. We also found that in red and green lentils, Ca^{2+} regulated these enzymes under saline conditions. SOD is the most effective antioxidant enzyme and it provides the first line of defense against ROS generation under various environmental stresses including salt stress (Gill and Tuteja., 2010). Significant increase in SOD activity under saline stress has been reported in several plants such as *Lycopersicon esculentum* (Gapinska et al., 2008), *Jerusalem artichoke* ((Xue et al., 2008)), *Chrysanthemum morifolium* (Hossain et al., 2004) and *L. culinaris* (Bandeoğlu et al., 2004). We found that Ca boosted SOD activity in red and green lentils under saline stress. APX plays an important role in scavenging particularly H_2O_2 in various organisms including plants. It has been shown that increased APX levels enhanced salt tolerance in many plants including *L. culinaris* (Bandeoğlu et al., 2004; Gill and Tuteja ., 2010). We have shown that Ca^{2+} significantly increased APX activity which was reduced by NaCl treatment in red and green lentils. Another important H_2O_2 scavenger is CAT, which has one of the highest turnover rates in all enzymes. It can convert approximately 6 million molecules of H_2O_2 to H_2 and O_2 per minute (Gill and Tuteja., 2010). We found that Ca^{2+} increased the activity of CAT which was reduced by NaCl treatment in red and green lentils. Our results suggest that in salt stressed red and green lentils, inhibition of MDA, H_2O_2 and proline contents may be due to the stimulatory effects of Ca^{2+} on the activities of SOD, APX and CAT.

In conclusion, this study indicated that Ca^{2+} ameliorated the inhibitory effects of NaCl on growth and photosynthesis by regulating the activities of pivotal antioxidant enzymes such as SOD, APX and CAT in red and green lentils. Further studies are needed to investigate the underlying molecular mechanisms of Ca^{2+} dependent protective effects under salt stress.

Conflict of Interest

The authors have not declared any conflict of interest.

REFERENCES

Ashraf MA, Ashraf M, Ali Q (2010). Response of two genetically diverse wheat cultivars to salt stress at different growth stages: leaf lipid peroxidation and phenolic contents. Pak. J. Bot. 42:559-56.

Arshi A, Ahmad A, Aref IM, Iqbal M (2010). Calcium interaction with salinity-induced effects on growth and metabolism of soybean (*Glycine max* L.) cultivars. J. Environ. Biol. 31:795-801.

Bandeoğlu E, Eyidoğan F, Yücel M, Öktem HA (2004). Antioxidant responses of shoots and roots of lentil to NaCl-salinity stress. Plant Growth Regulat. 42:69-77.

Bates L (1973). Rapid determination in free proline of water stress studies. Plant Soil 39:205.

Beyer WF, Fridovich I (1987). Assaying for superoxide dismutase activity some large consequences of minor changes in conditions. Anal. Biochem. 161:559-566.

Cha-um S, Singh HP, Samphumphuang T, Kirdmanee C (2012). Calcium-alleviated salt tolerance in indica rice (Oryza sativa L. spp. indica): Physiological and morphological changes. AJCS 6”176-182.

Faris MAE, Takruri HR, Issa AY (2013). Role of lentils (Lens culinaris L.) in human healthand nutrition: a review. Mediterr J. Nutr. Metab. 6:3–16.

Gapinska M, Sk1odowska M, Gabara B (2008). Effect of short- and long-term salinity on the activities of antioxidative enzymes and lipid peroxidation in tomato roots, Acta Physiol. Plant, 30:11-18.

Gill SS, Tuteja N (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol. Biochem. 48:909-930.

Hadia MR, Karimib N (2012). The role of calcium in plants' salt tolerance. J. Plant Nutr. 35:2037-2054.

Hare P, Cress WA Van SJ (1998). Dissecting the roles of osmolyte accumulation during stress. Plant Cell. Environ. 21:535-553.

Heath RL, Packer L (1968). Photoperoxidation in isolated chloroplasts. Arch Biochem. Biophys. 125:189–198.

Hilal M, Zenoff AM, Ponessa G, Moreno H, Massa EM (1998). Saline stress alters the temporal patterns of xylem differentiation and alternative oxidase expression in developing soybean roots. Plant Physiol. 117:695–701.

Hossain Z, Mandal AKA, Shukla R, Datta SK (2004). NaCl stress- its chromotoxic effects and antioxidant behaviour in roots of Chrysanthemum morifolium Rama. Plant Sci. 166:215-220.

Jana S, Choudhuri MA (1981). Glycolate metabolism of three submerged aquatic angiosperms during aging. Aquat. Bot. 12:345–354.

Katerji N, Hoorn JWV, Hamdy A, Mastrorilli M, Oweis T, Erskine W (2001). Response of two varieties of lentil to soil salinity. Agric. Water Manage. 47:179–190.

Kaya C, Ak BE, Higgs D (2003). Response of salt-stressed strawberry plants to supplementary calcium nitrate and/or potassium nitrate. J. Plant Nutr. 26:543–560.

Khan MN, Siddiqui MH, Mohammad F, Naeem M, Masroor M, Khan A (2010). Calcium chloride and gibberellic acid protect linseed (*Linum usitatissimum* L.) from NaCl stress by inducing antioxidative defense system and osmoprotectant accumulation. Acta Physiol. Plant 32:121-132.

Kökten K, Karaköy T, Bakoğlu A, Akçura M (2010). Determination of salinity tolerance of some lentil (*Lens culinaris* M.) varieties. J. Food, Agric. Environ. 8:140-143.

Laohavisit A, Siân LR, Shabala L, Chen C, Renato Colaço DDR, Swarbreck SM, Shaw E, Dark A, Shabala S, Shang Z, Davies JM (2013). Salinity-Induced Calcium Signaling and Root Adaptation in Arabidopsis Require the Calcium Regulatory Protein Annexin1. Plant Physiol. 163:253-262.

Lichtenthaler HK, Wellburn AR (1983) Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. Biochem. Soc. Transact. 11:591-592.

Lockhart J (2013). Salt of the earth: ethylene promotes salt tolerance by enhancing Na/K homeostasis. Plant Cell 25:3150. doi: 10.1105/tpc.113.250911. Epub.Sep 24

Manaa A, Faurobert M, Valot B, Bouchet JP, Grasselly D, Causse M, Ahmed HB (2013). Effect of salinity and calcium on tomato fruit proteome. OMICS. 17:338-352.

Munns R (2002). Comparative physiology of salt and water stress. Plant Cell Environ. 25:239–250.

Nakano Y, Asada K (1981) Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. Plant Cell Physiol. 221:867-880.

Parida AK Das AB (2005). Salt tolerance and salinity effects on plants: A review. Ecotoxicol. Environ. Safety 60:324–349.

Reddy VS, Reddy ASN (2004). Proteomics of calcium-signaling components in plants. Phytochemistry 65:1745–1776.

Romero-Aranda R, Soria T, Cuartero J (2001). Tomato plant-water uptake and plant-water relationships under saline growth conditions. Plant Sci. 160:265-272.

Serrano R, Mulet JM, Ríos JAG, Márquez de Larriona IF, Leube MP, Mendizabal I, Pascual-Ahuir A, Proft MRR, Montesinos C (1999). A glimpse of the mechanism of ion homeostasis during salt stress. J. Exp. Bot. 50:1023-1036.

Saha P, Chatterjee P, Biswas AK (2010). NaCl pretreatment alleviates salt stress by enhancement of antioxidant defense system and osmolyte accumulation in mungbean (*Vigna radiata* L. Wilczek). Indian. J. Exp. Biol. 48:593-600.

Sharma P, Jha AB, Dubey RS, Pessarakli M (2012). Reactive Oxygen Species, Oxidative Damage, and Antioxidative Defense Mechanism in Plants under Stressful Conditions. J. Bot. Article ID 217037, P. 26.

Sidari M, Santonoceto C, Anastasi U, Preiti G, Muscolo A (2008). Variations in four genotypes of lentil under NaCl-salinity stress. American J. Agric. Biol. Sci. 3:410-416.

Tavallali V, Rahemi M, Eshghi S, Kholdebarin B, Ramezanian A (2010). Zinc alleviates salt stress and increases antioxidant enzyme activity in the leaves of pistachio (*Pistacia vera* L. 'Badami') seedlings. Turk. J. Agric. For. 34:349-359 © TÜBİTAK doi:10.3906/tar-0905-10

Tsuganea K, Kobayashia K, Niwaa Y, Ohbab Y, Wadab K, Kobayashia H (1999). A Recessive Arabidopsis Mutant That Grows Photoautotrophically under Salt Stress Shows Enhanced Active Oxygen Detoxification. Plant Cell 11:1195-1206

Valderrama R, Corpas FJ, Carreras A, Gómez-Rodríguez MV, Chaki M, Pedrajas JR, Fernández-Ocaña A, Del Río LA, Barroso JB (2006). The dehydrogenase-mediated recycling of NADPH is a key antioxidant system against salt-induced oxidative stress in olive plants. Plant, Cell. Environ. 29:1449–1459.

Yadav S, Irfan M, Ahmad A, Hayat S (2011). Causes of salinity and plant manifestations to salt stress: a review. J. Environ. Biol. 32:667-685

Yang T, Poovaiah BW (2002). Hydrogen peroxide homeostasis: Activation of plant catalase by calcium/calmodulin. Proc. Natl. Acad. Sci. U.S.A. 99:4097–4102.

Xue YF, Liu L, Liu ZP, Mehta SK, Zhao GM (2008). Protective role of Ca against NaCl toxicity in Jerusalem artichoke by up-regulation of antioxidant enzymes. Pedosphere 18:766–774.

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