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Differential Response of Niger (*Guizotia abyssinica* Cass.) Cultivars to Salinity Stress in Relation to Seed Germination, Oxidative Stress, Osmotic Adjustment and Antioxidant Enzyme Activities

Patil Pradip Pandit[1] • Ghane Savaliram Goga[1] • Barmukh Rajkumar Baban[2] • Jaime A. Teixeira da Silva[3] • Nikam Tukaram Dayaram[1*]

[1] Department of Botany, University of Pune, Pune 411 007, M.S., India
[2] Modern college of Arts, Science and Commerce, Shivajinagar, Pune 411 005, MS., India
[3] Department of Horticulture, Faculty of Agriculture, Kagawa University, Ikenobe, Miki-cho, 761-0795, Japan

Corresponding author: * **tdnikam@unipune.ernet.in**

ABSTRACT

Guizotia abyssinica Cass. (niger, Asteraceae) is an important but neglected edible oil seed crop. It is cultivated in the Indian subcontinent, Ethiopia and East African countries. Meager information is available on its physiology as compared to other crops. The present investigation reports the influence of NaCl stress (0, 20, 40, 60, 80 and 100 mM) on seed germination, growth, chlorophyll content, osmolyte accumulation and antioxidant enzyme activity in four cultivars ('IGP-76', 'GA-10', 'No. 71' and 'IGPN-2004') of niger. The observations were recorded on the 7th day after salt treatment. Increasing salt stress greatly reduced the seed germination percentage in 'GA-10' than 'No. 71' and 'IGPN-2004', whereas 'IGP-76' was least affected. A similar pattern was observed for growth (shoot and root length), formation of biomass and total chlorophyll content. Maximum damage to the cellular membrane, as evidenced by a higher accumulation of malondialdehyde, occurred in 'GA-10', whereas least damage to cellular membranes was observed in 'IGP-76'. GB content and catalase activity were higher in 'IGP-76' than 'No.71', 'IGPN-2004' and 'GA-10'. Therefore, 'IGP-76' is a salt-tolerant cultivar and 'GA-10' is more sensitive to salt stress than 'No. 71' and 'IGPN-2004'.

Keywords: Asteraceae, edible oil crop, 'IGP-76', NaCl stress
Abbreviations: CAT, catalase; **Chl**, chlorophyll, **DW**, dry weight **FW**, fresh weight; **GB**, glycine betaine; **MDA**, malondialdehyde; **SOD**, superoxide dismutase

INTRODUCTION

The world population will be about 9 billion in 2050, with most growth in developing countries. Meeting the food needs of this expanding population would require dramatic increase in agricultural productivity and additional 1.6 billion ha in arable land (Sairam and Prakash 2005). However, making available additional cultivable land is a challenging task. The main constraints of agriculture and food production are abiotic and biotic stresses. The United Nations Environment Program estimates that approximately 20% of agricultural land and 50% of cropland in the world are salt-stressed (Flowers and Yeo 1995). FAO data indicates that soil salinization is increasing and influencing plant survival and crop yield. It changes the nutritional status of the plant species through osmotic inhibition and ion toxicity. In addition, it induces production of reactive oxygen species and creates oxidative stress. Finally, the stress disturbs the physiological and biological function of plant cell, leading to cell death.

Many researchers have attempted screening of salt-tolerant or salt-sensitive cultivars at seedlings growth stage (Asharaf 1999) in different species. The relation of various seedling growth parameters to seed yield and yield components under saline conditions are important for the development of salt-tolerant cultivars (Mujeeb *et al.* 2008). One of the most effective ways to overcome salinity problem is the introduction of salt-tolerant varieties.

Guizotia abyssinica (niger, Asteraceae) is an important oilseed crop mainly cultivated in Indian subcontinent, Ethiopia and East African countries (Nikam and Shitole 1993). The seed contains about 40% edible oil with fatty acid composition of 75-80% linoleic acid, 7-8% palmitic acid, stearic acid and 5-8% oleic acid (Dutta *et al.* 1994). The seeds are used for making 'chutney' and condiments (Nikam and Shitole 1993). Niger is pollinated mainly by insects (particularly bees) (Geleta *et al.* 2002) and is strictly outcrossing via self-incompatibility mechanism(s). Nemomissa *et al.* (1999) and Pradhan *et al.* (1995) studied the genetic variability and character association in niger and suggested that the improvement of niger is difficult since morphological characters are not discrete and consequently, varietal identification or genetic purity assessment is difficult. High diversity exists among Indian niger cultivars and RAPD markers were used to identify and maintain niger germplasm for crop improvement purposes (Nagella *et al.* 2008). Nine flavonoids were isolated from the aerial parts of niger and the structures of these compounds were identified (Kuo *et al.* 2007). It has been recognized as a semi-domesticated crop (Dempewolf *et al.* 2008) and shares many characteristics with its wild relatives. Dempewolf *et al.* (2010) sequenced the chloroplast genome of niger. Lipid composition suggests that niger seeds could be nutritionally considered as a new non-conventional supply of seed oils (Ramadan and Morsel 2002). The procedure for *Agrobacterium tumefaciens*-mediated transformation has been reported in niger (Murthy *et al.* 2003). Though an important oil seed crop, information available on physiological and biochemical studies in niger grown under salt stress is limited.

Being a rain fed crop, niger is exposed to varied types

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of environmental conditions such as salinity, drought, flooding and low nutrients which adversely affect plant growth and productivity. Literature on the effect of salinity on the germination of niger cultivars is sparse. Therefore, it becomes necessary to elucidate the salinity stress tolerance mechanism in niger to screen the cultivars for better agronomic performance and also to breed and improve the tolerant cultivars.

Therefore, the objective of the present study was to evaluate the salinity tolerance responses in the four cultivars ('IGP-76', 'GA-10', 'No.71' and 'IGPN-2004') of niger with reference to seed germination and growth, biomass production, pigment content, osmolytes accumulation and antioxidant enzyme activities at seedling stage.

MATERIALS AND METHODS

Seed source, salinity treatment and culture conditions

The certified seeds of *G. abyssinca* cvs. 'IGP-76', 'GA-10', 'No.71' and 'IGPN-2004' were procured from the Zonal Agricultural Research Station, Western Ghats, Igatpuri, Nasik, Maharashtra, India.

Seeds of each cultivar were surface sterilized with 0.1% (w/v) mercuric chloride ($HgCl_2$) for 5 min followed by three times rinsing in sterilized distilled water. The seeds were blotted dry on tissue paper. Twenty seeds of each cultivar were placed separately on sterile germination paper in polypropylene Petri dish (90 × 15 mm, Axygen, India). Initially the germination paper was moistened with 5 ml of various levels of NaCl (0, 20, 40, 60, 80 and 100 mM) after which 2 ml NaCl solution was added every day to maintain the moisture. The Petri dishes were incubated at 25 ± 2°C under 8 h photoperiod and the light intensity of about 30 µmol m^{-2} s^{-1} from cool white fluorescent tube lights (Champion 40W, Philips Electronics Ltd., India).

Seed germination and growth analysis

Data on percentage seed germination and growth parameters (shoot and root length) were recorded on 7th day after sowing. The fresh weight (FW) was measured immediately after removal of the seedling from the Petri dish. The dry weight (DW) was determined after drying the samples in hot air oven at 60°C for 48 h.

Chl content

Chlorophyll (chl) *a*, chl *b* and total chl contents were estimated following the method described by Arnon (1949). Fresh cotyledonary leaves (100 mg) were homogenised in 2 ml 80% pre-chilled acetone. The homogenate was centrifuged at 4000 × *g* for 5 min at room temperature. The supernatant was decanted and volume was adjusted to 10 ml with 80% acetone and the absorbance was measured at 663 and 645 nm on a UV-visible spectrophotometer (Shimadzu-6405). The concentration of chl *a*, chl *b* and total chl were expressed as mg g^{-1} FW.

Estimation of proline

Free proline content was determined according to the method of Bates *et al.* (1973). Fresh shoot tissue (0.5 g) was homogenized in 5 ml 3% (w/v) aqueous sulfosalicylic acid. The filtered homogenate (2.0 ml) was reacted with 2.0 ml each of acid ninhydrin and glacial acetic acid and incubated for 1 h at 100°C. The reaction was terminated in an ice bath and after brining the reaction mixture to room temperature; 4.0 ml toluene was added and mixed vigorously with a stirrer for 15 sec. The toluene phase was aspirated from the aqueous phase and warmed to room temperature. The absorbance was recorded at 520 nm using toluene as a blank. Proline content was expressed as µg of proline g^{-1} FW.

GB analysis

The GB was quantified following the method of Greive and Grattan (1983). Fresh shoot tissue (500 mg) was powdered in liquid nitrogen. The powder was mechanically shaken with 20 ml of deionised water at 25°C for 16 h. The samples were filtered and the extract (500 µl) was diluted (1:1) with 2 N H_2SO_4. The extract (500 µl) was cooled in an ice bath for 1 h and then mixed with 200 µl of KI-I_2 reagent. The tubes were stored at 0-4°C for 16 h followed by centrifugation at 10,000 × *g* for 15 min at 0-4°C. The per-iodide crystals were dissolved in 9.0 ml of 1,2-dichloroethane, and the absorbance was measured at 365 nm after 2 h on UV-visible spectrophotometer (Shimadzu-6405). The GB content was expressed as mg g^{-1} FW.

Lipid peroxidation

The degree of lipid peroxidation was measured in terms of malondialdehyde (MDA) content (Heath and Packer 1968). Fresh shoot tissue sample (250 mg) was ground to fine powder in liquid nitrogen and then homogenized in 5 ml of 5% trichloro-acetic acid (TCA). The homogenized mixture was centrifuged at 10000 × *g* for 10 min at 4°C. Two ml supernatant was mixed with 2 ml 0.67% thiobarbituric acid (TBA). The mixture was heated to 100°C for 30 min, and quickly cooled in an ice bath and centrifuged at 5000 × *g* for 1 min. Absorbance of the supernatant was measured at 532 nm keeping 0.25% TBA in 10% TCA as blank. The amount of non-specific absorption at 600 nm was subtracted. The extent of lipid peroxidation was expressed as mmol of MDA formed g^{-1} of FW using absorption coefficient of 155 $mmol^{-1}$ cm^{-1}.

Antioxidant enzyme assay

1. Extraction

Fresh shoot tissue samples (500 mg) were homogenized in 5 ml of ice cold 50 mM sodium phosphate buffer (pH 7.0) containing 0.1 mM EDTA and 1% (w/v) polyvinylpyrrolidone (PVP). The homogenate was filtrated through single layered cheesecloth and the filtrate was centrifuged at 15000 × *g* for 20 min at 4°C. An appropriate aliquot/dilution of the supernatant was used as crude preparation of antioxidant enzymes. Soluble proteins in the enzyme preparation were determined according to Bradford (1976) with bovine serum albumin (BSA) as a standard.

2. SOD (EC 1.15.1.1) assay

SOD activity was determined by measuring its ability to inhibit photochemical reduction of nitroblue tetrazolium chloride (NBT) (Becana *et al.* 1998). The reaction mixture consisted 50 mM phosphate buffer (pH 7.8) and 0.1 mM EDTA to which an oxygen generating system comprising 14.3 mM methionine, 82.5 µM NBT, 2.2 µM riboflavin and 100 µL of crude enzyme in a total volume of 3 ml was added. Riboflavin was added at the end and the contents were mixed thoroughly. The entire system was exposed to the light of about 30 µmol $m^{-2}s^{-1}$ intensity for 30 min. The reaction was arrested by switching off the light source. The light blank was also incubated in light and was formed with all the reactants except enzyme extract; whereas all the reactants without enzyme extract incubated in the dark constituted dark blank. The reduction in NBT was measured by monitoring the change in absorbance at 560 nm. The absorbance of light blank was used in calculation of enzyme units. One unit (U) SOD activity was defined as an amount that produced 50% inhibition of NBT reduction under specified conditions. The results were expressed as unit (U) SOD activity mg^{-1} protein g^{-1} FW.

3. CAT (EC 1.11.1.6) assay

CAT activity was measured by following the decomposition of hydrogen peroxide as described by Cakmak and Marschner (1992) with some modifications. The activity was measured in a reaction mixture (1.0 ml) containing 25 mM phosphate buffer (pH 7.0) and 15 mM H_2O_2. The reaction was initiated by adding 50 µl enzyme extract and the activity was determined by monitoring the decrease in absorbance at 240 nm for 2 min at the intervals of 15 sec. The slope of absorbance plotted against time interval was considered as ΔA and the enzyme activity was expressed as µKat of catalase activity mg^{-1} protein.

Table 1 Effect of NaCl on germination percentage, root length, shoot length and root/shoot ratio in niger (*G. abyssinica* Cass.).

Cultivar	NaCl (mM)	Germination (%)	Root (R) length (cm)	Shoot (S) length (cm)	R/S ratio
IGP-76	0	100 a	5.4 ± 0.3 a	3.8 ± 0.2 a	1.4
	20	90.0 b	4.3 ± 0.1 b	3.2 ± 0.3 b	1.3
	40	80.0 c	4.1 ± 0.09 c	2.9 ± 0.3 c	1.4
	60	73.3 d	3.7 ± 0.08 d	2.0 ± 2.9 d	1.8
	80	55.0 e	3.3 ± 0.07 e	1.8 ± 0.3 e	1.8
	100	43.3 f	2.6 ± 0.2 f	1.0 ± 0.1 f	2.6
GA-10	0	99.3 a	4.4 ± 0.2 a	3.0 ± 0.4 a	1.4
	20	81.6 b	3.8 ± 0.5 b	2.7 ± 0.4 b	1.4
	40	76.6 c	3.2 ± 0.6 c	1.9 ± 0.2 c	1.7
	60	71.6 d	2.7 ± 0.2 d	1.6 ± 0.2 d	1.7
	80	65.0 e	2.3 ± 0.3 e	1.0 ± 0.2 e	2.3
	100	35.0 f	1.8 ± 0.06 f	0.7 ± 0.1 f	2.5
No. 71	0	99.0 a	4.8 ± 0.5 a	3.1 ± 0.3 a	1.5
	20	88.3 b	4.4 ± 0.4 b	3.0 ± 0.3 b	1.5
	40	86.6 c	4.0 ± 0.3 c	2.2 ± 0.1 c	1.7
	60	71.6 d	2.9 ± 0.3 d	1.7 ± 0.4 d	1.7
	80	56.6 e	2.7 ± 0.3 e	1.3 ± 0.04 e	2.0
	100	40.0 f	2.4 ± 0.1 f	1.0 ± 0.02 f	2.4
IGPN-2004	0	98.3 a	5.2 ± 0.04 a	4.0 ± 0.02 a	1.3
	20	93.3 b	4.5 ± 0.02 b	3.8 ± 0.06 b	1.2
	40	86.6 c	4.1 ± 0.05 c	3.3 ± 0.04 c	1.2
	60	81.7 d	3.5 ± 0.04 d	3.0 ± 0.03 d	1.2
	80	70.0 e	2.9 ± 0.04 e	2.1 ± 0.04 e	1.4
	100	40.0 f	2.5 ± 0.04 f	0.9 ± 0.02 f	2.8

Results are mean of three replicate ± SE.
Values followed by similar letters do not differ significantly at $P \leq 0.05$ as per DMRT

Table 2 Effect of NaCl on fresh weight and dry weight of seedlings of niger (*G. abyssinica* Cass.).

NaCl (mM)	FW (mg) Mean ±SE			
	IGP-76	GA-10	No.71	IGPN2004
0	165.9 ± 0.5 a	166.5 ± 0.4 a	165.2 ± 0.3 a	164.7 ± 0.6 a
20	158.0 ± 0.6 b	149.8 ± 0.3 b	159.7 ± 0.3 b	143.5 ± 1.3 b
40	149.2 ± 0.4 c	139.9 ± 0.3 c	151.3 ± 0.4 c	135.4 ± 0.4 c
60	139.4 ± 0.4 d	129.8 ± 0.4 d	139.8 ± 0.3 d	129.8 ± 0.4 d
80	126.1 ± 0.9 e	98.9 ± 0.4 e	129.2 ± 0.3 e	125.7 ± 0.5 e
100	123.2 ± 0.5 f	85.6 ± 0.4 f	121.5 ± 0.4 f	120.0 ± 0.5 f
	DW (mg) Mean ±SE			
	IGP-76	GA-10	No.71	IGPN2004
0	32.3 ± 0.48 a	32.4 ± 0.37 a	32.5 ± 0.25 a	32.1 ± 0.21 a
20	31.0 ± 0.38 ab	29.9 ± 0.18 b	32.3 ± 0.22 a	30.5 ± 0.22 ab
40	30.6 ± 0.35 ab	29.3 ± 0.13 b	31. 8 ± 0.30 a	29.0 ± 0.32 b
60	29.9 ± 0.18 bc	29.2 ± 0.21 b	29.8 ± 0.08 b	28.9 ± 0.01 b
80	28.7 ± 0.33 cd	22.9 ± 0.24 c	28.9 ± 0.34 b	27.0 ± 0.16 c
100	27.9 ± 0.28 e	21.4 ± 0.18 c	27.9 ± 0.06 b	26.7 ± 0.25 c

Results are mean of three replicate ± SE.
Values followed by similar letters do not differ significantly at $P \leq 0.05$ as per DMRT

Statistical analyses

Each experiment was performed on 20 seeds. All the experiments were arranged in a completely randomized design (CRD). The experiments were repeated at least thrice with three replicates of each treatment. The data were analyzed for one-way analysis of variance (ANOVA) using SPSS 9.0. The treatment means were compared by using Duncan's Multiple Range Test (DMRT) at $P = 0.05$ (Duncan 1955).

RESULTS AND DISCUSSION

Influence of salinity on germination, growth and pigment content

In nature, genetic variations offer a valuable tool for the selection of cultivars with desirable traits, such as salt or drought tolerance and disease resistance (Misra and Dwivedi 2004). Seed germination is one of the most critical phases of plant life greatly influenced by various abiotic factors like salinity, temperature, moisture, etc. In the present investigation, seeds of four niger cultivars were subjected to various levels of salinity stress and the response was analyzed at the seedlings stage.

As compared to control, NaCl treatment significantly reduced the rate of seed germination over the entire range of concentration in all the four cultivars of niger. At the highest level of NaCl (100 mM), 57% reduction in germination was observed in 'IGP-76' whereas 'GA-10' showed about 65% reduction as compared to control. 'No. 71' and 'IGPN-2004' showed intermediate reduction in percentage germination (**Table 1**).

As compared to control, increasing levels of NaCl significantly reduced the growth rates measured in terms of shoot and root length in all the cultivars screened. Likewise, the biomass production (FW and DW of the seedlings) also decreased significantly with increasing level of NaCl (**Table 2**). 'GA-10' was most affected by treatment of 100 mM NaCl and showed about 49% reduced fresh weight and about 34% reduced dry weight as compared to control. The similar treatment in 'IGP-76' produced only 25.7 and 13.6% reduction respectively than their control. 'No. 71' and 'IGPN-2004' showed intermediate reduction in the fresh and dry weight. Sumithra *et al.* (2006) showed similar res-

Table 3 Effect of NaCl on chlorophyll *a*, chlorophyll *b* and total chlorophyll in niger (*G. abyssinica* Cass.).

NaCl (mM)	Chlorophyll *a* (mg g^{-1} FW) Mean ± SE			
	IGP-76	**GA-10**	**No.71**	**IGPN-2004**
0	0.49 ± 0.1 a	0.37 ± 0.1 a	0.49 ± 0.1 a	0.38 ± 0.1 a
20	0.39 ± 0.2 b	0.30 ± 0.2 b	0.46 ± 0.2 b	0.34 ± 0.2 b
40	0.38 ± 0.1 c	0.26 ± 0.2 c	0.38 ± 0.1 c	0.33 ± 0.1 c
60	0.36 ± 0.2 d	0.25 ± 0.3 d	0.32 ± 0.2 d	0.32 ± 0.2 d
80	0.35 ± 0.2 e	0.13 ± 0.1 e	0.30 ± 0.3 e	0.21 ± 0.1 e
100	0.30 ± 0.1 f	0.10 ± 0.2 f	0.23 ± 0.1 f	0.18 ± 0.2 f
	Chlorophyll *b* (mg g^{-1} FW) Mean ± SE			
0	0.18 ± 0.2 a	0.17 ± 0.1 a	0.15 ± 0.1 a	0.24 ± 0.1 a
20	0.18 ± 0.1 b	0.14 ± 0.1 b	0.14 ± 0.2 b	0.14 ± 0.1 b
40	0.17 ± 0.3 c	0.10 ± 0.2 c	0.13 ± 0.1 c	0.13 ± 0.1 c
60	0.15 ± 0.1 d	0.08 ± 0.1 d	0.12 ± 0.1 d	0.10 ± 0.1 d
80	0.12 ± 0.2 e	0.07 ± 0.3 e	0.11 ± 0.1 e	0.08 ± 0.2 e
100	0.10 ± 0.3 f	0.05 ± 0.1 f	0.05 ± 0.1 f	0.07 ± 0.2 f
	Total Chlorophyll (mg g^{-1} FW) Mean ± SE			
0	0.67 ± 0.01 a	0.54 ± 0.1 a	0.60 ± 0.1 a	0.49 ± 0.1 a
20	0.53 ± 0.02 b	0.41 ± 0.1 b	0.55 ± 0.2 b	0.46 ± 0.2 b
40	0.50 ± 0.3 c	0.40 ± 0.2 c	0.51 ± 0.1 c	0.44 ± 0.2 c
60	0.49 ± 0.1 d	0.34 ± 0.3 d	0.44 ± 0.2 d	0.43 ± 0.1 d
80	0.45 ± 0.1 e	0.22 ± 0.1 e	0.41 ± 0.3 e	0.35 ± 0.4 e
100	0.40 ± 0.2 f	0.15 ± 0.1 f	0.29 ± 0.1 f	0.29 ± 0.1 f

Results are mean of three replicate ± SE.
Values followed by similar letters do not differ significantly at $P \leq 0.05$ as per DMRT

ponses in *Vigna radiata* cultivars subjected to various levels of NaCl. The FW and DW of shoots and roots gradually declined with the increase in NaCl stress from 100 to 300 mM. Compared to the control, the salt sensitive cultivar 'CO 4' showed a 56% decline while salt-tolerant 'Pusa Bold' exhibited a 43% decline in FW at a higher level of NaCl (300 mM).

Reduced germination percentages in salt-sensitive cultivars (< 20%), moderately tolerant cultivars (20-25%) and tolerant cultivars (>25%) of safflower (*Carthamus tinctorius* L.) have also been reported (Ezaz *et al.* 2007).

The reduction in germination percentage, growth and biomass might be associated with the accumulation of Na^+ in the salt sensitive cultivars, which alter the normal metabolism and functional state of the plant, resulting in physiological stress (Gasper *et al.* 2002). Jaleel *et al.* (2006) also reported trend of decreasing germination percentage and growth with increasing concentrations of NaCl stress in *Catharanthus roseus.* The reduction in FW and DW in all the cultivars of niger are suggestive of the physiological stress generated due to increased concentrations of NaCl.

Salinity severely affected chl *a*, chl *b* and total chl content in all cultivars of niger (**Table 3**). A significant reduction of about 40% in total chl content was observed in the seedlings of 'IGP-76' exposed to 100 mM NaCl as compared to control. Likewise, significant reduction (73%) in total chl content was also recorded in 'GA-10' followed by reasonable decline in 'No. 71' (52%) and 'IGPN-2004' (41%) at 100 mM NaCl as compared to their respective control. Thus, 'IGP-76' appears to be resistant to salt stress as compared to 'No. 71' and 'IGPN-2004', whereas 'GA-10' is most susceptible. The reduced chl content in salt stressed niger cultivars resulted in declined growth and biomass content. These results are in agreement with the reduced chl content observed in wheat genotypes under salt stress (Sairam *et al.* 2002). The decline in chl content is proportionate to the salinity levels, the time of exposure to salt and the species – salt tolerant or salt-sensitive (Siler *et al.* 2007).

Osmotic adjustment to salt stress through accumulation of osmolytes

Osmotic adjustment, wherein accumulation of inorganic ions (Na^+) or organic osmolytes i.e. proline and GB decrease the water potential of the cell without an accompanying decrease in cell turgor is a well-known phenomenon adapted by plants in response to salt stress (Ashraf 2004). Apart from osmotic adjustment, the osmolytes play an important role in detoxification of reactive oxygen species, protection of membrane integrity and stabilization of enzymes and proteins (Yancey *et al.* 1982; Bohnert and Jensen 1996). The accumulation of osmolytes in response to salt stress was significantly higher in salt tolerant lines as compared to salt sensitive (Ashraf and Foolad 2007).

The osmotic adjustment to salt stress in response to various levels of NaCl in niger was mediated by accumulation of organic osmolytes - proline (**Fig. 1**) and GB (**Fig. 2**). Higher levels of NaCl significantly accumulated both the osmolytes in all the four cultivars as compared to their control. 'IGP-76' showed the highest accumulation of proline (104.1-413.7 mg/g FW) at all the levels of NaCl as compared to its control (67.7 mg/g FW); whereas GA-10 showed least (86.5-383.5 mg/g FW) and 'IGPN-2004' and 'No. 71' showed moderate content of proline at all the concentrations of NaCl. Likewise, GB accumulation was higher in 'IGP-76' (1.98 m/g FW), least in 'GA-10' (1.79 m/g FW), and intermediate in 'IGPN-2004' and 'No. 71'.

Accumulation of higher proline content in salt-tolerant genotypes as compared to salt-sensitive ones was also reported in other crop species such as wheat (Sairam *et al.*

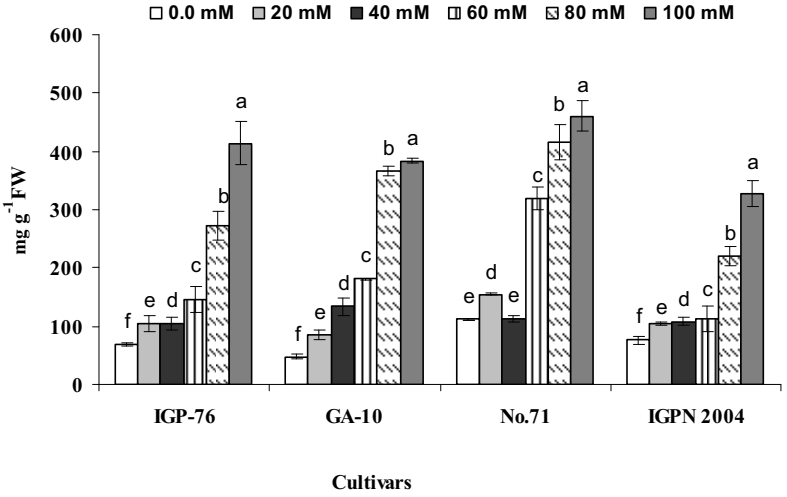

Fig. 1 Effect of salt stress on proline in niger (*G. abyssinica*). The values represent the mean ± SE. Values followed by similar letters do not differ significantly at $P \leq 0.05$ as per DMRT (n=6).

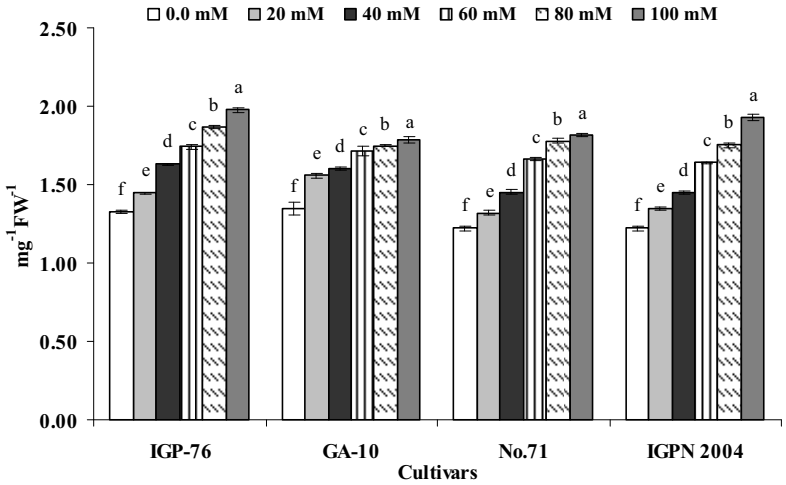

Fig. 2 Effect of salt stress on glycine betaine in niger (*G. abyssinica*). The values represent the mean ± SE. Values followed by similar letters do not differ significantly at $P \leq 0.05$ as per DMRT (n=6).

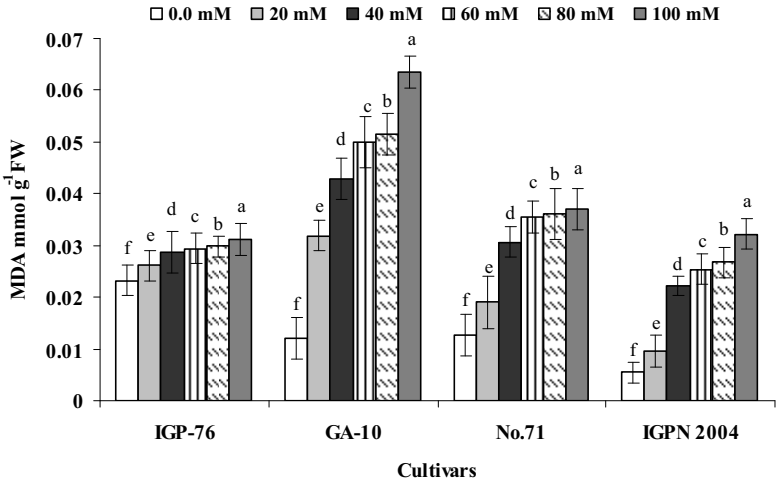

Fig. 3 Effect of salt stress on lipid peroxidation in niger (*G. abyssinica*). The values represent the mean ± SE. Values followed by similar letters do not differ significantly at $P \leq 0.05$ as per DMRT (n=6).

2002) and sorghum (Jogeswar *et al.* 2006). Similarly, the accumulation of GB in response to salt stress has been reported in tolerant genotypes and not in sensitive genotypes of sorghum (Yang *et al*. 2003). The relative concentrations of GB varied both among and within species, GB levels increased with seedling age and/or salinization in GB accumulating genotypes of sorghum. Total quaternary ammonium compound levels in the betaine fraction of 240 sorghum genotypes screened ranged from as low as 0.1 µmol g^{-1} FW to as much as 33 µmol g^{-1} FW (Yang *et al*. 2003). At a higher level (200 mM) of NaCl, the accumulation of GB in green gram (*Phaseolus aureus*) was 2-fold more in tolerant genotype 'T-44' and 1.3-fold more in sensitive genotype 'SML-32' than the control (Misra and Gupta 2005). Similarly, as compared to the control, there was a 10 and 6-fold higher accumulation of GB in salt-tolerant cultivar 'S1' and salt-sensitive cultivar 'ATP', respectively at a higher NaCl level (1.5%) in *Morus alba* L. (Kumar *et al*. 2003). There was a 56 and 35% increase in GB in salt-tolerant *Vigna radiata* cultivar ('Pusa Bold') and salt-sensitive 'CO 4', respectively at higher NaCl level (300 mM) (Sumithra *et al*. 2006). In the tomato seedling subjected to NaCl stress (0 to 300 mM), the content of proline increased with increasing concentration of NaCl (Li *et al.* 2009).

Effect of salt stress on oxidative damage to membrane lipids

The oxidative stress generated in response to salt stress revealed degree of damage to the membrane lipids in terms of peroxidation as a result of increased levels of MDA. The extent of oxidative stress was significantly higher as compared to control in all the cultivars screened. 'GA-10' was more susceptible to oxidative damage as revealed by the highest lipid peroxidation (0.0635 mg/g FW) at higher (100 mM) levels of NaCl followed by 'IGPN-2004' (0.0322 mg/g FW) and 'No.71'. (0.037 mg/g FW) (**Fig. 3**). Interestingly, 'IGP-76' showed least damage to membrane lipids under salt stress as revealed by least lipid peroxidation (0.0311 mg/g FW). The higher lipid peroxidation at higher levels of NaCl leads to excess production of reactive oxygen species (ROS).

Salinity induces oxidative stresses in addition to osmotic stress in plants, and lipid peroxidation in terms of MDA content has been frequently used as an indicator of oxidative stress. In the present investigation, lipid peroxidation under salt stress was more significant in salt-sensitive cultivar ('GA-10') as compared to salt-tolerant ('IGP-76'), whereas 'IGPN 2004' and 'No. 71' showed a moderate increase in MDA content. Our results are concurrent with those on salt-sensitive *V. radiata* (Sumithra *et al.* 2006) and tomato (Shalata and Neumann 2001) cultivars. In *V. radiata*, at a higher NaCl level (300 mM), the salt sensitive cultivar ('CO 4') showed 57% higher lipid peroxidation activity than the salt-tolerant cultivar, 'Pusa Bold'. Similarly, there

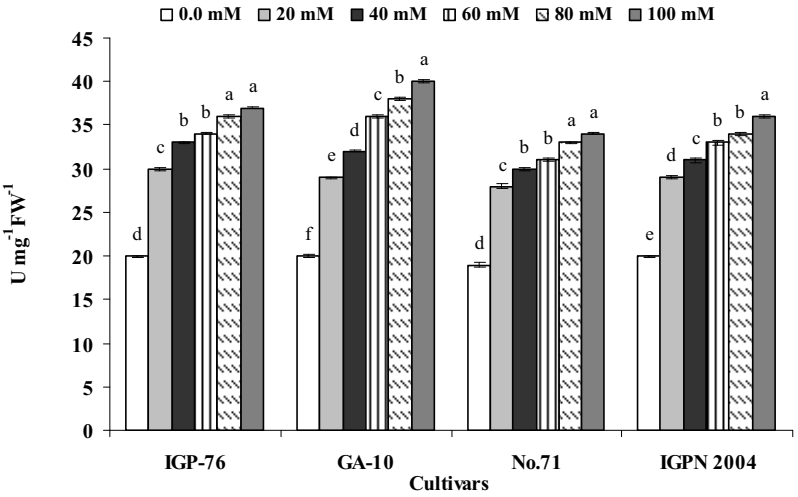

Fig. 4 Effect of salt stress on SOD in niger (*G. abyssinica*). The values represent the mean ± SE. Values followed by similar letters do not differ significantly at $P \leq 0.05$ as per DMRT (n=6).

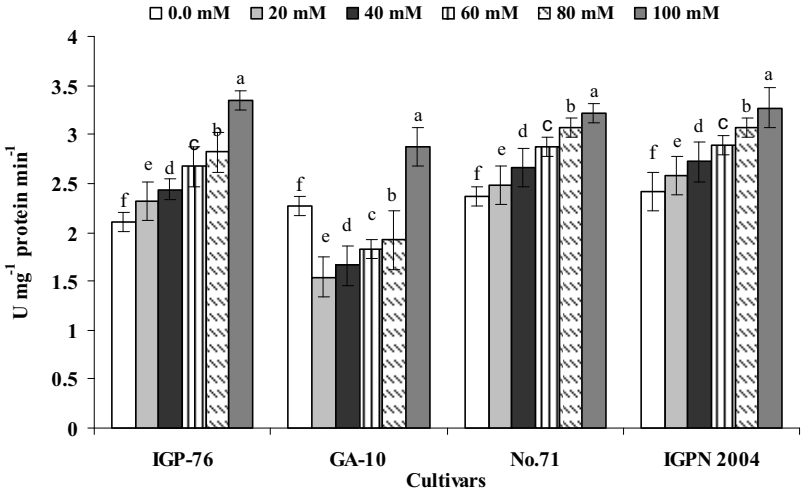

Fig. 5 Effect of salt stress on CAT in niger (*G. abyssinica*). The values represent the mean ± SE. Values followed by similar letters do not differ significantly at $P \leq 0.05$ as per DMRT (n=6).

was a progressive increase in the accumulation of lipid peroxidation products, in the form of thiobarbituric acid reactive substances (TBARS), in the roots, stems and leaves of salt-stressed seedlings of tomato (Shalata and Neumann 2001).

The increase in lipid peroxidation was also observed in rice (*Oryza sativa*) seedlings under drought stress (Gao *et al.* 2008). Salinity impacts in terms of lipid peroxidation were more pronounced in primary roots of the *Zea mays* L. 'Aristo' even 6 weeks after final treatment concentrations (34, 68 and 102 mM NaCl) were reached, indicating more sensitivity of this variety (Hajlaoui *et al.* 2009).The lower peroxi-dation of membrane lipids under salt stress describes the higher tolerance and adaptation of cultivar to stressed condition as compared to sensitive cultivars. Therefore a possibility of the cell membranes playing a role in plant salt tolerance has been suggested (Mansour and Salama 2004).

Antioxidant enzyme responses to salt stress

The homeostasis in plant cells is disturbed due to impact of various abiotic and biotic stresses and further enhances the production of ROS in plants. In addition, increased peroxidation of membrane lipid is related to increased ROS generation. Thus, salt stress resistance may depend, at least in part, on scavenging of highly toxic ROS through the regulation of antioxidant defense system. The defense mechanism includes antioxidant compounds (glutathione and ascorbate) and several antioxidant enzymes such as catalase, ascorbate peroxidase, guaiacol peroxidase, glutathione reductase and superoxide dismutase, etc. Because of oxidative damage created by excess salt concentrations, the ROS generation increased significantly as compared to control in all the cultivars of niger. SOD dismutates the excessively produced superoxide radicals and converts them to H_2O_2. In the present investigation, SOD activity increased significantly with increase in the concentrations of NaCl as compared to control (19-20 U mg^{-1} FW) and it was the highest at 100 mM NaCl (34-40 U mg^{-1} FW) in all the cultivars of niger. Likewise, CAT, a H_2O_2-decomposing enzyme, showed significantly higher activity at all concentrations of NaCl in all the cultivars screened as compared to control (**Fig. 5**). However, its activity was highest in 'IGP-76' (3.35 U mg^{-1} protein min^{-1}) than in the other cultivars (2.87-3.22 U mg^{-1} protein min^{-1}). In general, relatively more tolerant cultivar ('IGP-76') showed higher activity of antioxidant enzymes to tune the fine balance, whereas the least ('GA-10') and moderately tolerant ('IGPN-2004', 'No.71') cultivars showed fair activity of both these enzymes. On the contrary, a significant increase in SOD and CAT activities with the increasing severity of NaCl stress in the leaves of salt-tolerant Jerusalem Artichoke 'Dafeng' grown under NaCl stress (75, 150, and 225 mM NaCl) for 7 days was reported, whereas no significant change was observed in the salt-sensitive cultivar 'Wuxi' (Xue and Liu 2008). SOD is an important antioxidant enzyme and is the first line of defense against oxidative stress in plants. It plays an important role in determining the concentration of O_2^- and H_2O_2 in plants

and hence performs a key role in the defense mechanism against free radical toxicity (Bowler *et al.* 1992). In the present work, H_2O_2 was efficiently degraded as evident from higher CAT activities in salt-stressed tissues as compared to control in all the four cultivars (**Fig. 5**). In addition, the fine balance between SOD (**Fig. 4**) and CAT (**Fig. 5**) activities was observed at all the concentrations of NaCl in niger cultivars. Similar fine tuning of CAT and SOD activities was reported by Sumithra *et al.* (2006) in cultivars of *Vigna radiata* and in wheat (Sairam *et al.* 2002) genotypes under salt stress conditions. In *Medicago sativa* L. 'Xinmu No. 1' subjected to 200 mM NaCl treatment, reduced lipid peroxidation coupled to higher enzymatic activity of SOD and CAT in its shoots and roots were observed, indicating that this cultivar's tolerance to salt stress during germination is associated with enhanced activity of antioxidant enzymes (Wang *et al.* 2009). Likewise, the activity of antioxidant enzymes catalase, and superoxide dismutase in the salt-tolerant cultivars of *Zea mays* L. ('SC 129' and 'SC 13') increased markedly during salinity stress (0-250 mM NaCl), while they were mostly decreased by salinity stress in the salt sensitive cultivar ('SC 155') (Azooz *et al.* 2009). In tomato seedlings subjected to NaCl stress (0 to 300 mM), SOD and CAT activities increased with increasing concentration of NaCl (Li *et al.* 2009). Therefore, we suggest that the combined activities of SOD and CAT play a key role in the removal of ROS in niger cultivars, thus minimizing the cellular damage caused by ROS under salt stressed conditions (Gosset *et al.* 1994; Acar *et al.* 2001; Chaitanya *et al.* 2002).

CONCLUSION

The present investigation reveals differential responses on seed germination, growth, biomass, pigment content, osmolytes accumulation and antioxidant enzyme activities during salt stress in four niger cultivars that could be associated with differences in tolerance to salinity. Among the four cultivars, 'IGP-76' was the most and 'GA-10' the least tolerant. 'IGPN-2004' and 'No.71' showed a moderate response to salt stress tolerance. The differences in the tolerance level in the four niger cultivars was associated with osmotic adjustments through higher accumulation of proline and GB content as well as higher antioxidant enzyme activities in salt tolerant cultivars ('IGP-76') as compared to salt sensitive ('GA-10') and moderately tolerant cultivars ('IGPN-2004' and 'No. 71') of niger. Therefore, 'IGP-76' is a superior cultivar in combating salinity stress.

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