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VARIATIONS IN ACCESSIONS OF SUNFLOWER AND SAFFLOWER UNDER STRESS CONDITION

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Abstract

Two sunflower i.e., Helio & NuSun, and 2 safflower accessions i.e. Spiny & Non-Spiny differing in salt sensitivity, were examined for compatible solutes and some enzyme activities involved in seed germination i.e. α-amylase, β-amylase and α-glucosidase, under control and different levels of sea salt concentrations i.e. 0, 0.2, 0.4, 0.6 and 0.8% having ECiw = 0.5, 3.4, 6.1, 8.6 and 10.8 dS/m respectively. The inhibitory effects of salts differed among all the accessions tested. In Helio and Non-Spiny cultivars the increase in salt concentration reduced germination percentage and lower relative water content, and also decreased the endogenous levels of proline, total soluble sugars and activities of the main enzymes involved in the germination process. In contrast, seeds of NuSun and Spiny cultivars accumulated higher proline and total soluble sugar concentrations in response to salt stress, which improved their water status and the enzyme activities involved in the process of germination. Differences in response of the different accessions of sunflower and safflower to salt stress may be due to the accumulation of compatible solutes in their seeds.

Introduction

Salinization is a worldwide problem, particularly acute in semi-arid areas which use lots of irrigation water, are poorly drained, and never get well flushed. Globally salinization obviously reduces crop productivity worldwide. Though salts are a common and necessary component of soil and essential plant nutrient, but its presence in excess can cause reduction in availability of water, accumulation of sodium ions and imbalance uptake of mineral that affect seed germination and plant metabolism. In response of all these factors plants show morphological, physiological and metabolic modifications e.g. decrease in seed germination, shoot and root length, alterations in the cell membranes integrity, inhibition of different enzymatic activities involved in various metabolic process (Khan *et al.,* 2009). Sunflower and safflower are considered as major edible oil yielding crops for human and as a source of food for animal consumption. These crops are classified as moderately tolerant to salinity, although the threshold of the two species is nearly identical, but the rate of yield decline above the threshold is much greater for safflower. Selection of resistant crops appears as an arduous and perilous task and therefore plant breeders are in search of fast, economical and reliable ways to assess the salt-resistance of selected crop.

Seed germination is the first growth stage and a basic phase of the plant to limit its development under saline stress (Zapata *et al.,* 2003) and the determination of germination potential of seeds in saline stress could be an effective parameter for several reasons. First, at germination stage resistance to salinity stress was shown to be a heritable multi-genic trait and the overall trait is determined by a number of sub-traits these sub-traits could enhance the ability to minimize the net accumulation of Na^+/ or Cl^- ion and maximize the K^+ uptake under high salinity level thus it could be efficient criteria for the selection of salt-resistant breeds (Mano & Takeda, 1997; Flowers, 2004). Second, seeds and young seedlings usually face much higher salinities than vigorously growing plants because germination usually occurs in surface soils which accumulate soluble salts due to the evaporation and capillary rise of water. A considerable variability for salt tolerance has been observed by many workers among and even within the species (Almansouri *et al.,* 2001; Zapata *et al.,* 2004, Shereen *et al.,* 2011). To improve the production of edible oil it is the need of the day that marginal lands should be used for the production of oil seed crops. One way to use these lands is reclamation which is costly, and the other approach is to grow salt tolerant species and cultivars. Therefore present study was conducted with the objective to investigate the salt tolerance of two genotypes of sunflower and two of safflower, which would allow crop breeders to cultivate these crops on saline soils or with saline water which often occur in the semi-arid areas.

The accumulation of compatible solutes such as proline, glycine betaine and total sugars, the production of stress proteins and the expression of different sets of genes are part of the plant defense system against salinity (Sairam & Tyagi, 2004). Solute accumulation under stress contributes osmoregulation in cells to stabilize the production of enzyme / protein and maintain turgor in growing organs (Cushman, 2001; Sanchez *et al.,* 2004)). In the present study, two genotypes of sunflower i.e., Helio and NuSun and two of safflower i.e., Spiny and Non-Spiny with different salinity tolerance were studied to determine the factors which are responsible for failure of germination. To achieve this objective, the effects of different sea salt concentrations were evaluated on important biochemical processes related with seed germination.

Materials and Methods

Accessions of safflower germplasm (i.e. Spiny and Non-Spiny) were obtained from National Agricultural Research Center, Islamabad, and of sunflower (i.e. Helio and NuSun) from Global Chemical Company Karachi, Pakistan.

Seed germination: In order to study the effects of salinity stress on germination in sunflower and safflower accessions an experiment was conducted in factorial form, using a completely randomized design with five replications. Well stored (20 ± 1°C) seeds of each sunflower and safflower accessions were surface-sterilized by soaking in 30% (v/v) H_2O_2 for 20 min, then rinsed and soaked in distilled water for 1 h to remove the traces of sterilizing agent. 20 seeds of each cultivar were placed on a filter paper in 9 cm Petri dishes containing 3 cm^3 of distilled water (control), or 0, 0.2, 0.4, 0.6 and 0.8% of respective salt solution having ECiw = 0.5, 3.4, 6.1, 8.6 and 10.8 dS/m respectively. Sea salt solutions were prepared by adding a required amount of sea salt into per liter of water. The Petri dishes were sealed with parafilm to prevent loss of moisture by evaporation and then care kept in a humidity chamber at a temperature of 25 ± 1 °C in the dark. The seeds were considered germinated when there was radical protrusion through the seed coat. For the determination of dry weight, 10 seeds of each cultivar, were taken out and were dried at 70 °C in an oven till there is no decrease in weight.

Enzyme assays: Alpha-, beta-amylase and alpha-glucosidase activities in the crude extracts of each cultivar of sunflower and safflower were determined. The seeds of each cultivar, in deionised water (control) and treated with different concentrations of sea salt solutions (0, 0.2, 0.4, 0.6 and 0.8%) were homogenized in a chilled mortar with distilled water 1:4 (w/v) and centrifuged at 14000 g for 30 min. The supernatants were filtered through a single layer of muslin cloth and were used for the estimation of α-amylase (EC 3.2.1.1) according to the method of Coombe *et al.,* (1967), β–amylase (EC 3.2.1.2) and α-glucosidase (EC 3.2.1.20) with the method of Bergmeyer *et al.,* (1983).

Content of compatible solutes: Extraction and determination of proline contents was performed according to the method of Bates *et al.,* (1973). Seeds were hand-homogenized in 3% of sulfosalicylic acid and centrifuged at 3000 g at 4°C for 10 min. The supernatants were used for proline estimation.
The total soluble sugars were determined with the Anthrone method (Yemn & Willis, 1954).

Statistical analysis: Data were analyzed separately for each genotype by one way procedure of ANOVA ($p \leq 0.05$) according to a completely randomized design with five replicates. Treatment means were compared using the Duncan's Multiple Range Test ($p<0.05$) (Duncans, 1955).

Results

Germination of both the crops cultivars started after 24 h of sowing. Germination percentage was recorded higher than 98% in control seeds. While the seeds treated with different concentrations of saline solutions showed delay in the germination that was different among the cultivars. At 48 and 72 h the germination percentage of Helio sunflower and Non-Spiny safflower under salinity was significantly lower than Spiny safflower and NuSun sunflower. However, at highest salinity level i.e., EC 10.8dS/m, final germination percentage was significantly reduced in Helio (52%) and more in Non-Spiny (60%) cultivars, while under control condition almost 100 % germination were recorded for both cultivars (Table 1).

Table 1. Germination percentage in sunflower and safflower cultivars in control and under different level of salt concentrations that had been exposed for 24, 48 and 72 h.

Salinity (dS/m)	Cultivars	24 h	48 h	72 h
0.5	Helio	98.0	99.9	99.9
	NuSun	100	100	100
	Spiny	100	100	100
	Non-Spiny	98.9	99.9	99.9
3.4	Helio	99.0	99.5	100
	NuSun	100	100	100
	Spiny	100	100	100
	Non-Spiny	99.0	99.5	99.9
6.1	Helio	93.5	94.0	95.0
	NuSun	98.0	98.5	99.0
	Spiny	98.0	98.6	99.0
	Non-Spiny	94.0	94.0	94.4
8.6	Helio	85.0	85.4	85.9
	NuSun	92.0	92.8	93.0
	Spiny	91.6	92.0	92.3
	Non-Spiny	84.2	85.0	85.0
10.8	Helio	47.0	47.0	47.2
	NuSun	79.0	79.5	80.0
	Spiny	71.5	71.5	72.2
	Non-Spiny	38.4	39.0	39.0

Helio and NuSun are sunflower and Spiny and Non-spiny are safflower cultivars

The cultivars Spiny and NuSun exhibited a fair degree of salt tolerance by showing germination under saline condition more than the other ones i.e., 72 and 80% respectively (Table 1). The proline content of Spiny and NuSun cultivars were enhanced at different salt concentrations (Fig. 2). The highest amount of proline was observed at EC 8.6 and 10.8dS/m for both cultivars. Both cultivars accumulated almost two times more proline than Helio and Non-Spiny cultivar (Fig. 2). With the increasing level of salinity Spiny and NuSun cultivar showed a gradual increase in total soluble carbohydrate while Helio and Non-Spiny cultivars showed a lesser degree of total soluble carbohydrate accumulation under salt stress (Fig. 1).

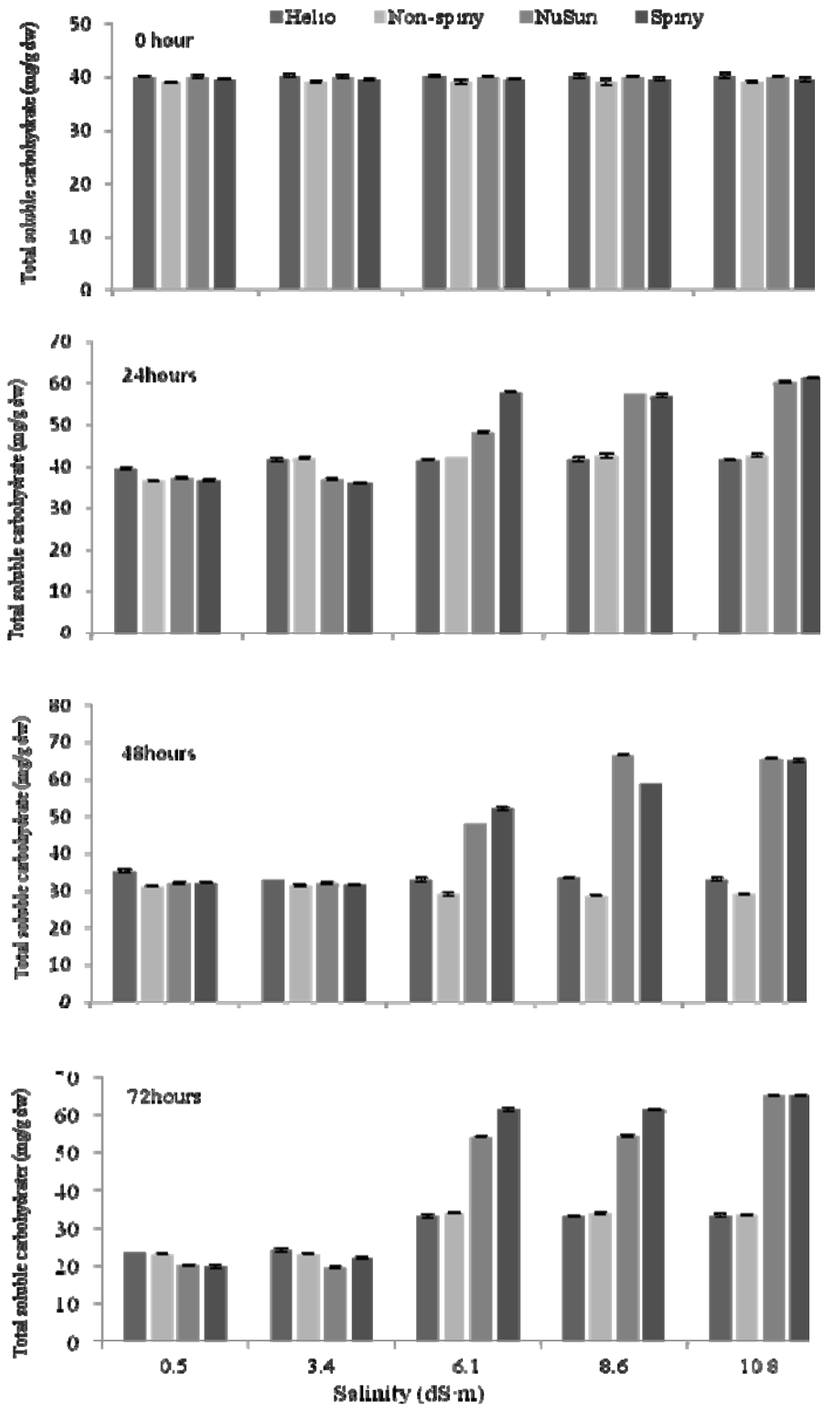

Fig. 1. Total soluble carbohydrate in Helio, NuSun sunflower and Spiny , Non-Spiny safflower cultivars seeds under different salinity levels at 0, 24, 48 and 72 hours. Vertical bars shows standard error of the mean (n=5).

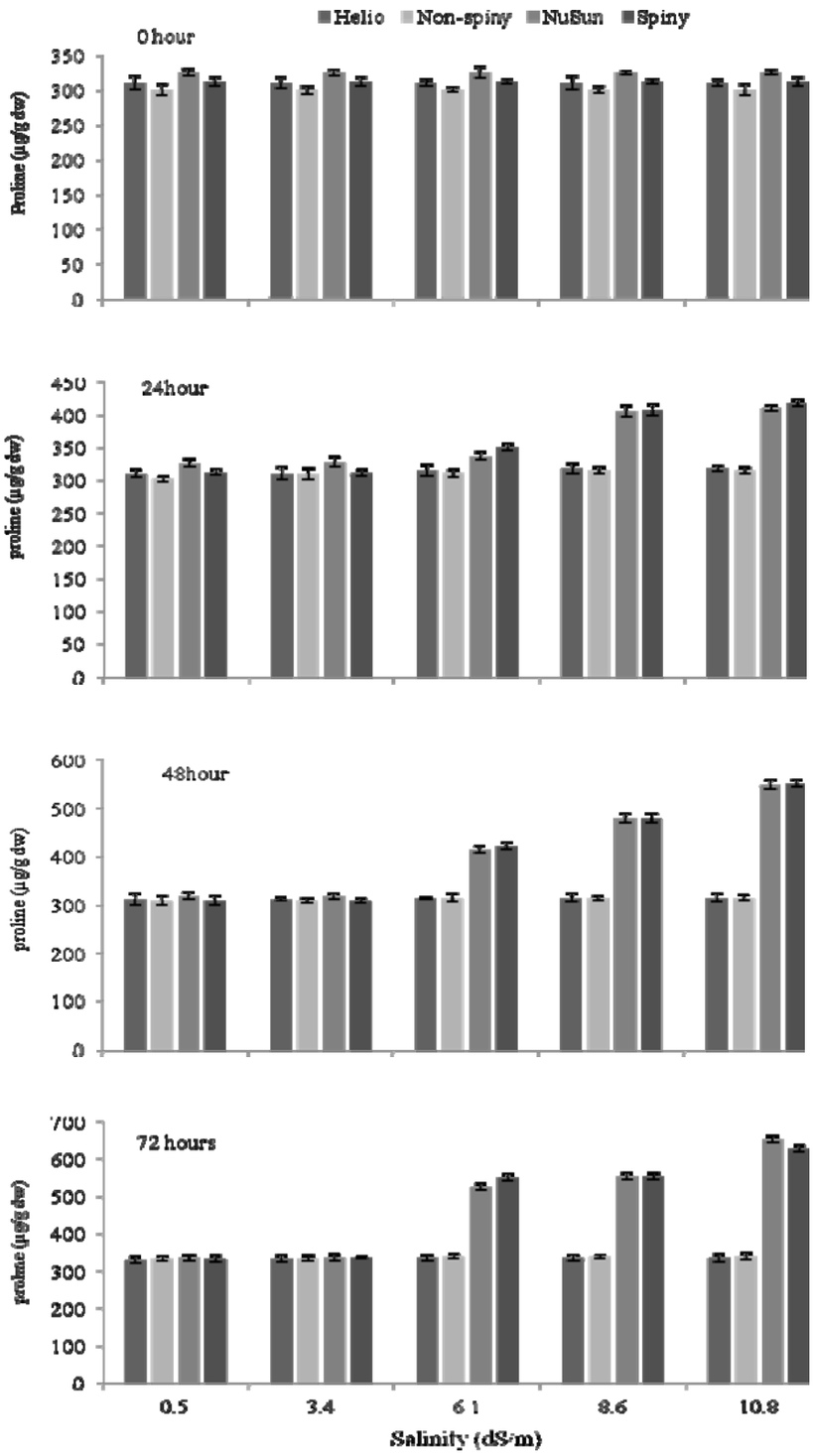

Fig. 2. Proline content in Helio, NuSun sunflower and Spiny, Non-Spiny safflower cultivars seeds under different salinity levels at 0, 24, 48 and 72 hours. Vertical bars shows standard error of the mean (n=5).

The activity of the enzymes α-amylase, β-amylase and α-glucosidase decreased with the salinity but in a dose-dependent manner. The effects of salinity differ among cultivars and were more pronounced in seeds of Helio and Non-Spiny cultivars. β-amylase and α-glucosidase activities decreased with the increase in salt concentration. The activities of β-amylase and α-glucosidase in stressed seeds of Spiny and NuSun were higher as compared to those observed in Helio and Non-Spiny genotypes (Tables 2-4).

Table 2. α-Amylase, β-amylase and α-glucosidase activities (µmoles of reducing sugars $min^{-1}g^{-1}$ fw) in sunflower and safflower cultivars in control and under different level of salt concentrations that had been exposed for 24 h.

Salinity (dS/m)	Cultivars	α-amylase	β-amylase	α-glucosidase
0.5	Helio	16.13d	0.77ab	0.281b
	NuSun	26.21a	1.02a	0.305a
	Spiny	26.33a	0.98b	0.312a
	Non-Spiny	18.30c	0.72ab	0.230ab
3.4	Helio	15.40d	0.40c	0.278b
	NuSun	26.55a	1.17a	0.094d
	Spiny	26.35a	1.44a	0.289b
	Non-Spiny	5.50ef	0.25d	0.278b
6.1	Helio	14.01cd	0.44c	0.194bc
	NuSun	23.03b	1.03a	0.292b
	Spiny	24.93b	1.14a	0.290b
	Non-Spiny	5.05ef	0.22d	0.043d
8.6	Helio	11.40de	0.21d	0.092d
	NuSun	19.14c	1.12a	0.189c
	Spiny	18.38c	1.11a	0.188c
	Non-Spiny	2.01f	0.10e	0.033de
10.8	Helio	8.14e	0.17cd	0.080c
	NuSun	18.06c	1.15a	0.101cd
	Spiny	15.98d	0.83b	0.097d
	Non-Spiny	2.00f	0.09e	0.012e

Different letter(s) indicate significant differences among treatments at 5% level of significance in Duncan's Multiple Range Test
Helio and NuSun are sunflower and Spiny and Non-spiny are safflower cultivars

Table 3. α-Amylase, β-amylase and α-glucosidase activities (µmoles of reducing sugars $min^{-1}g^{-1}$ fw) in sunflower and safflower cultivars in control and under different level of salt concentrations that had been exposed for 48 h.

Salinity (dS/m)	Cultivars	α-amylase	β-amylase	α-glucosidase
0.5	Helio	16.50bc	0.94c	0.280ab
	NuSun	26.99a	1.39a	0.331a
	Spiny	26.00a	1.31a	0.308a
	Non-Spiny	15.60bc	0.83c	0.229b
3.4	Helio	16.40bc	0.50d	0.295ab
	NuSun	26.71a	1.20ab	0.301a
	Spiny	27.15a	1.63a	0.293ab
	Non-Spiny	4.55e	0.30de	0.100d
6.1	Helio	15.11bc	0.52d	0.192c
	NuSun	21.03b	1.18ab	0.296ab
	Spiny	23.49b	1.21ab	0.293ab
	Non-Spiny	4.60e	0.26de	0.050ef
8.6	Helio	13.25d	0.30de	0.096d
	NuSun	19.44c	1.01d	0.201bc
	Spiny	20.12b	1.23b	0.194c
	Non-Spiny	2.11f	0.14e	0.041e
10.8	Helio	6.15ef	0.21d	0.090d
	NuSun	15.87bc	1.16ab	0.102d
	Spiny	14.05d	0.90dc	0.095d
	Non-Spiny	2.14f	0.11e	0.017f

Different letter(s) indicate significant differences among treatments at 5% level of significance in Duncan's Multiple Range Test
Helio and NuSun are sunflower and Spiny and Non-spiny are safflower cultivars

Table 4. α-Amylase, β-amylase and α-glucosidase activities (μmoles of reducing sugars $min^{-1}g^{-1}$ fw) in sunflower and safflower cultivars in control and under different level of salt concentrations that had been exposed for 72 h.

Salinity (dS/m)	Cultivars	α-amylase	β-amylase	α-glucosidase
0.5	Helio	16.53b	1.17c	0.297b
	NuSun	24.98a	1.75a	0.348a
	Spiny	26.12a	1.47ab	0.328a
	Non-spiny	12.10bc	0.98d	0.136cd
3.4	Helio	17.44b	0.57de	0.303ab
	NuSun	24.60a	1.46ab	0.304ab
	Spiny	25.02a	1.75a	0.300ab
	Non-Spiny	1.09d	0.37e	0.104d
6.1	Helio	15.40b	0.59de	0.212c
	NuSun	21.43ab	1.33b	0.302ab
	Spiny	25.40a	1.35b	0.300ab
	Non-Spiny	1.05d	0.31ef	0.055de
8.6	Helio	13.71bc	0.30ef	0.102d
	NuSun	14.78bc	1.17c	0.211c
	Spiny	21.85ab	1.35b	0.201c
	Non-Spiny	1.04d	0.21f	0.042e
10.8	Helio	7.81c	0.28	0.098d
	NuSun	16.76b	1.15c	0.110d
	Spiny	15.91b	0.98d	0.103d
	Non-Spiny	1.05d	0.15f	0.022e

Different letter(s) indicate significant differences among treatments at 5% level of significance in Duncan's Multiple Range Test
Helio and NuSun are sunflower and Spiny and Non-spiny are safflower cultivars

Discussion

The existence of genetic variability within the species of cultivars offers a fair tool to study the mechanism of salt tolerance. In present study the results showed a decrease in germination of seeds with increasing levels of salinity in all studied accessions of sunflower and safflower. The magnitudes of such decrease in the cultivars Non-Spiny was in minor extent, and in Helio were more as compared to that of cultivars NuSun and Spiny particularly at high salinity concentrations. The germination process consists of imbibitions, metabolism and initiation of radical growth which lead to radical emergence. For the hydrolysis of stored substrates a hydration level is required for the synthesis of hydrolytic enzymes which are responsible for hydrolysis. The hydrolyzed products formed are used in synthesis of seedling tissue and radical elongation (Ramagopal, 1990). Salt induced inhibition of seed germination has been attributed to osmotic stress or to specific ion toxicity that affect the synthesis of hydrolytic enzymes limiting the hydrolysis of food reserves from storage tissues as well as to impaired translocation of food reserves from storage tissue to developing embryo axis (Ghoulam & Fares 2001; Lacerda *et al.,* 2003; Sidari, 2008).

The presence of salt at low concentrations could have contributed to a decrease in the internal osmotic potential of germinating structures due to ions penetration (Dodd & Donovan, 1999; Almansouri *et al*., 2001), leading to water uptake and initiation of germination processes. Present results show a lower content of total soluble carbohydrate and proline in presence of the highest salt concentration in Non-Spiny and Helio compared to NuSun and Spiny cultivars, suggesting that salt tolerance ability of these two last cultivars appears to be associated to the accumulation of compatible solutes, which being hydrophilic in nature improved their water status. Salt stress inhibits different enzymatic activities which limit the mobilization of starchy endosperm reserves in several species (Almansouri *et al.,* 1999; Ahmad *et al.,* 2006). Simultaneous activities of α-amylase, β-amylase and α-glucosidase result in starch mobilization in germinating seeds. Starch degradation in germinating seeds is initiated by α-amylase (Yamasaki, 2003) that produces soluble oligosaccharides which in turn hydrolyzed by β-amylase to liberate maltose. Maltose is broken down by α-glucosidase into main respiratory substrate, glucose (Sidari, 2008).

A correlation was found in germination performance with α-amylase by many workers in contrast, Das & Sen-Mandi (1992) demonstrated greater importance of β-amylase compared to α-amylase, during the early hours of germination in wheat scutella. Nandi *et al.,* (1995) demonstrated that β-amylase activity can easily be measurable immediately before visible germination becomes evident, whereas α-amylase activity is initiated at later stage of germination, suggesting that α-amylase affects rate of seedling growth while β-amylase activity is linked with initiation of germination. Therefore it can be manipulated that β-amylase is an essential enzyme for germination. In present investigation high salt concentrations adversely affected each enzyme activity in all genotypes studied. β-amylase activity was always significantly lower in Non-Spiny and Helio compared to their own control and salt treated Spiny and NuSun at every concentration used. When Non-Spiny and Helio were treated with low salt concentrations β-amylase activity at 72 h was lower than that detected at 24h in their own control or in Spiny and NuSun salt treated seeds at all concentration used, suggesting that this enzyme

could be related to salt sensitivity. The variation in response to salt stress sensitivity of all the genotypes studied may be associated to the ability of osmoregulation under stress, which cause a strong decrease in water content affecting the hydrolytic enzyme activities, particularly the levels of β-amylase. β-amylase is probably synthesized during imbibitions, that enhances seed vigor and bring about an enhancement in β-amylase activity (Nandi *et al.,* 1995). The data in the present investigation provide support for the view that the higher β-amylase activity in Spiny and NuSun cv. might be the reason for the major seed germination in presence of sea salt salinity.

In can be concluded that cultivars of sunflower i.e. NuSun and of safflower i.e., Spiny could be cultivated in such environments where salinity of the soils is a common constraint and the breeders could utilize both the cultivars of sunflower and safflower in breeding programs to improve the saline resistance of the species.

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