International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2019; 7(5): 508-514

Received: 19-07-2019
Accepted: 21-08-2019

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Influence of seed priming on seed germination, seedling growth, peroxidase activity, proline and total soluble sugar content of pearl millet (*Pennisetum glaucum* L.) under salinity stress

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Abstract
The experiment was conducted to investigate the effect of seed priming on seed germination and salinity tolerance of pearl millet seedlings. A completely randomized design in a 2x2x5 factorial scheme with four replications of 100 seeds each was used. Seeds of two pearl millet accessions, IP 14294 (tolerant) and IP 17862 (susceptible) were primed using homobrassinolide, triacontanol, salicylic acid, potassium nitrate and water (hydropriming). Primed seeds were subjected to salt stress of 0 and 150 mM NaCl. Salicylic acid priming improved the salinity tolerance in both the genotypes significantly by increasing the peroxidase activity (0.57 µmol ml^{-1} min^{-1}), proline (4.22 µmol g^{-1}) and total soluble sugar content (16.25 mg g^{-1}) as compared to hydropriming. Significantly highest seed germination (85.44%) was observed in salicylic acid primed seeds and lowest was recorded in hydroprimed seeds (80.63%). However, remaining chemicals also had positive effect on both the genotypes compared to hydroprimg.

Keywords: Pearl millet, salinity, seed priming, salicylic acid

Introduction
Pearl millet [*Pennisetum glaucum* (L.) Syn. *Cenchrus americanus* (Morrone), (2n=14)] is a native of Sahel zone of West Africa, that belongs to botanical family *Poaceae*. In India it is called as bajra or bajri, which is a low price food grain crop and a central component of food and fodder of the rural poor in dry areas with very limited rainfall. It is usually grown under most adverse agro-climatic conditions where other crop fails to produce economic yields. Soil salinity hampers pearl millet productivity to a greater extent by delaying seed germination and severely affecting subsequent growth throughout plant life cycle (Ashraf and Idrees, 1992) [1]. Efficient strategies are required for effective utilization of saline lands for crop cultivation. Development of salt tolerant plants through conventional breeding is very slow due to the complexity of salt tolerance and lack of reliable traits for selection. Among the strategies used to mitigate salt stress-induced adverse effects, seed priming with salts or plant growth regulators are cited as most appropriate, efficient and economic techniques to enhance the rate and the uniformity of germination (Ashraf *et al.*, 2008) [2] in saline soils.

All seed priming chemicals used in the experiment enhances the plant metabolism in the crop plants to withstand even under salinity stress. Salicylic acid is an endogenous plant growth regulator which has been found to generate a wide range of metabolic and physiological responses in plants thereby affecting their growth and development (Hayat *et al.*, 2010) [17]. Potassium plays an important role in balancing membrane potential and turgor, activating enzymes and regulating osmotic pressure (Kaya *et al.*, 2009) [21] in salt stressed plants. Previous studies revealed that low concentrations of KNO_3 could alleviate the NaCl induced reduction in seed germination in certain grass species (Neid and Biesboer, 2005) [28]. The activation of seedling growth by homobrassinolide under salinity stress was associated with enhanced levels of nucleic acids, soluble proteins and free proline.

With this view the role of these priming agents viz., homobrassinolide, triacontanol, salicylic acid and potassium nitrate for improving vigour under salt stress conditions and beneficial effects in various crops, the present study was conducted to evaluate and compare the effects among the seed priming agents for improving the germination, seedling growth and biochemical activity under salinity stress in pearl millet.

Material and Methods

The experiment was conducted at Department of Seed Science and Technology, College of Agriculture, University of Agricultural Sciences, Raichur during 2019. Seeds of two pearl millet accessions, IP 14294 (salinity tolerant) and IP 17862 (salinity susceptible), were obtained from the pearl millet minicore collection of gene bank, ICRISAT, Hyderabad. The seeds were primed with four different chemicals viz., homobrassinolide (3 µM), triacontanol (10 µM), salicylic acid (100 µM), potassium nitrate (0.5%) and water (hydropriming) for 8 hours @ 1:3 seeds to solution ratio. After that, seeds were removed from the solutions and shade dried at room temperature to bring back to its original moisture content for assessing the germination, seedling growth, vigour and biochemical parameters .

For creating salt stress, 8.76 g of NaCl was dissolved in one litre of water, to get the required salt concentration (150 mM NaCl) and the germination papers were soaked in this salt solution. The primed seeds of tolerant and sensitive genotypes were subjected to germination test by following between paper method (ISTA, 2013) [18], using germination papers soaked in salt solution. Another set of primed seeds were put for germination in papers soaked in distilled water (0 mM NaCl) which was used as control. Details of the treatments imposed were enlisted in Table 1.

Table 1: Treatments assigned in the study

Genotype (G)	Salinity level (S)	Seed priming (P)
G_1: IP 14294 G_2: IP 17862	S_1: 0 mM NaCl (control) S_2: 150 mM NaCl	P_1: Homobrassinolide @ 3 µM P_2: Triacontanol @ 10 µM P_3: Salicylic acid @ 100 µM P_4: Potassium nitrate @ 0.5% P_5: Hydropriming

Germination

Four replicates of 100 seeds each were germinated in a germination chamber maintained at 25 ± 2 ºC temperature and 90 ± 5 per cent relative humidity. Then the final count was taken on 7th day. The numbers of normal seedlings from each replication were counted and the mean germination was expressed in percentage.

Seedling growth parameters

At the time of germination count in roll towel method, ten normal seedlings were selected at random from each replication and used for measuring the root and shoot length. The values were calculated and expressed in centimetre. The seedlings used for growth measurement were placed in a butter paper cover and dried in shade for 24 h and then kept in a hot air oven maintained at 70 ºC for 24 h. Dry weight was recorded and the mean values were expressed in milligram. The seedling vigour index-I computed by employing the formula given by Abdul-Baki and Anderson, 1973, and the mean values were expressed in whole number.

SVI-I = Germination (%) × Mean seedling length (cm)

Biochemical parameters

Peroxidase activity

Peroxidase activity was measured according to Fielding and Hall (1978) [14]. It was estimated by recording the increase in the absorbance of oxidized guaiacol at 470 nm and was expressed as micromole of guaiacol oxidized per millilitre of reaction mixture per minute at 25 ºC.

Proline content in seedlings

The proline content in the seedlings was estimated as per the procedure given by Bates *et al.* (1973) [3] and expressed in µmol g^{-1} fresh weight (FW).

Total soluble sugars

Total soluble carbohydrates (TSC) concentration was estimated by taking absorbance at 490 nm, as described by Dubois *et al.* (1956) [6] using glucose as a standard. TSC was presented as mg g^{-1} fresh weight (FW).

Statistical analysis

The data collected from the experiments were analysed statistically by the procedure suggested by Sundarajan *et al.* (1972) [37]. The design used for analysing the data was three factorial completely randomised design (FCRD) in which the three factors includes genotype, salinity level and seed priming. Whenever F test was found significant, the critical difference (CD) values were calculated and the treatment means were compared at one per cent level of probability (p = 0.01).

Results and Discussion

Germination (%) and seedling growth

The seed germination and seedling characteristics are the most useful criteria used for selecting salt tolerance in plants (Boubaker, M., 1996). In the present study, significant differences were observed in these parameters due to accessions, salinity levels and priming treatments.

Among the two accessions, the germination (%) was significantly higher (88.88%), in IP 14294, the tolerant genotype compared to IP 17862, (76.15%) (Table 2). There was 17 per cent higher germination in IP 14294 compared to IP 17862. Some genotypes may possess superior inherent genetic characteristics that could assist in identification of superior genes for salt tolerance in pearl millet for improving its productivity (Mukhopadhyay, 2005) [26]. The reduction in germination will be at a lesser rate in such genotypes compared to the sensitive ones (Yakubu *et al*., 2010) [40].

While comparing the salinity levels, S_1 (0mM; control) recorded significantly higher seed germination (87.40%) compared to S_2 (150 mM NaCl) (77.63%). There was 11.2 per cent reduction in germination under salinity compared to control. This might be due to hyper-osmotic stress and toxic effects of sodium and chloride ions on germinating seeds in a saline environment which delay or inhibit germination (Farsiani and Ghobadi, 2009) [11]. The decrease in seed germination under salinity is in agreement with Yakubu *et al.* (2010) [40] in pearl millet and Gill *et al.* (2003) [15] in sorghum.

Seed priming with homobrassinolide, triacontanol, salicylic acid and KNO_3 enhanced the seed germination under saline as well as non-saline conditions compared to hydropriming. However, seed priming with salicylic acid @ 100µM recorded significantly higher germination (85.44%) over hydroprimed seeds (80.63%). The results suggests that salicylic acid possibly induces the genes encoding salt resistance and acts on germination by increasing the physiological activity and mobilization of reserved food

material necessary for growth. An increase in these traits may due to the role of SA in increasing oxygen and nutrients uptake and activity of α-amylase in seeds (Alamri *et al.*, 2018). Similarly, Kaydan *et al.* (2007) [22] reported increased seedling emergence by salicylic acid under saline conditions in wheat.

Seedling growth

Significantly higher root length, shoot length and seedling dry weight, (19.16 cm, 12.31 cm and 82.08 mg, respectively) were observed in tolerant genotype, IP 14294 compared to IP 17862 (Table 2). This points that the depressive effect of salinity was negligible in case of IP 14294 with respect to root and shoot growth as well as accumulation of dry matter. The higher germination rate and seedling length was attributed to significantly higher vigour index (2807) for salt tolerant genotype (Table 2). On the other hand the sensitive ones had lower germination rate and seedling length, ultimately had a lower vigour index (1868). The findings were in agreement with Yakubu *et al.* (2010) [40] who reported that the effect of salinity on growth of pearl millet varied among the varieties. And also differences in growth among *Phaseolus* species under saline condition were reported by Jeannette *et al.* (2002) [20]. Salt tolerance at seedling stage appears to be controlled by more than one gene and is highly influenced by salt concentration (Jeannette *et al.*, 2002) [20].

Under saline condition (150 mM NaCl), significant reduction was observed in all the growth parameters including root length, shoot length, seedling dry weight and seedling vigour index. Under salinity stress root length, shoot length, seedling dry weight and seedling vigour index were 16.34 cm, 8.74 cm, 60.45 mg and 1975, respectively where as in control it was 18.73 cm, 11.97 cm, 77.10 mg and 2700, respectively (Table 2). The reduction in root and shoot length may be due to toxic effects of the Na^+ and Cl^- used as well as unbalanced nutrient uptake by the seedlings (Jamil *et al.*, 2006) [19]. The dry weight is the consequence of plant physiological and biological activity. It was found that under salt stress the photosynthetic rate was reduced markedly, spent huge energy for salt removal mechanism, reduced transportation of compatible nutrient, arrested cell division and enlargement, decreased root and shoot length and ultimately reduction of plant growth and accumulation of dry matter was noticed.

All the four chemical used for seed priming (homobrassinolide, triacontanol, salicylic acid and potassium nitrate) had positive effects on seedling growth as well as improved the vigour of the seedlings compared to hydropriming. However, salicylic acid @ 100 µM recorded significantly higher root length, shoot length, seedling dry weight and seedling vigour index, (18.37 cm, 10.72 cm, 72.75 cm and 2513, respectively) compared to other treatments (Table 2). The ill effect due to increased accumulation of Na^+ and Cl^- ions under salt stress might be reduced by salicylic acid priming. Increase in root and shoot length might be due to the role of salicylic acid in nutrients mobilization, also accumulation of abscisic acid and indole-3-acetic acid, resulting in improved protection and promoting effects of salicylic acid (Sakhabutdinova *et al.*, 2003) [32]. Salicylic acid treatment increases the growth of radicle cells by both division and expansion of meristem cells (Boukraa *et al.*, 2013) [4] which resulted in increasing the root length. Salicylic acid has ameliorating and growth inducing effects under stress and non-stress conditions by decreasing over-production of ROS in roots. Increase in seedling dry weight could be attributed to increased CO_2 assimilation, photosynthetic rate and increased mineral uptake by stressed plant under salicylic acid treatment (Khan *et al.*, 2003) [23]. Application of salicylic acid improved the parameters such as germination percentage, root length, shoot length and seedling dry weight under both conditions. As germination percentage and seedling length positively correlates with seedling vigour index, it will ultimately result in improved seedling vigour.

Biochemical parameters

Peroxidase activity

Salinity leads to oxidative stress in plants due to the production of Reactive Oxygen Species (ROS) such as the super oxide radical, hydrogen peroxide and hydroxyl radical. Oxidative stress is one of the major limiting factors in plant productivity. ROS generated during metabolic processes damage cellular functions and consequently lead to disease, senescence and cell death. Plants have evolved an efficient defence system by which the ROS is scavenged by antioxidant enzymes such as peroxidase (POX).

The tolerant genotype, IP 14294 recorded 27 per cent more peroxidase activity (0.57 µmol ml^{-1} min^{-1}) than the susceptible one, IP 17862 (0.45 µmol ml^{-1} min^{-1}) (Fig. 1) The higher enzyme activity may be one of the reasons for successful establishment of tolerant varieties under saline condition. Salt- tolerant plants often have higher POD activity. Sreenivasulu *et al.* (1999) [36] reported that exposure of cultivars to salinity resulted in changes in the induction of total peroxidase activity which vary between cultivars.

In this study, peroxidase activity was significantly higher (0.40 µmol ml^{-1} min^{-1}) under salt stress, (150 mM NaCl) over control (0.62 µmol ml^{-1} min^{-1}) (Fig.1). It is thought that increasing the antioxidant enzyme levels under salinity is an effective strategy to confer salt tolerance. Peroxidase enzyme involved not only in scavenging H_2O_2 but also in plant growth, development, lignification, suberization, and cross-linking of cell wall compounds which prevents the entry of more ions (Passardi *et al.*, 2005) [30]. Similar results were also found by Sese *et al.* (1998) [33] in rice and Shalata and Tal (1998) [34] in tomato.

In the present study, pearl millet seeds primed with salicylic acid @ 100 µM recorded high peroxidase enzyme activity (0.57 µmol ml^{-1} min^{-1}) compared to hydropriming (0.46 µmol ml^{-1} min^{-1}) indicating that salicylic acid greatly activate defence system in order to alleviate oxidative damage induced by salt stress (Fig. 1). However, peroxidase activity of KNO_3 primed seeds (0.55 µmol ml^{-1} min^{-1}) were on par with salicylic acid primed seeds. Increase in the activity of antioxidant enzymes in the seedlings of pearl millet treated with salicylic acid led to an up regulation of the genes controlling the synthesis of these enzymes or an increased activation of constitutive enzymes pools in the seedlings under stress conditions (El-Khallal *et al.*, 2009) [7]. Thus, SA treated plants have high resistance to the oxidative damage as compared with un-treated plants and the results are in line with Sakhabutdinova *et al.* (2003) [32] in wheat.

Proline content

Proline is the most common compatible solute that occurs in a wide variety of plants. Increased levels of proline accumulated in plants correlate with enhanced salt tolerance (Munns and Tester, 2008) [27].

The proline content recorded was significantly higher (4.69 µmol g^{-1} FW) in tolerant genotype, IP 14294 compared to the sensitive one, IP 17862, (2.84 µmol g^{-1} FW) (Fig. 2). Accumulation of endogenous free proline in plant tissues

exposed to salt stress has been documented in tolerant genotypes (Fedina *et al.,* 2002) [12]. The significant accumulation of proline in tolerant genotypes of rice seedlings may play an important role in osmotic adjustment that helps to maintain relative water content and reduce lipid peroxidation under salt stress (Chutipaijit *et al.,* 2010).

This study showed that accumulation of proline occurred to a significantly larger extend (6.10 µmol g^{-1} FW) at higher salinity level (150 mM NaCl) compared to control (1.44 µmol g^{-1} FW). Proline accumulation in salt-stressed plants is a primary defence response to maintain osmotic pressure in a cell (Gomes *et al.,* 2010) [16]. Wanichananan *et al.* (2003) [39] found that the proline content of rice seedlings was affected by the presence of NaCl in the growth medium and the proline content positively correlated with the NaCl.

Regarding seed priming, salicylic acid @ 100 µM proved best, which lead proline content of about 4.22 µmol g^{-1} FW, whereas P_1 (hydro priming) recorded significantly less proline (3.17 µmol g^{-1} FW) (Fig. 2). According to other studies, the proline accumulation by salicylic acid treatment has been noted in wheat, oat, bean and tomato under oxidative stresses (Tasgin *et al.,* 2006) [38]. Salicylic acid pre-treatment activates the process of defense mechanisms and make plants to tolerate stresses by modulating levels of osmolytes like proline., Fahad & Bano (2012) [10] observed a significant increase in leaf proline content in maize plants treated with SA under salinity.

Total soluble sugars

Total soluble sugars are forms of carbohydrates such as fructose, glucose and reducing sugar which generally utilized for growth and maintenance of the osmotic homeostasis of cells. These are important solutes that synthesized and accumulated in cytosol under salt stress. Here, the TSS content was significantly higher (17.89 mg g^{-1} FW) in tolerant genotype compared to the sensitive one, IP 17862, (13.10 mg g^{-1} FW) (Fig. 3). Higher TSS content in the tolerant genotype probably caused better osmotic adjustment and maintained turgor for growth under salinity. Similar results were also reported by Nemati *et al.* (2011) [29] in rice.

In this study S_1 (0 mM NaCl; control) has recorded significantly lowest (9.91 mg g^{-1} FW) TSS content compared to S_2 (150 mM NaCl) which recorded 21.08 mg g^{-1} FW of TSS (Fig. 3). Higher soluble sugars under salinity stress, is effective to balance osmotic pressure. The plant cell for escaping from plasmolysis creation during salt stress should be changed and from macro molecule to micro molecule. The accumulation of sugar at stress conditions acts as a protective mechanism which prevents the sodium entry via cell (Fernando *et al.,* 2000) [13]. The results were in confirmation with the findings of Zahra *et al.* (2010) [41] in tomato, Khodary (2004) [24] in maize and El tayeb (2005) [8] in oats.

Regarding the seed priming, salicylic acid @ 100 µM caused a significant increase (16.25 mg g^{-1} FW) in the content of soluble sugars compared to that P_5, hydroprimed seedlings (14.60 mg g^{-1} FW) (Fig. 3). It was observed that salt stress increases the level of reducing sugars (sucrose and fructose) within the cell in a number of species. Increase in soluble sugar level by application of salicylic acid in salt stressed maize plants were reported (Khodary, 2004) [24]. The major role played by these sugars in stress mitigation involves osmo protection, carbon storage and scavenging of reactive oxygen species. It is also supposed that salicylic acid treatment deranges the enzymatic system of polysaccharide hydrolysis (Khodary, 2004) [24]. Similar findings were also reported in okra (Esan and Olaiya, 2016) [9], and salvia (Sahar *et al.,* 2011) [31].

Table 2: Effect of seed priming on seed germination (%), and seedling growth parameters of IP 14294 and IP 17862 at 0 mM (control) and 150 mM NaCl (saline condition)

Treatment	Germination (%)	Root length (cm)	Shoot length (cm)	SDW (mg)	Seedling vigour index
Genotype (G)					
G_1	88.88	19.16	12.31	82.08	2807
G_2	76.15	15.90	8.39	55.48	1868
S.Em±	0.32	0.09	0.09	0.53	14
C.D @ 1%	0.91	0.25	0.24	1.50	39
Salinity level (S)					
S_1	87.40	18.73	11.97	77.10	2700
S_2	77.63	16.34	8.74	60.45	1975
S.Em±	0.32	0.09	0.09	0.53	14
C.D @ 1%	0.91	0.25	0.24	1.50	39
Seed priming (P)					
P_1	81.94	17.84	10.34	69.44	2345
P_2	81.38	17.17	10.21	67.06	2265
P_3	85.44	18.37	10.72	72.75	2513
P_4	83.19	18.14	10.51	71.06	2417
P_5	80.63	16.13	9.99	63.56	2148
S.Em±	0.46	0.14	0.14	0.84	22
C.D @ 1%	1.44	0.35	0.38	2.37	62
Interactions					
Genotype × Salinity level (G×S)					
S.Em±	0.51	0.12	0.12	0.75	20
C.D @ 1%	1.29	0.39	0.34	2.12	56
Genotype × Seed priming (G×P)					
S.Em±	0.72	0.20	0.19	1.19	31
C.D @ 1%	NS	0.55	NS	NS	NS
Salinity level × Seed priming (S×P)					
S.Em±	0.72	0.20	0.19	1.19	31
C.D @ 1%	NS	NS	NS	NS	NS
Genotype ×Salinity level × Seed priming (G×S×P)					
S.Em±	1.02	0.28	0.27	1.06	43
C.D @ 1%	NS	NS	NS	NS	NS

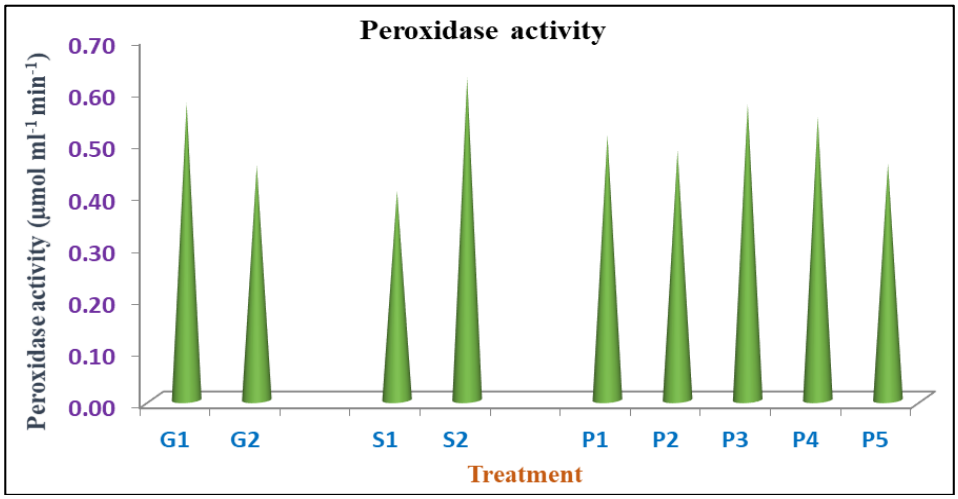

Fig 1: Influence of seed priming on peroxidase activity of IP 14294 and IP 17862 at 0 mM and 150 mM NaCl concentrations (G_1: IP 14794, G_2: IP 17862; S_1: 0 mM, S_2: 150 mM; P_1: Hombrassinolide @ 3µM, P_2: Triacontanol @ 10 µM, P_3: Salicylic acid @ 100 µM, P_4: KNO_3 @ 0.5%, P_5: Hydropriming)

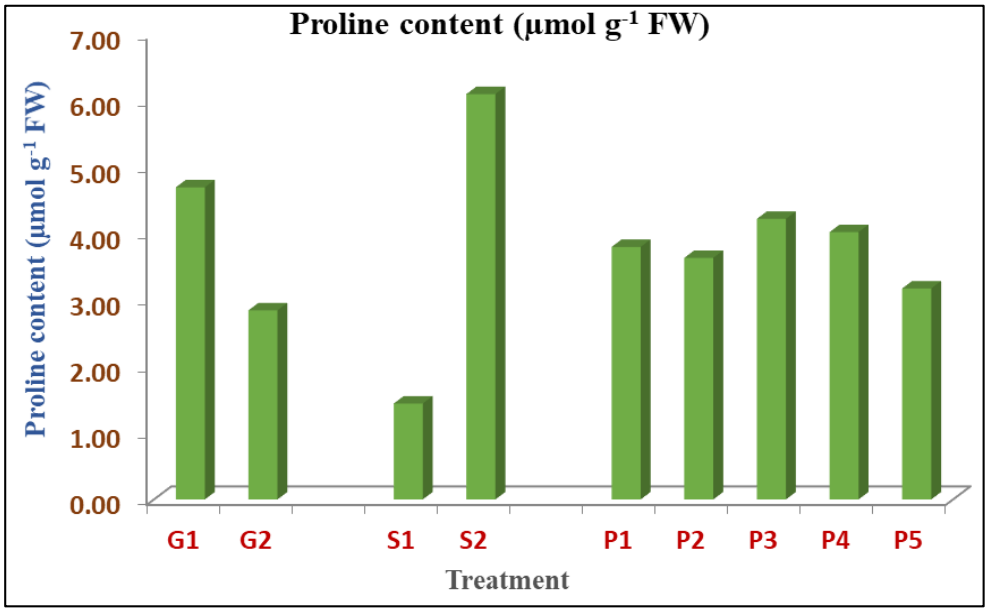

Fig 2: Influence of seed priming on proline content of IP 14294 and IP 17862 at 0 mM and 150 mM NaCl concentrations (G_1: IP 14794, G_2: IP 17862; S_1: 0 mM, S_2: 150 mM; P_1: Hombrassinolide @ 3µM, P_2: Triacontanol @ 10 µM, P_3: Salicylic acid @ 100 µM, P_4: KNO_3 @ 0.5%, P_5: Hydropriming)

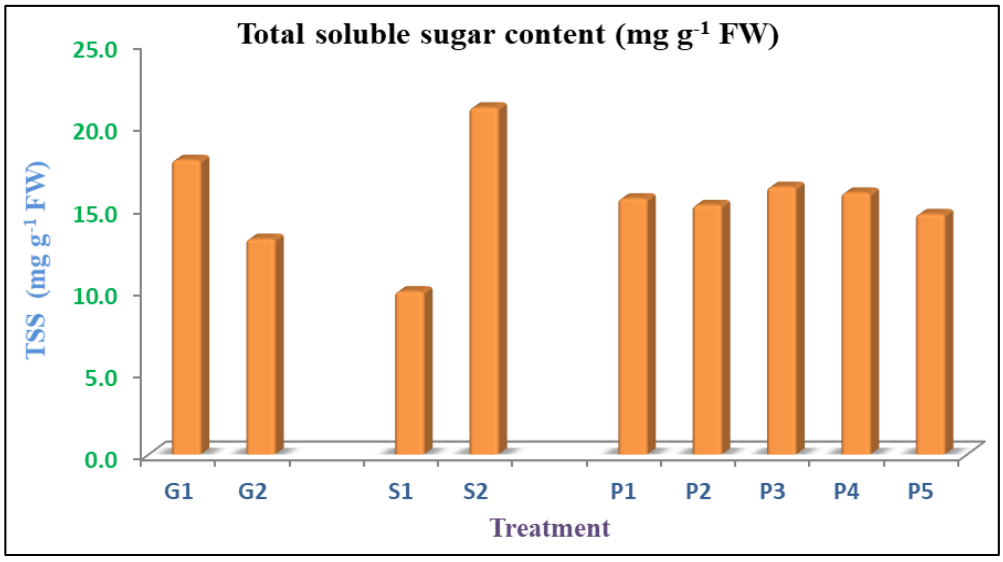

Fig 3: Influence of seed priming on TSS content of IP 14294 and IP 17862 at 0 mM and 150 mM NaCl concentrations (G_1: IP 14794, G_2: IP 17862; S_1: 0 mM, S_2: 150 mM; P_1: Hombrassinolide @ 3µM, P_2: Triacontanol @ 10 µM, P_3: Salicylic acid @ 100 µM, P_4: KNO_3 @ 0.5%, P_5: Hydropriming)

Conclusion

In conclusion, the results of this study signify the role of salicylic acid in regulating the salt response of pearl millet and suggest that salicylic acid acts as a potential growth enhancer to improve seed germination and seedling growth. The salt tolerant accession IP 14294 performed better than IP 17862 under salt stress conditions. Salicylic acid triggered reduction in oxidative stress in seedlings subjected to salt stress by increasing the activity of antioxidant enzymes such as peroxidase and catalase. In the present study, the soluble sugar and proline content plays an important role in the osmotic adjustment in stressed plants primed with salicylic acid. Hence, priming pearl millet seeds with salicylic acid @ 100 µM for 8 hours can improve the germination (%) and seedling vigour of the genotypes under higher salinity level (150 mM NaCl).

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