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A quantitative assessment of germination parameters: the case of *Capsicum annuum* l.

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Abstract

Increasing salinity stress may provide crops a great competitive environment to survive. The aim of the present study was to evaluate the effect of salinity (60mM & 80mM NaCl) on germination of osmo-primed (PEG 4000), hydro-primed (12 hour duration) and thermo-primed (4℃) sweet pepper seeds (*Capsicum annuum* L.) of two varieties namely Mercury and Ganga. The influence of different priming treatments was determined by applying 60mM and 80mM NaCl solutions to primed and unprimed seeds. The applied salt concentrations were chosen based on results relating to germination ability of seeds, obtained in the vegetative stage. Results revealed that germination percentage, germination performance and vigor of Ganga variety were influence by thermo-priming (4℃) as compared to non-primed seeds. Seeds mean germination time was greatly reduced by osmo-priming in variety Mercury at 80mM NaCl treatment in comparison to non-primed seeds. Water content and water use efficiency were also enhanced by all the given priming treatments. Response of both varieties was different. The non-primed and untreated Mercury seeds exhibit the poor performance by inhibiting the root-shoot ratio, shoot moisture content, leaf moisture content, root moisture content and reducing leaf area ratio as compared to primed seeds that increased both the length and weight of plant whereas; Ganga variety had a negative response to these parameters under primed and NaCl treatments. Similarly, total plant biomass was recorded least both in primed and non-primed seeds under salinity stress, suggesting that salinity reduce both fresh and dry mass of plant whereas; different priming strategies could contribute seed germination during stressful environment. The growth performance of test specie confirmed the degree of resistivity to various levels of induced sodium chloride stress indicating successful demonstration of priming technique will help agricultural industry to improve seed quality, vigor, germination rate and biomass prooduction.

Keywords: Hydropriming, Osmo-priming, Thermopriming, *Capsicum annuum* L, Salinity.

1. Introduction

Plants are continuously facing many adverse situations both in natural and agricultural conditions. Sudden changes in climatic and environmental conditions cause different abiotic stresses or even a small change in environment can induce expression or repression of hundreds of genes in plants which lead to decrease in crop production (Ayaydin et al 2015; Vijaya kumari *et al.* 2016) [5, 43]. Globally the main cause to farm production loss is due to some abiotic stresses, among which salinity stress is the most frequent abiotic factor for plants (Grigorova *et al.* 2011) [17]. It is stated that on earth the entire area is fourteen billion ha and 6.6 billion ha of this are semi-arid and arid part whereas; 1 billion ha of this region is salt affected. Such as there is about 22% of entire arable and 33% of land devoted to agriculture is saline that is increasing 10% annually by area. In Pakistan the area affected by salinity is 6174.5 thousand hectares (Jamil *et al.* 2011) [21] whereas; the productive land was being damaged at a rate of about 40000 hectares annually, so salinity is becoming a serious problem for crop productivity both in arid and semi-arid regions (Plazek *et al.* 2013) [34].

Salinity stress due to large ion present in the soil may cause different morphological changes in plant that delay germination, poor stand establishment, high seedling mortality, stunted growth, lower yields and affecting plant breeding (Wojtyla *et al.* 2001; Golezani *et al.* 2011) [46, 15]. Salinity could also bring many physiological and biochemical abnormalities by inducing toxic radical formation due to accumulation of large number of Na^+ and Cl^- ions such that the first visual symptoms of osmotic stress are leaf wilting and rolling due to stomatal closure which leads to leaf death by desiccation, lower the rate of photosynthesis that generate reactive oxygen species (ROS), loss of turgor, accumulation of anthocyanins, nutritional imbalance, reduce enzymatic activity, inhibition of cell elongation, damage cell membrane and nucleic acid structure (Plazek *et al.* 2013; Bres *et al.* 2016) [34, 7]. Reactive oxygen species (ROS) formation by high sodium ions concentration including superoxide radicals in anion form (O_{-2}), singlet oxygen ($^1O_2$), generation of peroxide of hydrogen (H_2O_2) and hydroxyl ion (-OH) cause oxidation of proteins, amino acids, nucleic acids, lipid peroxidation, damage cell membrane and even cell death may occur (Grebosz *et al.* 2014) [16].

The biochemical strategies to salinity stress within defense response of plants are various such as selective build-up or exclusion of salt ions, control of ions uptake by roots and their transport into leaves, changes in structure of cell membrane, compartmentation of ions, osmolytes synthesis, as well as activation of plant antioxidant defense system (Munz and Tester, 2008) [27]. Though development of salt-tolerant crops or desalination of soil by leaching excessive salts is not enough to overcome this problem (Gamal *et al.* 2012) [10]. The most considerable criterion of salt tolerance is a production of biomass, which is significantly declined due to soil salinity (Plazik *et al.* 2013). To enhance the resistivity of plants to salt stress, the use of many agricultural practices has been introduced recently to ameliorate soil stresses like salinity on plant growth has received a greater attention. In Pakistan, increasing the productivity of chilies per unit area as well as expanding the cultivated area in newly reclaimed lands is the major important national target. Increasing productivity per unit area, particularly in saline media, could be achieved by cultivating high yielded cultivars along with importing agronomical practices. As a result, seed treatment technology (priming) is often more applicable and better technique of plant tolerance to the environmental stresse. Seed priming is a pre-germination seed treatment in which seeds are soaked in water or any osmotic solution for improvement of seed quality that increase germination rate and uniformity. Seeds pre-germination treatment has been used to enhance germination, stimulate vegetative growth, decrease time to seed germination, ameliorate stand establishment, greater emergence, repairs damage of aged seeds, enhance plant vigor, better osmotic adjustment, rapid flowering and harvesting that improve grain production production (Nasri *et al.* 2011; Ghiyasi *et al.* 2008; Mouradi *et al.* 2016) [29, 13, 25]. It is estimated that osmopriming is germination activation process by significant oxidative metabolism like increasing the activity of antioxidant enzymes superoxide dismutase (SOD) and peroxidase (POD), glutathione reductase in aged seeds or by the activation of ATPase, enhance α- amylase and acid phosphatase activities, synthesis of RNA and protein (Roy *et al.* 2013 ; aPiwowarczyk *et al.* 2017) [36].

Sweet pepper (*Capsicum annuum* L.) is a Solanaceous agricultural important cash crop, both an economical and health promoting vegetable for consuming the nutritional value of its fruit worldwide, formation of basic antioxidant compounds particularly, polyphenols (e.g., capsaicin) ascorbic acid (vitamin C), flavonoids and carotenoids (Cagno *et al.* 2009) [17]. In Pakistan, pepper a summer vegetable is grown in southern parts of Punjab and Sindh. It is reported that about 4-8 weeks old seedlings can be transplanted when they attained a size of about 15-24 cm (Naz *et al.* 2006) [30]. Global production of chilli fruit loss has been reported up to 50% by the attack of some pathogens (*Colletotrichum capsisa*) and abiotic factors that proved to be cause wilting in chilli plants (Sattar *et al.* 2016) [39].

In this investigation an attempt is made to account the degree of resistivity of two sweet pepper cultivars (Mercury & Ganga) against salinity stress of 60mM NaCl and 80mM NaCl solution under pre-liminary study (Plazik *et al.*, 2013) because of large Na^+ and Cl^- ions accumulation in northern and southern regions of Pakistan reduced pepper productivity. A short-term experiment was conducted based on assumptions that soaking of seeds in osmotin like polyethylene glycol (osmo-priming), in pure water (hydropriming) and at low temperature (thermopriming) has shown to be an elementary, inexpensive and impregnable method for expanding the capability of seeds to increase seedling establishment, vigor growth and biomass production during induced salinity stress proposed to induce resistivity in test specie.

Materials and Methods

Site description

Field experiments were conducted at University of Peshawar (34° 1' 33.3012" N and 71° 33' 36.4860" E.), Pakistan, during the 2018 growing seasons. Peshawar lies in Iranian plateau area of the tropical climate. The weather ranges between 5°C (in January) and 39°C (in June) with mean annual rainfall of about 513 mm. Most of the rainfall occurs during monsoon months (Julye & August), while wheat seasons (November & April) receive nearly 350 mm rainfall. Soil samples (0-30 cm) from the experimental site were collected prior to sowing for soil physico-chemical analyses. Soil textural class as determined by hydrometer method (Gee and Bauder, 1982) [12] was silt loam. Averaged across two years, the chemical properties of the soil were: EC 2.41ds/m, pH 6.0 (McLean, 1982) [23], organic C 20.5 g/kg (Nelson and Sommers, 1982), N content 2.05g/kg (Keeney and Nelson, 1982) along with P available 7.5 mg/kg(Jackson, 1973) and K available 90.5 mg/kg (Hanway and Heidel, 1952) [18].

Experimental Design

The seeds of *Capsicum annuum* L. two varieties (Mercury & Ganga) were obtained from National Agriculture Research Centre (NARC) Pakistan. This is a high productive variety with an average weight of 50-60g four lobed fruit that is dark green in color. Harvesting period is from 70-75 days of transplanting. Nursery with 3 meter length and 1.5 meter width was occupied with earthen pots in which seeds were sown (20cm height, 18cm upper/lower diameter and 2cm thickness) contain 2kg soil and silt in the ratio of 2:1 with farm yard well rotten manure. The data set was as Randomized Complete Block Design (RCBD) with two cultivars (Mecury & Ganga) and four factor levels including osmopriming with polyethylene glycol (-0.2 MPa) for 1h (Mouradi *et al.* 2016) [25] hrdopriming with distilled water for 12h (Islam *et al.* 2015) and 1h thermopriming at 4℃ (Afzal *et al.* 2006). Intact seeds homogeneous and identical in size and color free from wrinkles were surface sterilized with 70% ethanol and 0.1 % mercuric chloride at room temperature (Warwate *et al.*, 2017) [45] and then sown in earthen pots. Watering was done regularly to maintain proper moistening of seedlings during the whole period of growing. About 3-liter water per pot was used during the entire experiment till the plants were cultivated. Pots were kept free from weeds by hand weeding to expose seedlings to sunlight for better growth. After a week of germination, plants were thinned to three per pot. One set of experiment was subjected to saline treatment (60mM & 80mM NaCl) to 15 days old plants whereas; the second set was taken into control or untreated. Three replications per treatment were taken and salt treatment was applied by direct induction of 10ml salt solution (NaCl) to each pot. All the standard practices for pot experiment from time to time were done for good and disease or pest free crop during the entire experiment. Specimens were collected for the determination of various growth parameters.

Physio-chemical analysis of rhizospheric soil

Soil pH and Soil electrical conductivity (EC)

Mckeague (1978); Mclean (1982) [23] method was used for soil pH finding. Take 10g of soil and 10ml of water by process it through standardized mixing and then filtered it with Whatman # 42 filter paper. pH meter used for pH determination while EC of soil was estimated through electrical conductivity meter in micro Siemens per centimeter.

Growth measurements

At the end of experimental period, growth measurements for the plants for each saline level (60mM & 80mM) was taken after treatment. The three replicates taken for each treatment, were used to determine the mean of each measurement. The measurements were taken by using the following formulas:

Absolute growth rate (AGR)

Absolute growth rate was calculated according to the formula as described by (Ghule *et al.* 2013) [14].

$$\text{AGR (plant height)} = \frac{H_2 - H}{t_2 - t_1}$$

Eq. 1

$$\text{AGR- (dry matter)} = \frac{W_2 - W_1}{t_2 - t_1}$$

Eq. 2

Where H_1, and H_2 refers to plant height (cm). W_1 and W_2 refer to plant dry matter weight (g) during the time t_1 to t_2,

Relative growth rate (RGR)

This parameter was determined by the by the method as per suggested by (Ghule *et al.* 2013) [14]. Relative growth weight refers to growth of plant per unit dry weigh.

$$\text{RGR} = \frac{(\log_e W_2 - \log_e W_1)}{t_2 - t_1}$$

Eq. 3

Hence W_1 and W2 is dry weight (g) of plant at time t1 and t2 whereas; the interval in days, $\log_e$ is Natural logarithms (Logarithms to the base of 2.3026). Relative growth rate is expressed in gram/plant/day.

Net assimilation rate (NAR)

Net assimilation rate was reported by the proposed method of (Ghule *et al.* 2013) [14]. This parameter refers to rate of any increase in dry matter of plant per unit of its assimilatory surface per unit time.

$$\text{NAR} = \frac{W_2 - W_1 \text{ x } (\text{loge } A_2 - \text{loge } A_1)\ (\text{g/cm /day})}{t_2 - t_1 \quad A_2 \text{- } A_1}$$

Eq. 4

Where, A_1 and A_2 are the leaf surface areas (cm^2) and W_1, W_2 are total dry matter (g) of plant at time t_1 and t_2.

Time to 50% emergence (T_{50})

Time to 50% emergence was determined as suggested by (Vujosevic *et al.* 2018) [44]. For such determination days were counted treatment from the day of sowing till 50% seeds have been emerged.

$$T_{50} = \frac{t_i + (N/2 - ni)\ (t_j - t_i)}{(n_j - n_i)}$$

Eq. 5

Where "N" represents the number of final emerged seeds, nj and ni are the cumulative number of seeds emerged by adjacent counts at times tj and ti, respectively, when $ni < N/2 < nj$.

Timson germination index (TGI)

Timson germination index denoted the number of seeds germinated per day. This was calculated by the method as peer describe by (Al-Ansari & Ksiksi, 2018) [4].

$$\text{TGI} = \sum G/T$$

Eq. 6

Where G is the percentage of seed germinated per day, and T is the germination period.

Leaf area index (LAI)

Leaf area index Leaf mean area has been calculated as per formula reported by (Shah *et al.* 2017) [40] by collecting three leaves per pot randomly for measuring their width and length.

$$\text{LAI} = \frac{\text{Leaf area (cm)}^2}{\text{Land area (cm)}^2}$$

Eq. 7

Crop growth rate (CGR)

This has been calculated by the formula proposed by (Shah *et al.* 2017) [40]. Dry weight was determined by keeping the samples for drying in an oven at 30℃ for three days.

$$\text{CGR} = \frac{W_2 - W_1}{T_2 - T_1} \text{ x } \frac{1\ (\text{g/m}^2\text{/d})}{\text{Land area}}$$

Eq. 8

Where W_1 and W_2 denotes plant dry mass at time T_1 and T_2, respectively

Relative water contents (RWC)

This parameter has been reported by the method established by (Shah *et al.* 2017) [40].

$$\text{RWC} = \frac{Wf - Wd}{Ws - Wd} \text{ x100 (\%)}$$

Eq. 9

Where Wf is leaf fresh weight and Wd is leaf dry weight Saturated weight of leaves (Ws) was measured after floating leaves in water for 18h.

Leaf area ratio (LAR)

Leaf area ratio was reported as per formula suggested by (Shah *et al.* 2017) [40]. Leaf area was measured by taking length and width by help scale while plant dry mass (g) was determined by keeping the samples in an oven at 30℃ for 72 hr duration.

$$\text{Leaf area ratio} = \frac{\text{Leaf area}}{\text{Final plant dry weight}}$$

Eq. 10

Root-shoot ratio (RSR)

Root-shoot ratio has been determined by the proposed formula of (Chuyong & Acidri, 2017) [9]. Dry weights of root and shoot in both treatments (60Mm and 80mM) were taken by keeping the samples in an oven for 72 hrs.

$$\text{Root-shoot ratio} = \frac{\text{Root dry mass}}{\text{Shoot dry mass}}$$

Eq. 11

Water use efficiency (WUE)

Chuyong and Acidri (2017) [9] reported the formula for the determination of water use efficiency of plant. Total biomass was taken by adding the dry mass of root, stem and leaf of plant (Chuyong & Acidri, 2017) [9].

$$\text{Water use efficiency} = \frac{\text{Total water used during the experiment (ml)}}{\text{Total biomass (g)}}$$

Eq. 12

Field capacity percentage (FCP)

The field capacity of rhizospheric soil was measured by the following formula of (Ullah *et al.* 2016) [42].

$$\text{Field capacity Percentage} = \frac{\text{Weight of wet soil} - \text{Weight of dry soil}}{\text{Weight of dry soil}} \times 100$$

Eq. 13

Percent moisture content

Percent moisture content of leaf, root, shoot and soil was determined by the estimated method of (Ullah *et al.* 2016) [42].

$$\text{Percent moisture content} = \frac{\text{Wet weight of sample} - \text{Dry weight of sample}}{\text{Dry weight of sample}}$$

Eq. 14

Seed vigor index-I (SVI-I)

Seed vigor index regarding the seedling's length has been reported by the method established by (Bina & Bostani, 2017) [6].

Seed vigor index
= Seedling length (cm) x Seed germination % age Eq. 15

Seed vigor index-II (SVI-II)

Seed vigor index regarding the seedlings dry weight has been reported by the method established by (Sharma, 2016) [41].

Seed vigor index =Seedling dry weight (mg)
x Seed germination % age Eq. 16

Statistical analysis

The statistical analysis was four factors factorial, arranged in a randomized completely block design, with three replicates of each variety and 10 seeds per replicate. The first factor was control, the second factor was osmotic potential level (0.2 MPa) in PEG 4000 solution, the third factor was 12-hour hydropriming and the fourth factor was thermopriming at 4℃. Data for various germination parameters was determined by Statistix 10. Mean separation and standard deviation were performed.

Results

The findings obtained from the present study where growth parameters and yield of *Capsicum annuum* L. as influenced by various priming techniques under different salt treatments have been studied. Results in Table 1 showed, timson germination (TG), vigor index-I (VI-I), vigor index-II (VI-II), leaf area ratio (LAR), leaf area index (LAI), time to 50% germination (TG50%). According to the results, maximum TG was recorded in 12hr hydroprimed seed with 60mM NaCl concentration in Mercury. VI-I significantly increased during 12hr thermopriming while VI-II indicated maximum result in thermoprimed seeds with 60mM NaCl concentration in Mercury variety. Similarly, Ganga variety represented maximum TG value in thermoprimed (4°C) seeds with 80mM NaCl treatment, VI-I was greatly influenced by 4℃ thermopriming and maximum VI-II was reported in thermoprimed (4°C) seeds with 60mM NaCl treatment in similar variety. Consequently, TG, VI-I and VI-II significantly reduced in 12hr hydropriming in Mercury variety. Similar trend was recorded in Ganga variety except TG that is negatively affected by induced 60mM NaCl treatment in 12hr hydropriming. Moreover; LAR results indicated maximum value in 12hr hydroprimed seeds with 60mM NaCl concentration in both varieties. LAI reported maximum value up to great extent in osmoprimed (PEG) seeds with induced 60mM NaCl concentration in Mercury and 12hr hydroprimed seeds with 80mM NaCl treatment in Ganga cultivar. TG50% reported maximum with applied 60mM NaCl concentration in Mercury and osmoprimed (PEG) Ganga variety seeds under same treatment. LAR, LAI and TG50% indicated minimum values in osmoprimed (PEG) and thermoprimed (4°C) seeds by induced 80mM NaCl treatment except thermoprimed (4°C) seeds that reported decreased leaf area ratio by applied 60mM NaCl concentration while maximum reduction (LAR) recorded in control of Mercury cultivar. Same parameters in Ganga variety were strongly affected by increasing NaCl concentration (80mM NaCl) during hydropriming.

According to the results presented in Table 2, indicated that relative water content (RWC) was calculated maximum in thermoprimed (4°C) seeds with 80mM NaCl concentration in Mercury variety and 80mM NaCl treatment in Ganga variety under similar priming technique. Same parameter showed minimum value in Mercury variety under control environment and 60mM NaCl treatment in Ganga variety, indicated that priming technique proved to be affective against induced saline treatment with respect to unprimed seeds with induced NaCl treatment. Plant water use efficiency (WUE) was recorded maximum in 12hr hydropriming at applied 60mM NaCl concentration in both varieties whereas; minimum results were measured in control condition of Mercury variety and 80mM NaCl treatment in Ganga variety. Similarly, absolute growth rate (AGR-I) with respect to plant dry mass calculated maximum in 12hr hydropriming while minimum at 80M NaCl concentration in osmoprimed (PEG) and thermoprimed (4°C) seeds in both varieties. Absolute growth rate (AGR-II) related to plant height indicated maximum result in osmoprimed (PEG) seeds with 80Mm NaCl in Mercury cultivar and 60mM NaCl treatment in Ganga variety. However; minimum results reported in thermoprimed (4°C) seeds with 60mM NaCl concentration in Mercury variety and 12hr hydroprimed seeds of Ganga variety at 80mM NaCl treatment. Results of growth parameters (AGR-I & AGR-II) clearly showed weight and height of plant were greatly affected by increasing the concentration of NaCl solution from 60mM to 80mM created a significant stress condition. Consequently, relative growth rate (RGR), Net assimilation rate (NAR) and crop growth rate (CGR) indicated maximum value in thermoprimed (4°C) seeds of Mercury variety and induced 80mM NaCl concentration in Ganga cultivar except CGR that reported maximum in control seeds. Simultaneously, minimum value for the same parameters (RGR, NAR, CGR) were recorded in 12hr hydropriming at 60mM NaCl concentration in Mercury variety and thermoprimed (4°C) seeds with 80mM NaCl treatment in Ganga variety.

Root-shoot ratio (RSR) and root moisture content (RMC) of plant in Mercury variety as shown Table 3**,** indicated a strong positive/maximum response by increasing the amount of NaCl solution from 60mM to 80mM both in 12hr hydroprimed and osmoprimed (PEG) seeds. Similar results reported in Ganga variety thermoprimed (4°C) seeds with 60mM NaCl concentration. A very significant decrease of RSR & RMC was observed in Mercury variety under control condition and osmoprimed (PEG) seeds of Ganga variety in the same applied treatment. Moreover, shoot moisture content (SMC) reported maximum at 12hr hydropriming in Mercury variety. Same parameter was recorded maximum at 12hr hydropriming by induced 60mM NaCl treatment in Ganga cultivar. Minimum value of SMC was indicated in control, osmoprimed (PEG) and 12hr hydroprimed seeds at 60mM NaCl concentration in Mercury variety whereas; Ganga cultivar was less affected with maximum salinity stress (80mM NaCl). Leaf moisture content (LMC) reported maximum in both varieties along with maximum salinity stress indicated that priming has been proved to be affective against induced salinity stress. Minimum value was observed in control of Mercury variety and 60mM NaCl applied to Ganga cultivar for the same parameter. Plant total biomass (TBM) was recorded maximum in control and negatively affected with 80mm NaCl concentration in osmoprimed (PEG) and 12hr hydroprimed seeds in both varieties. Similarly, root moisture content (RMC) indicated maximum value with 80mM NaCl concentration in thermoprimed (4°C) seeds of Mercury variety and 60mM NaCl in Ganga variety with similar priming technique.

Discussion

Among all the environmental factors, salinity severely affects the growth, development, physiology and production of plant. To survive under such adverse condition, use of seed priming technique can be a great approach against salt tolerance to cope with several environmental stresses. Plant resistance at stage of germination determined to be the major aspects of salt tolerance for great germination and uniformity. Only seed germination does not denote that a species has a great ability of germination, but other aspects related to germination including germination rate, speed of emergence, emergence percentage, seed vigor and its establishment are involved in measuring the germination ability of a seed. Similarly, the association of these different germination parameters with each other differentially gives us an idea and a great deal for understanding and studying the problems related to germination in different plant species under different situation. Hence, the present study aim is to find out the ability of seed priming through PEG-4000 (osmo-priming), water (hydro-priming) and chilling temperature at 4℃ (thermo-priming) to assess the performance of germination of two sweet pepper cultivars (Mercury & Ganga) against salt tolerance under two different concentrations of NaCl solution (60mM & 80mM) at seed germination stage and cultivation stage. According to our results (Table-1) timson germination, vigor index, leaf area ratio and time required to 50% germination appeared to be the most descriptive parameters that increased significantly up to great tolerance level against salinity (Bina & Bostani, 2017; Noumani, *et al.* 2011) [6, 32]. These parameters enhanced by both hydro-priming and thermo-priming with a least difference among different priming conditions and saline treatments. Leaf area ratio and leaf area index reported decreased under thermo-priming condition with increased saline solution but enhancement of the same parameters occurred during hydro-priming under similar concentration of saline solution in Ganga variety that proved to be sensitive to chilling temperature. According to Abdolahpou & Lotfi (2014) [1] reported that leaf area reduced under salinity in salt sensitive cultivars (Abdolahpour & Lotfi, 2014) [1].

Seedlings germinated by hydro-priming and thermopriming (Table 2) represented high relative water content and a water use efficiency for both specie during induced salinity stress as compared to control condition that is negatively affected by salinity stress (Mouradi *et al.* 2016) [25]. Net assimilation Rate, crop growth rate indicted different performance for the same treatment. This may be due to different priming technique and their duration period but generally found decreased by increasing saline treatment. Nascimento (2013) [28] concluded that priming enhances germination rate and plant total germination by improving germination response to longer duration of seed priming (Nascimento *et al.* 2013) [28]. Our results agree with the findings of Ramzan *et al.* (2010) [35]; Mirlotfi *et al.* (2015) [24]; Ghule *et al.* (2013) [14].

Similarly, root-shoot ratio (Table 3) during induced salinity greatly increased while shoot moisture content was found minimum suggested that salinity negatively affect the moisture content of shoot by decreasing the mass (Gebreegziabher & Qufa, 2016) [11]. Sardoei and Mohammadi (2014) [38] investigated that growth of shoot and root are greatly affected by salt stress (Sardoei & Mohammadi, 2014) [38]. Soil moisture content and percent field capacity in sensitive variety (Mercury) was reduced by increasing salinity in priming condition while decreased in untreated control condition in “Ganga” specie concluded that salinity affecting this parameter both in unprimed and primed seeds. On the contrary root moisture along its mass was significantly enhanced by induced maximum salinity in priming situation leaf moisture content show no significant changes while total biomass of plant was observed maximum in control and minimum under saline condition. According to Al-Abdoulhadi (2011) [3]; Ramzan (2010) [35] investigated that total biomass of plant (root, stem, leaf) significantly decrease with increase salinity levels (Abdolahpour & Lotfi, 2014; Ramzan *et al*. 2010) [1, 35].

Table 1: Effect of hydropriming, osmo-priming and thermopriming on timson germination, vigor index-I, vigor index-II, leaf area ratio, leaf area index and time to 50% germination under salinity stress

Varieties	Treatments		TG	VI-I	VI-II	LAR	LAI	TG50%
Mercury	Control		1.70±0.26	1656.0±270	541.7±19	35.879±20.9	1.70±0.58	2.50±0.0
	Salinity	60mM NaCl	2.06±1.02	1760.3±923	610.8±82	91.15±38.28	2.08±0.25	2.72±0.3
		80 mM NaCl	1.60±0.43	1470.7±693	765.6±90	61.12±8.224	2.11±0.27	2.50±0.0
	Osmo-priming	PEG	1.33±0.64	1483.0±680	490.2±54	73.51±28.94	1.87±0.55	2.50±0.0
		PEG+60mM NaCl	1.33±0.23	1480.0±680	529.8±34	100.02±62.2	2.52±0.96	2.50±0.0
		PEG+80mM NaCl	1.06±0.92	1276.0±110	563±146	69.06±17.31	1.44±1.25	2.50±0.0
	Hydropriming	12hrs	0.53±0.25	682.00±317	416.6±94	103.36±67.0	2.18±0.91	2.50±0.0

		12hrs+60mM NaCl	2.10±0.81	1546.3±397	410.7±41	136.42±20.7	2.21±0.26	2.50±0.0
		12hrs+80mM NaCl	1.73±0.92	1671.0±721	645.4±99	83.96±17.03	2.04±0.82	2.71±0.3
	Thermopriming	4℃	1.56±0.83	1895.7±320	1068±73	48.78±7.575	2.17±0.19	2.50±0.0
		4℃+60mM NaCl	1.20±0.52	1110.7±226	1483±26	86.93±30.17	1.68±0.11	2.66±0.2
		4℃+80mM NaCl	1.86±0.58	1398.3±364	572±102	65.20±13.48	1.94±0.42	2.50±0.0
Ganga	Control		1.30±0.30	1243.7±532	539±350	65.68±21.90	2.13±0.41	2.50±0.0
	Salinity	60mM NaCl	1.13±0.72	971.33±586	436.0±91	92.24±30.79	1.79±0.47	2.50±0.0
		80 mM NaCl	1.76±0.37	520.33±671	412.4±27	67.10±93.20	2.13±0.67	2.69±0.3
	Osmo-priming	PEG	1.26±0.23	1605.3±948	614.0±11	66.31±31.20	1.65±0.12	2.50±0.0
		PEG+60mM NaCl	1.43±0.15	1646.7±359	472.3±55	87.23±33.63	1.90±0.25	2.69±0.3
		PEG+80mM NaCl	1.30±0.69	1606.7±500	469.3±59	88.97±15.19	1.91±0.38	2.50±0.0
	Hydropriming	12hrs	0.73±0.47	510.33±353	196.8±36	86.47±42.70	1.58±0.53	2.50±0.0
		12hrs+60mM NaCl	0.60±0.10	891.33±105	309.2±30	129.85±26.6	1.83±0.75	2.50±0.0
		12hrs+80mM NaCl	1.36±0.41	1189.7±168	454.1±47	120.9±39.88	2.26±0.29	2.50±0.0
	Thermopriming	4℃	1.63±0.85	1606.0±301	473.7±16	108.01±53.7	1.80±0.53	2.50±0.0
		4℃+60mM NaCl	1.76±0.41	1436.0±399	349±780	120.95±45.3	1.74±0.56	2.50±0.0
		4℃+80mM NaCl	1.80±0.20	1203.0±244	1282±37	64.100±18.3	1.08±0.32	2.50±0.0

TG= Timson Germination, VI-I= Vigor Index-I, VI-II= Vigor Index-II, LAR= Leaf Area Ratio, LAI= Leaf Area Index, TG50%= Time to 50% Germination

Table 2: Effect of hydropriming, osmo-priming and thermopriming on relative water content, water use efficiency, absolute growth rate (plant dry weight), absolute growth rate (plant height), relative growth rate, net assimilation rate and crop growth rate under salinity stress

Varieties	Treatments		RWC	WUE	AGR-I	AGR-II	RGR	NAR	CGR
Mercury	Control		155±138.2	3531.8±216	0.70±0.192	0.071±0.020	0.12±0.01	0.12±0.01	3.10±2.6
	Salinity	60 mM NaCl	241±8.34	7134.0±223	0.58±0.094	0.167±0.10	0.14±0.04	0.14±0.04	3.59±1.1
		80 mM NaCl	232±2.57	4904.7±108	0.73±0.216	0.162±0.09	0.20±0.03	0.20±0.03	4.96±8.3
	Osmo-priming	PEG	231±12.9	6371.5±874	0.68±0.087	0.066±0.09	0.14±8.34	0.14±8.34	3.58±2.0
		PEG+60mM NaCl	241±2.8	6221.4±158	0.76±0.202	0.169±0.11	0.15±0.02	0.15±0.02	3.69±6.7
		PEG+80mM NaCl	220±24.5	5351.7±163	0.52±0.094	0.207±0.35	0.14±0.12	0.21±0.04	3.41±3.0
	Hydropriming	12hrs	241±5.3	7482.0±186	0.94±0.088	0.067±0.08	0.23±0.12	0.23±0.12	5.56±3.0
		12hrs+60mM NaCl	227±21.0	10507.0±976	0.84±0.148	0.149±0.07	0.09±0.02	0.09±0.02	2.19±5.0
		12hrs+80mM NaCl	235±15.5	7196.2±155	0.77±0.178	0.195±7.21	0.15±0.05	0.15±0.05	3.67±1.2
	Thermopriming	4℃	233±12.0	3758.6±576	0.80±0.066	0.117±0.02	0.26±0.06	0.26±0.06	6.36±1.5
		4℃+60mM NaCl	228±20.1	8643.5±323	0.63±0.553	0.052±0.05	0.15±0.06	0.15±0.06	3.67±1.5
		4℃+80mM NaCl	242±11.6	5864.8±205	0.82±0.151	0.075±0.03	0.17±0.06	0.17±0.06	4.16±1.6
Ganga	Control		159±204	5099.1±108	0.71±0.090	0.088±0.03	0.17±0.02	0.17±0.02	4.30±5.5
	Salinity	60 mM NaCl	221±11.6	9424.2±494	0.70±0.170	0.122±0.04	0.14±0.08	0.14±0.08	3.59±1.9
		80 mM NaCl	74.4±154	4179.7±504	0.82±0.071	0.113±0.07	0.56±0.42	0.56±0.42	0.01±0.0
	Osmo-priming	PEG	242±9.8	6627.7±293	0.78±0.166	0.100±0.06	0.16±0.06	0.16±0.06	3.87±1.6
		PEG+60mM NaCl	237±13.0	7667.4±318	0.84±0.220	0.072±0.05	0.13±0.06	0.13±0.06	3.20±1.4
		PEG+80mM NaCl	234.±3.0	8103.7±298	0.73±0.093	0.080±0.044	0.11±0.02	0.11±0.02	2.67±7.0
	Hydropriming	12hrs	150±150	8701.9±226	0.88±0.074	0.115±0.05	0.12±0.02	0.12±0.02	3.04±6.0
		12hrs+60mM NaCl	209±8.13	14021.0±891	0.87±0.105	0.036±0.03	0.15±0.09	0.15±0.09	3.78±2.2
		12hrs+80mM NaCl	241±23.1	9215.4±384	0.84±0.116	0.020±8.78	0.15±0.06	0.15±0.06	3.82±1.5
	Thermopriming	4℃	223±9.7	9755.9±242	0.80±0.241	0.121±0.10	0.11±0.01	0.11±0.01	2.70±2.9
		4℃+60mM NaCl	281±103	13467.0±982	0.76±0.174	0.115±0.04	0.12±0.05	0.12±0.05	3.09±1.3
		4℃+80mM NaCl	237±8.06	10043.0±207	0.61±0.173	0.044±0.04	0.08±0.02	0.08±0.02	2.16±6.1

RWC=Relative Water Content, WUE= Water Use Efficiency, AGR-I= Absolute Growth Rate (plant dry weight), AGR-II= Absolute Growth Rate (plant height), RGR= Relative Growth Rate, NAR= Net Assimilation Rate, CGR= Crop Growth Rate

Table 3: Effect of hydropriming, osmo-priming and thermopriming on root-shoot ratio, shoot moisture content, leaf moisture content, root moisture content, soil moisture content, % field capacity and total biomass under salinity stress

Varieties	Treatments		RSR	SMC	LMC	RMC	SMC	%FC	TB
Mercury	Control		7.26±11	84.67±3.62	99.95±0.04	57.8±22	17.40±4.6	21.31±6.6	0.35±0.32
	Salinity	60 mM NaCl	52.1±72	86.60±2.15	99.98±2.13	83.2±17	10.80±13	13.91±18	0.07±8.48
		80 mM NaCl	16.8±50	85.73±1.68	99.98±7.30	83.6±10	17.86±9.2	22.86±14	0.10±0.01
	Osmo-priming	PEG	14.3±30	85.29±2.33	99.97±4.03	84.9±4.1	22.83±2.4	29.67±4.1	0.08±0.03
		PEG+60mM NaCl	52.7±66	84.73±2.01	99.98±7.15	84.2±1.1	23.13±5.1	30.49±8.9	0.08±0.02
		PEG+80mM NaCl	25.6±22	86.17±1.27	99.99±8.61	88.6±8.1	43.13±49	17.34±5.0	0.03±0.04
	Hydropriming	12hrs	11.7±80	90.19±6.79	99.98±1.30	88.1±1.8	15.73±3.2	18.78±4.5	0.09±0.01
		12hrs+60mM NaCl	10.9±20	84.06±5.27	99.99±7.06	85.9±3.4	15.50±2.6	18.42±3.8	0.04±0.02
		12hrs+80mM NaCl	14.3±0.9	86.64±2.29	99.98±4.51	86.7±2.7	8.96±6.61	10.25±8.3	0.07±0.04
	Thermopriming	4℃	21.6±12	84.93±0.93	99.98±3.24	90.1±6.5	24.90±3.1	33.30±5.5	0.11±0.01
		4℃+60mM NaCl	25.7±30	87.82±1.57	99.99±6.30	97.2±4.6	14.76±0.8	17.33±1.1	0.06±0.05
		4℃+80mM NaCl	20.7±80	84.56±0.42	99.98±3.01	99.9±3.4	17.53±7.3	21.93±11	0.09±0.05
Ganga	Control		9.78±30	86.57±2.29	99.96±0.03	56.1±19	8.26±5.77	9.30±6.95	0.27±0.19
	Salinity	60 mM NaCl	24.4±12	86.42±4.61	99.99±4.15	70.3±26	20.70±4.7	26.41±7.8	0.17±0.13

		80 mM NaCl	23.9±11	85.45±0.69	99.93±0.04	92.5±12	33.2±24.1	66.07±68	0.05±0.03
	Osmo-priming	PEG	9.16±20	86.61±0.28	99.98±2.44	52.8±45	22.13±4.6	28.72±7.6	0.10±0.02
		PEG+60mM NaCl	24.7±13	83.83±3.96	99.98±3.26	76.7±6.0	17.66±8.4	22.3±13.0	0.07±0.03
		PEG+80mM NaCl	17.6±13	83.6±1.62	99.98±8.39	90.7±10	16.16±7.9	20.00±11	0.07±0.02
	Hydropriming	12hrs	16.5±40	90.18±0.99	99.96±0.04	83.7±4.0	15.00±2.6	17.72±3.6	0.13±0.14
		12hrs+60mM NaCl	24.3±30	90.85±0.61	99.99±3.27	92.0±3.1	15.96±3.6	19.14±4.9	0.05±0.01
		12hrs+80mM NaCl	22.9±60	88.62±1.42	99.98±5.63	86.1±1.9	14.46±3.4	17.04±4.7	0.01±0.03
	Thermopriming	4℃	44.4±22	85.75±3.24	99.99±3.10	82.1±27	16.73±5.2	20.41±7.4	0.03±0.02
		4℃+60mM NaCl	47.0±54	89.88±2.28	99.98±0.01	99.9±00	10.46±6.2	12.04±7.5	0.05±0.04
		4℃+80mM NaCl	22.2±11	83.16±2.45	99.98±2.19	96.2±6.3	23.33±2.6	30.53±4.4	0.04±0.02

RSR= Root-Shoot Ratio, SMC= Shoot Moisture Content, LMC= Leaf Moisture Content, RMC= Root Moisture Content,
SMC= Soil Moisture Content, %FC=% Field Capacity, TB= Total Biomass

Conclusion

It has been concluded that under salinity stress, germination of sweet pepper thermo-primed seeds with 4℃ were of maximum amplitude. Osmo-priming with PG-4000 at 0.2 MPa improved seedling establishment and field performance. Hydropriming with 12-hour duration was also helpful in reducing poor germination but proved to be less effective than thermo and osmopriming. Our findings simply revealed that priming is a simple, useful and cheap technique for agricultural industry for improving seed quality and more affective results could expected by increasing the duration of priming and alleviate the harmful effects of salinity stress.

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