Screening of tomato genotypes using osmopriming with PEG 6000 under salinity stress

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Abstract: The aim of this study was to elucidate the effects of osmopriming with PEG 6000 on five tomato genotypes viz. Kashi Vishesh, Kashi Amrit, Kashi Anupam, Kashi Hemant and Kashi Sarad, under salinity stress. Seeds were primed with varying concentration of PEG 6000 i.e. (-0.5, -1.0, -1.5, -2.0 MPa) and along with one hydroprimed for 48 hours at 25°C. Dry tomato seeds served as control (non-primed). Treated seeds of all varieties were sown in Petri dishes supplied with three different concentrations of salt solution i.e. 4, 8 and 12 dSm-1 and along with one control. Results of variance analysis made clear that different osmotic potential and priming duration had significant effect on germination percentage, vigor index and dry weight. Of the five tomato genotypes, Kashi Hemant primed with -0.5 Mpa showed highest per cent germination (71 %), seedling length (18 cm), seed vigor index (994) and dry weight (14 mg) at 4 dSm-1 as compared to 8 dSm-1, 12 dSm-1 and control respectively. The results revealed that the same variety has immense potential to tolerate salinity stress. Furthermore, it was also observed that primed seeds exhibited better stress tolerance than non-primed seeds.

Key words: Tomato, seed priming, osmopriming, polyethylene glycol, salinity stress, germination, vigor index

Introduction

Tomato (Solanum esculentum L.) is one of the most important horticultural crop worldwide, India ranks second in production after China, accounting for about 11% of the world tomato production (Indian horticulture database, 2011). On a global scale, it contributes for about 15% of total vegetable production or about 160 million tonnes produced in 2011, (FAO, 2014). Tomato crop yield and quality is depending on seeds and its rapid and synchronous seed germination potential. However, decrease in seed vigor due to storage, might reduced the seed germination (Coolbear et al., 1984) and non-uniformity in seed vigor (Ismail et al., 2005) and effects of abiotic stress (Wang et al., 2011). Salinity has become a great threat to tomato growth (Chen et al., 2009), thus, poor and non-uniform seed germination as well as salinity stress might become limiting factors for synchronized seed germination and seedling establishment in tomato. Plant anatomy, physiology and morphology were also affects by soil salinity (Greenway and Munns, 1980; Munns, 2002; Ashraf and Harris, 2004; Sharaf-Al-Tardeh and Naimm Iraki, 2013). However, seed priming techniques is a low cost and effective treatment to improve seed germination and seedling vigor. Thus, understanding to the physiology of salt tolerance in plants became important for the effective solution of the problem of salinity in agricultural and horticultural soils (Larcher, 1994). Under various environmental stresses such as salinity, high and low temperatures as well as water deficiency, osmopriming leads to cellular, sub-cellular and molecular changes in seeds and consequently promotes seed vigor during germination and emergence in different plant species promotes (Argerich and Bradford, 1989; Abdulrahmani, 2007). At present, the seed priming is being extensively used to improve seed germination and seedling emergence in various crop species (Alvarodo et al., 1987; Farahani et al., 2011) and seed priming is basically a physiological process in which the seeds are presoaked before planting which then allows partial imbibitions though preventing the seed germination (Parera and Cantliffe, 1994) and controls seeds hydration and drying to their original moisture content (Zang et al., 2012). However, Rivas et al., (1984) found improved germination rates in Jalapeno and Tabasco tomato seeds primed with PEG-6000. In tomato, previous studies have reported that priming improved seed germination under drought (Mauromicale and Cavallaro, 1985) and low temperature stress (Mauromicale and Cavallaro, 1997; Ozbingol et al., 1998) and it improved seed storability (Liu et al., 1996). Jumsoon et al., (1996) found that primed seeds had higher percentage germination than unprimed seeds at 15°C or 20°C under both water and saline stress. There is evidence that seed osmopriming increased salinity tolerance of tomato (Lycopersicon esculentum Mill.), sunflower (Helianthus annus L.), bean (Phaseolus vulgaris L.), (Khadri et al., 2007; Damirkaya et al., 2006; Jumsoon et al., 1996).

In fact, seed priming is a technique applied before germination in which seeds are partially hydrated to a point where all necessary metabolic activities occurred for germination but radicle emergence is prohibited. This technique is used for improvement of germination speed, germination vigor, seedling establishment and

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yield. Improvement in priming is affected by some factors such as plant species, water potential form of priming factor, priming duration, temperature, vigor and seed primed storage condition. There are different treatments for seed priming and polyethylene glycol (PEG) is one of the most prevalent treatment. This treatment does not have any physiological responses to seed and in order to having more molecular weight cannot pass the cell wall and so it be used for water potential adjustment in germination experiments. Seed priming has been successfully employed for many crops and species where rapid, uniform and complete seedling emergence are essential. One practical drawback in many species is that primed seeds can often suffer from more rapid deterioration under normal storage conditions. Increased attention to drying protocols after priming, and storing primed seeds in conditions where \( T + \% \text{RH} \) is equal to or less than 80 will decrease storage concerns.

The objectives of this study were to explore the beneficial effects of osmopriming using PEG as priming reagent on salt tolerance of tomato genotypes. Therefore, several seed vigor traits such as germination percentage, seedling length (cm), seedling dry weight (mg) and vigor index were analyzed under salinity conditions, respectively. These results may provide valuable information for improving salt tolerance of tomato genotypes under stress conditions by PEG 6000 osmopriming treatment.

Materials and Methods

The present investigation was carried out in the laboratory of Indian Institute of Vegetable Research (IIVR) Varanasi, India, with five varieties of cultivated tomato (Solanum esculentum L.) released from IIVR, namely Kashi Vishesh, Kashi Amrit, Kashi Anupam, Kashi Hemant and Kashi Sarad. The seeds of five tomato genotypes were primed with varying concentration of PEG-6000 viz. (-0.5, -1.0, -1.5, -2.0 MPa) and hydropriming for 48 hours at 25°C and dry tomato seeds were considered as control (non-primed). After thorough washing out of chemical from the treated seeds by the double distilled water, were sown equidistantly (25 seeds Petri dishes \(^{-1}\)) in each Petri dishes containing two layers of Watman no. 1 filter paper. Treated seeds were supplied with three different concentrations of salt solution \( \text{i.e., 4, 8 and 12 dSm}^{-1} \) (Electrical conductivity) and distilled water for hydroprimed and dry seeds with three replications and observed for different morphological parameters such as seed germination test, seedling length, seedling dry weight and seedling vigor. Seeds were allowed to grow into seedling for two weeks in growth chamber at 20°C. Germination percentage was calculated by the following formula. Germination \( \% = \frac{\text{Number of seed germinated}}{\text{Total number of seeds in Petri dishes}} \times 100. \) Seedling length was measured in centimeter by scale from root tip to shoot tip for ten seedlings and was averaged. Dry weight of ten seedlings was weighed in gram and was averaged to obtain average seedling dry weight. Vigor index was calculated using per cent germination and seedling length according to the equation given by Orchard T. (1977). Seedling vigor index (SVI) was calculated using formula, \( \text{SVI} = \frac{[\text{Seedling length (cm)} \times \text{germination } \%]}{100}. \) All these experiments were conducted in factorial completely randomized block design (FCRD). Experimental data were analyzed using the analysis of variance as per standard statistical procedures as described by Panse and Sukhatme (1967). Critical difference values were calculated at 1% probability level.

Effects of priming on per cent germination: The data indicated significant differences in per cent germination among five tomato genotypes across osmopriming treatments and salinity levels (Fig. 1). The interaction effects between genotype, treatment and salinity levels were also found to be significant. Among genotypes, Kashi Hemant showed the highest germination percentage and Kashi Vishesh showed the least germination percentage across different treatments and salinity levels including control. Among the treatments osmopriming with PEG-6000 (-0.5 MPa) resulted in highest per cent germination followed by hydro-primed seeds for all genotypes at all salinity levels. The above treatments were significantly superior in respect to per cent germination as compared to dry seed sowing or absolute control. The least per cent germination was observed in PEG-6000 (-2.0 MPa). As the salinity level increased there was decrease in per cent germination observed for all the genotypes and treatments of osmopriming. Kashi Hemant primed with PEG 6000 (-0.5MPa) showed highest per cent germination of 71 per cent under salinity levels of 4 dSm\(^{-1}\) as compared to 8 and 12 dSm\(^{-1}\) i.e., 40 and 30 per cent respectively.

Results and Discussion

Effects of priming on seedling length: There was significant decrease in seedling length observed as there was increase in salinity levels for all osmopriming treatments and genotypes (Fig. 2). Seed primed with -0.5 MPa was found to be the best treatment and reported significantly highest seedling length compared to other treatments at all salinity levels and in all genotypes. The significantly highest seedling length was reported for Kashi Hemant (18 cm) followed by Kashi Amrit (16 cm), while Kashi Vishesh (5 cm), was measured lesser seedling length. The interaction of genotype, treatment and salinity levels for seedling length was found to be non-significant.

Effects of priming on dry weight of seedling: There was significant decrease in dry weight of seedlings with increasing salinity level in all tomato genotypes at all osmopriming treatments (Fig. 3). Among the treatments, -0.5 MPa primed seeds showed significantly higher dry weight of seedlings compared to other treatments except in Kashi Amrit, Kashi Vishesh and Kashi Sarad where priming with distilled water was found to be superior or on par with -0.5 MPa. Among the genotypes, Kashi Hemant primed with -0.5 MPa showed maximum dry weight at 4dSm\(^{-1}\) (14 g).

Effects of priming on vigor Index: Mean seedling vigor index for all treatments and salinity levels was found to be highest for Kashi Hemant (Fig. 4). Among genotypes, Kashi Hemant primed with -0.5 MPa showed higher vigor index i.e. 994, 400 and 237 under salt condition at 4, 8 and 12 electrical conductivity (EC) respectively. While other genotypes showed significantly lower vigor index values. For all the genotypes, priming with -0.5 MPa followed...
Fig. 1: Shows the per cent germination (%) of five tomato genotypes under different osmopriming treatments (PEG-6000) at different salinity levels grown in petridishes.

Fig. 2: Represents the seedling length (cm) of five tomato genotypes under different osmopriming treatments (PEG-6000) at different salinity levels grown in petridishes.

Fig. 3: Shows the dry weight (mg) of five tomato genotypes under different osmopriming treatments (PEG-6000) at different salinity levels grown in petridishes.

Fig. 4: Shows the vigor index of five tomato genotypes under different osmopriming treatments (PEG-6000) at different salinity levels grown in petridishes.

(in all fig: Display error bar with 1% value. T<sub>0</sub>=Dry control, T<sub>1</sub>=Hydro primed, T<sub>2</sub>= -0.5MPa, T<sub>3</sub>= -1.0 MPa, T<sub>4</sub>= -1.5 MPa, T<sub>5</sub>= -2.0 MPa)
by distilled water primed performed better than other treatments under salt condition. Vigor index decreased with increased salinity for all genotypes and all seed priming treatments. Kashi Vishesh showed the poorest vigor index i.e. 98, 32 and 22 at 4, 8 and 12 dSm\(^{-1}\) (EC) levels of salinity respectively and was most susceptible among five genotypes.

Soil salinity is a major environmental constraint to crop productivity worldwide. One alternative approach to alleviate adverse effects of salt stress on plants involves treatment of seed with different inorganic or organic chemicals. Strogonov (1964) for the first time proposed that salt tolerance of plants could be enhanced by treatment of seed with salt solution prior to sowing. Such controlled imbibition of seed followed by dehydration was referred to seed priming. Primed seed would then germinate more rapidly than unprimed seed when placed in an appropriate germination environment. Rapid seed germination and uniform field emergence are essential to the establishment of successful crops under both saline and non-saline conditions. Haigh (1988) reported priming in tomato (*Lycopersicon esculentum*), resulted in more rapid imbibition, increased the extensibility of radicle cell walls, and weakened the endosperm, which together shortened the lag phase between imbibition and radicle emergence.

In the present study, five tomato genotypes viz. Kashi Vishesh, Kashi Amrit, Kashi Anupam, Kashi Hemant and Kashi Sharad were used for study the effect of seed osmopriming with PEG-6000, showed that there was increase in per cent germination, seedling length, dry weight and vigor index in primed seeds as compared to non-primed seeds and distilled water primed seeds (hydroprimed). The best performances for above attributes were observed in -0.5 MPa primed seeds. Osmopriming with PEG-6000 at -0.5 MPa also imparted salt tolerance in tomato genotypes which performed better under different salinity levels as compared to non-primed seeds. Kashi Hemant primed with -0.5 MPa showed better performance for per cent germination under salt condition compared to other genotypes at same salinity level and hence reveals immense potential to tolerate under salinity condition. The poor per cent germination, seedling length, seedling dry weight and vigor index in non-primed seeds might be due to decrease in water uptake by the seeds due to low water potential of the germination medium, improper activation of enzyme activities (Ashraf et al., 2002; Guerrier, 1988), impediment of inorganic nutrients mobility to developing tissues (Ashraf and Wahid, 2000), nitrogen metabolism interruption (Dell’ Aquila and Spada, 1993), plant growth regulators level become imbalance (Khan and Rizvi, 1994), decrease in hydrolysis and utilization of food reserves (Ahmad and Bano, 1992) etc.

The salinity tolerance in primed seeds may be due to higher potential of these plants for osmosis regulation. It has been proposed that priming causes considerable invigoration of the dry seeds (Heydecker and Coolbear, 1978), which results from the initiation of metabolic processes that normally take place during imbibitions and are fixed by subsequent drying (Hanson, 1973). There was decreasing per cent germination, seedling length at decreasing osmotic potential of PEG-6000 i.e. -1.0MPa, -1.5MPa and -2.0MPa. It may be due to decreased availability of water and hence non proper initiation of germination events.

In our findings, even hydropriming significantly improved germination, seedling growth and seedling vigor under both stress and non stress conditions. Similar findings indicating hydropriming as suitable seed invigoration treatment under drought and saline conditions have been reported by Jannmohammadi et al., 2008. Several workers have collaborated our research findings (Ghiyasi et al., 2008, Soulangue and Levantard, 2008; Thirusenduraselvi and Jerlin, 2009, Umair et al., 2010). Khalil et al., (1997) reported that plant raised from seed preconditioned in PEG-8000 exhibited rapid germination and higher shoot length and dry weight compared to plants raised from untreated seeds which supports our findings. In tomato, a space is developed in the primed seed that facilitates water uptake, there by accelerating the speed of germination (Argerich and Bradford, 1989). In tomato, priming stimulates the synthesis of nuclear DNA in radical tip cells (Liu et al., 1997). Priming induces the expression of aquaporins, resulting in increased germination under stress conditions (Gao et al., 1999). Osmopriming may contribute the rapid seed germination by affecting active oxygen metabolism and a rapid increase in the respiratory intensity, which were associated with an increased in germination vigor (Jie et al., 2002). Seed osmoconditioned with PEG 6000 showed increased activity of amylase and dehydrogenase and improved germination under non-saline conditions (Singh et al., 1999). The rate of mobilization of stored reserves was also altered by osmopriming which is crucial for growth and development of the embryo (Capron et al., 2000).

It is evident from our experiment that considerable gain in terms of seed germination, seedling length, seedling dry weight and vigor index has been achieved by osmopriming with PEG 6000 at -0.5MPa, similar findings have been reported by (Hur, 1991) who worked on Italian ryegrass and sorghum, where seeds primed with 20 per cent PEG 8000 for 2 days at 10\(^{\circ}\)C exhibited increased in per cent germination, germination rate, seedling establishment and dry matter production under various stress conditions such as drought, waterlogged, cold and salt stresses. Kashi Hemant primed at -0.5MPa demonstrated better performance under varying salt concentrations of 4, 8 and 12 dSm\(^{-1}\) (EC). Thus, osmopriming of seeds may lead to its enhanced performance under saline conditions. Among five genotypes, Kashi Hemant primed with PEG 6000, -0.5MPa showed best performance as the per cent germination, seed vigor index, dry weight, were recorded maximum compared to other genotypes and showed better performance under salt condition of 4, 8 and 12 EC, while remaining varieties showed poor performance compared to Kashi Hemant. Thus, the results revealed that Kashi Hemant primed with -0.5MPa have immense potential for cultivation on the saline soil. Therefore, the osmopriming by PEG has positive response under stress conditions. Further studies of this genotype on biochemical and molecular aspects may elucidate its tolerance potential under salt stress at gene level and also impart
ideas for characterization and isolation of gene for its better performance.

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References