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# SODIUM NITROPRUSSIDE (SNP) SEED PRIMING IMPROVES SEED GERMINATION AND SEEDLING GROWTH IN WHEAT (*TRITICUM AESTIVUM L.*) UNDER OSMOTIC STRESS INDUCED BY POLYETHYLENE GLYCOL

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## ABSTRACT

Effects of seed priming with sodium nitroprusside (SNP) on seed germination and seedling growth under osmotic stress induced by polyethylene glycol (PEG) were investigated. Seed priming treatments were hydropriming, soaking seeds in aerated distilled water for 8 h and SNP priming, soaking seeds in an aerated solution of 75, 100 and 125 µM SNP for 8 h. Seeds were germinated in petri plates under osmotic stress induced by 15 % PEG- 6000. Final germination percentage (FGP) significantly increased by SNP priming treatments. SNP priming also reduced the mean germination time (h) (MGT) of the seeds. The germination energy, vigor index and germination index significantly increased by SNP priming. All SNP priming treatments effectively improved the germination rate, shoot length and root length. Hydropriming of seeds reduced the MGT and improved the germination rate as compared with non-primed seeds. However, hydropriming was unable to improve the FGP, germination energy, germination index, vigor index, root and shoot lengths. This means observed enhancements in the seed germination and seeding growth were due to SNP priming not because of only seed soaking. We concluded that SNP seed priming can accelerate the seed germination and seedling growth under osmotic stress resulting in improved tolerance.

**Keywords:** osmotic stress, germination test, shoot length, root length, water-deficit stress

## INTRODUCTION

Seed priming is a form of seed preparation in which seeds are pre-soaked before planting (Ahmad *et al*., 2012). Seed priming is a simple, low cost and effective approach for enhancement of seed germination, early seedling growth and yield under stressed and non-stressed conditions. Seed priming with different chemicals, ions, organic compounds, hormones and antioxidants has been reported to enhance the salt tolerance in wheat (Hameed *et al*., 2010). Further, germination and seedling vigor has been reported to be enhanced by priming with polyamines in tomato cultivars (Afzal *et al*., 2009).

Recently, sodium nitroprusside (SNP) has been reported to act as germination promoter in wheat. SNP enhanced the germination responsive genes in wheat to enhance germination. Actually the S-nitrosylation process acts as a regulatory switch for seed germination in wheat (Sen, 2010). SNP has also been reported to enhance the seed vigor index in wheat under salt stress by enhancing germination promoting proteins (Duan *et al*., 2007). Moreover, rice seeds primed with SNP germinated well and showed high performance under salt stressed condition (Noman *et al*., 2010). However, improvement of osmotic stress tolerance by this new priming agent has not been investigated.

Osmotic stress resulted in rapid decline in growth of most of the plants (Flowers, 2004). Osmotic stress resulted in reduction of leaf chlorosis, antioxidants, plant growth and development and hormonal imbalance (Iqbal and Ashraf, 2010; Ashraf *et al*., 2010). But reduction in growth depends upon the duration and level of stress and plant tissue types (Meloni *et al*., 2003). Similarly, many scientists has been reported the reduced growth of leaves and stems, leaf area, number of tillers, development of new leaves, lateral buds, branches formation and continued root growth under osmotic stress (Munns and Tester, 2008; Taiz and Zeiger, 2006). Poly ethylene glycol (PEG) is most commonly used to create osmotic stress in plants because it is not naturally produced in the plant tissue nether penetrate into cell from the media. PEG eventually destroys the normal emergence, growth, biochemical attributes and yield of wheat (Pei *et al.*, 2010).

In this view, in present work we tested the use of sodium nitroprusside as seed priming agent for improvement of osmotic stress tolerance in wheat. The effect of seed priming with different concentrations of SNP on seed germination attributes and early seedling growth under osmotic stress induced by PEG was tested.

## MATERIALS AND METHODS:

### Experimental details and seed priming

The spring wheat (*Triticum aestivum* L. cv. AARI-2011) seeds were obtained from wheat section, Ayub Agriculture Research Institute (AARI), Faisalabad, Pakistan. Seed priming treatments used in the study were

hydropriming, soaking seed in aerated distilled water for 8 h and sodium nitroprusside (SNP) priming, soaking seeds in an aerated solution of 75, 100 and 125 µM SNP for 8 h. After priming treatments, seeds were given three washings with water and re-dried near to original weight with forced air under shade at 26 ±2 $^{o}$C.

**Germination test:**

Germination potential of the primed and non-primed wheat seeds was estimated in accordance with the International Rules for Seed Testing by Association of Official Seeds Analyst (AOSA) (Anonymous. 1990). To test seed germination and seedling vigor under osmotic stress, four replicates of 25 seeds were germinated in 12 cm diameter petri dishes at 25°C. To test the effect of priming treatments under osmotic stress condition, five ml of 15 % polyethylene glycol (PEG-6000) solution (-3.0 Mpa) was applied in each petri dish to impose stress. Similarly, four replicates of 25 seeds were germinated in 12 cm diameter petri dishes at 25°C in these studies. A seed was scored as germinated when coleoptile and radicle lengths reached 2-3 mm. Counts of germinating seeds were made twice a day at different time intervals (20, 28, 44, 52, 68, 76, 92 and 200 h), starting on the first day of imbibition, and terminated when maximum germination was achieved.

**Mean germination time:**

Mean germination time (MGT) was calculated according to the formula of Ellis and Roberts (1981).

$MGT=\sum D_n / \sum n$

Where n is the number of seeds, which were germinated on day D and D is the number of days counted from the beginning of germination.

**Final germination percentage:**

Final germination percentage was measured according to following formula.

$$FGP= \frac{\text{No of seeds germinated on final day}}{\text{Total no of seeds sown}} \times 100$$

**Germination index:**

Germination index (GI) was calculated as described in the Association of official Seed Analysts (AOSA) (Anonymous. 1983) as the following formulae.

$$GI= \frac{\text{number of germinated seeds}}{\text{Days of first count}} + \text{----------} + \frac{\text{number of germinated seeds}}{\text{days of final count}}$$

**Energy of germination:**

Energy of germination was recorded 4$^{th}$ day after planting. It is the percentage of germinated seeds 4 days after planting relative to the total number of seeds tested (Ruan *et al.*, 2002).

**Growth response:**

For growth response, seedlings were allowed to continue growth after collecting the data for germination Ten day old seedlings were then harvested for comparison of growth under osmotic stress after seed priming treatments. Root and shoot length was measured by spreading them on a scale calibrated in cm.

**Statistical analysis of data**

Significance of data was tested by analysis of variance and Tukey (HSD) Test at $p<0.05$ and where applicable at $p<0.01$ using XL-STAT software. Values presented in graphs are mean ± SD.

**RESULTS**

Effects of seed priming with different SNP concentrations and hydropriming were tested by comparing with non-primed seeds under osmotic stress induced by PEG. Final germination percentage was significantly increased after SNP priming treatments (Fig. 1). Highest germination percentage (95 %) was observed after priming with 100 µM SNP. However, SNP induced increase in the final germination percentage was statistically same for all three tested concentrations. Hydropriming (soaking of seeds in distilled water) was not able to improve the final

germination percentage. This clearly indicates that observed enhancement in the final germination percentage was not because of seed soaking treatment but due to the influence of SNP. For non-primed seeds, mean germination time (MGT) was significantly longer as compared with the primed seeds. All the priming treatments significantly reduced the germination time. Seed priming with varying levels of SNP more promptly reduced the MGT of the seeds. All the concentrations of SNP reduced the MGT to same extent with non-significant diffidence. Hydropriming of seeds also reduced the MGT as compared with non-primed control seeds. However, hydropriming induced reduction in the MGT was less as compared to that induced by SNP priming treatments. These observations provide evidence that tested seed priming treatments can accelerate the seed germination under osmotic stress condition that shows an improvement in the stress tolerance.

The germination energy (%) was also calculated for primed and non-primed seeds under osmotic stress induced by PEG. Interestingly, the germination energy (%) significantly increased after seed priming with SNP (Fig. 2). Most prominent increase in germination energy was induced by 100 µM and 125 µM SNP priming treatments. In contrast, hydropriming of seeds was unable to improve the germination energy under osmotic stress as level remained same as in non-primed control seeds. Similarly, all SNP priming treatments significantly improved the germination index under osmotic stress conditions as compared to non-primed seeds. Seed priming with 100 µM SNP induced highest increase in germination index as compared to non-primed control seeds. Hydropriming treatment was unable to improve the germination index under osmotic stress condition. Vigor index was also significantly improved by SNP priming treatments as compared to non-primed control seeds under osmotic stress. All SNP treatments were equally effective in improving the vigor index as level was statistically same for all. Hydropriming was not able to improve the vigor index under osmotic stress as compared to non-primed control seeds.

The effect of seed priming treatments on germination rate of seeds under osmotic stress was also monitored. It was observed that 100 and 125 µM SNP treatments were equally effective in improving the seed germination rate under osmotic stress (Fig. 3). Seed priming with 75 µM SNP also improved the germination rate, however, degree of improvement was comparatively low on three time intervals as induced by higher SNP concentrations. Hydropriming also improved the germination rate as compared to non-primed control seeds. However, hydropriming induced improvement in germination rate was less as compared to seed priming with SNP. In case of hydroprimed seeds, germination percentage was low as compared to SNP primed seeds on almost all time intervals.

Effect of seed priming treatments on seedling growth under osmotic stress induced by PEG was also measured in terms of root and shoot growth. All concentrations of SNP equally and significantly increased the shoot length under osmotic stress (Fig. 4). However, hydropriming of seeds could not improve the shoot length of seedlings under osmotic stress. Similarly, it was also true for root length under osmotic stress which was increased by all SNP priming treatments. Seed priming with 75 µM SNP induced highest increase in the root length of the seedlings. However, all SNP concentrations induced statistically same increase in the root length. In contrast, hydropriming of seeds could not improve the root length of seedlings under osmotic stress.

## DISCUSSION

Rapid and uniform seed germination followed by good seedling emergence is key factors in better and synchronized crop establishment. Seeds are particularly vulnerable to stresses encountered between sowing and seedling establishment (Carter and Chesson, 1996). Pre-sowing seed treatments have been shown to enhance emergence percentage and stand establishment under non-stressed conditions (Khan, 1992; Afzal *et al*., 2005) and have potential in stress full environments (Hameed *et al*., 2010). Similarly in present study, seed priming with SNP enhanced the all germination attributes under osmotic stress. Sodium nitroprusside (SNP) has been reported as a NO donor (Bethke *et al*., 2004). Nitric oxide (NO) has been proved as an important signaling molecule that regulates a series of physiological processes in both animals and plants (Besson-Bard *et al*., 2008; Crawford and Guo, 2005). Moreover, it has been reported to promote seed germination of various crops under abiotic stresses (Noman *et al*., 2010, Bethke *et al*., 2007) and can enhance germination rate in wheat seedlings under salt stress (Zheng *et al*., 2009). SNP has also been reported as germination promoter in wheat that activated the germination responsive genes and resulted in enhanced seed vigor index under abiotic stresses (Sen, 2010; Duan *et al*., 2007). Present observations are in accordance with previous reports as the germination rate was also improved by SNP seed priming under osmotic stress. There is possibility that similar germination responsive genes may be activated as a result of SNP seed priming under osmotic stress.

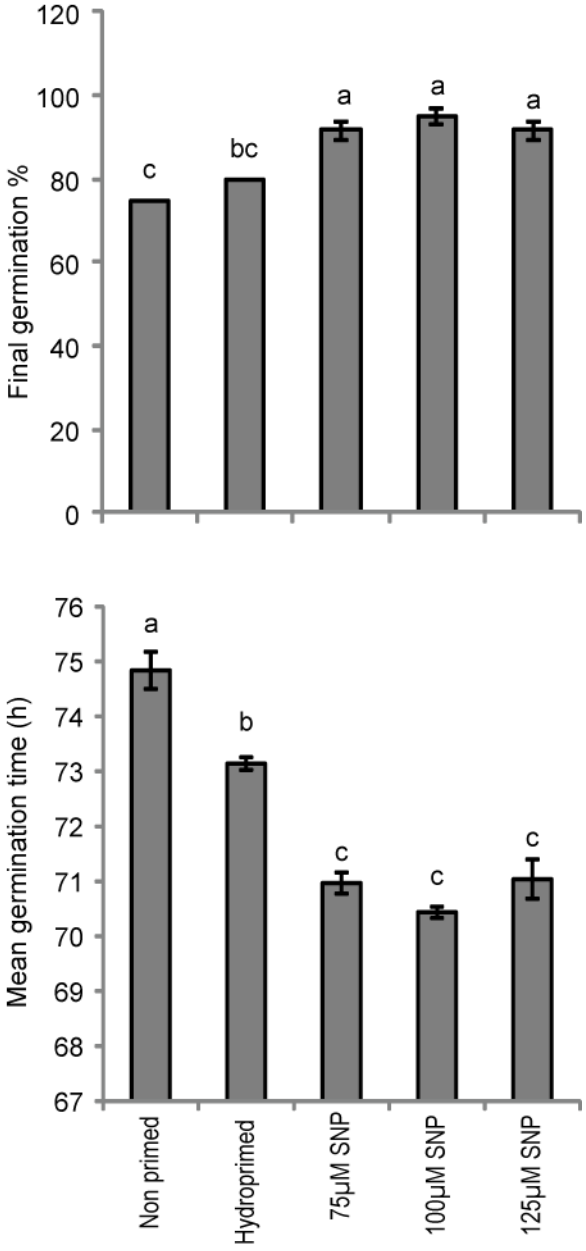


Fig. 1. Effect of sodium nitroprusside seed priming on final germination % and mean germination time under water-deficit stress induced by PEG.

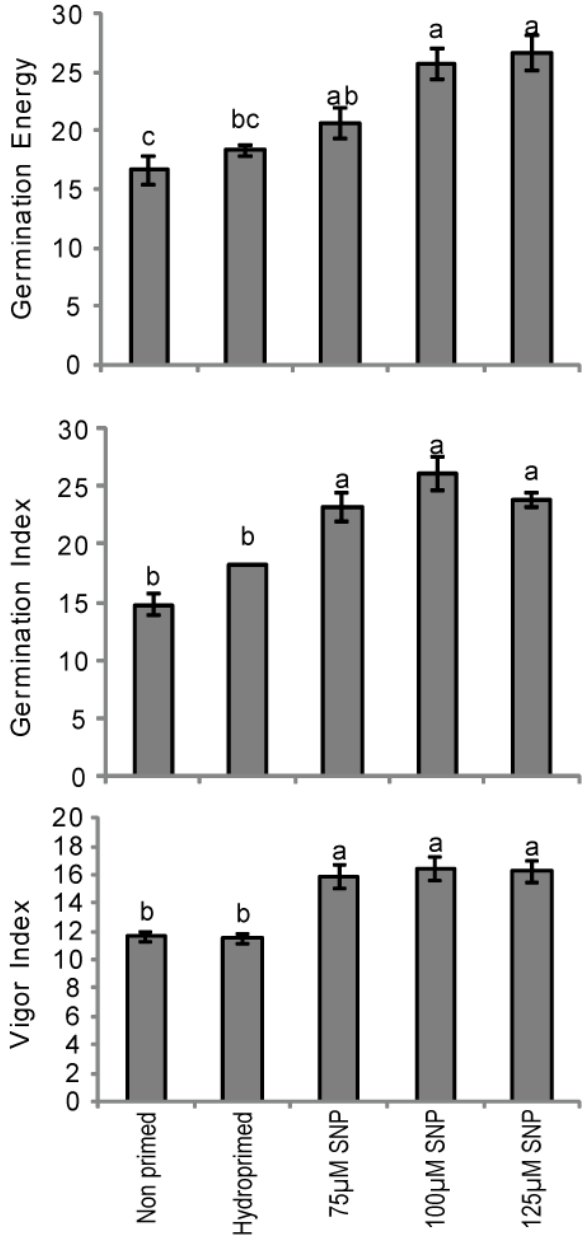


Fig. 2. Effect of sodium nitroprusside seed priming on germination energy, germination and vigor index under water-deficit stress induced by PEG.

Seed priming with SNP improved the final germination percentage, shoot and root growth under osmotic stress in present study. Similarly, improvement in germination percent, emergence and seedling stand by seed priming techniques has been reported (Basra *et al*., 2003). Actually, seed priming induces a set of biochemical changes in the seed that are required for initiating the germination process. These process or changes include activation of enzymes, dormancy breaking, hydrolysis or metabolism of germination inhibitors and imbibitions (Ajouri *et al*., 2004). Some or all these process that precede the germination are triggered by seed priming and also persist following the re-drying the seeds (Asgedom and Becker, 2001). Thus upon sowing, primed seed can rapidly imbibe and revive the seed metabolism, resulting in higher germination percentage (Rowse, 1995). Similar mechanisms seem to operate in the SNP primed seeds in present study that resulted in the higher germination percentage and

rapid seedling growth under osmotic stress. Seed priming most likely allowed some repairs of damaged to membrane caused by deterioration (Ruan *et al*., 2002).

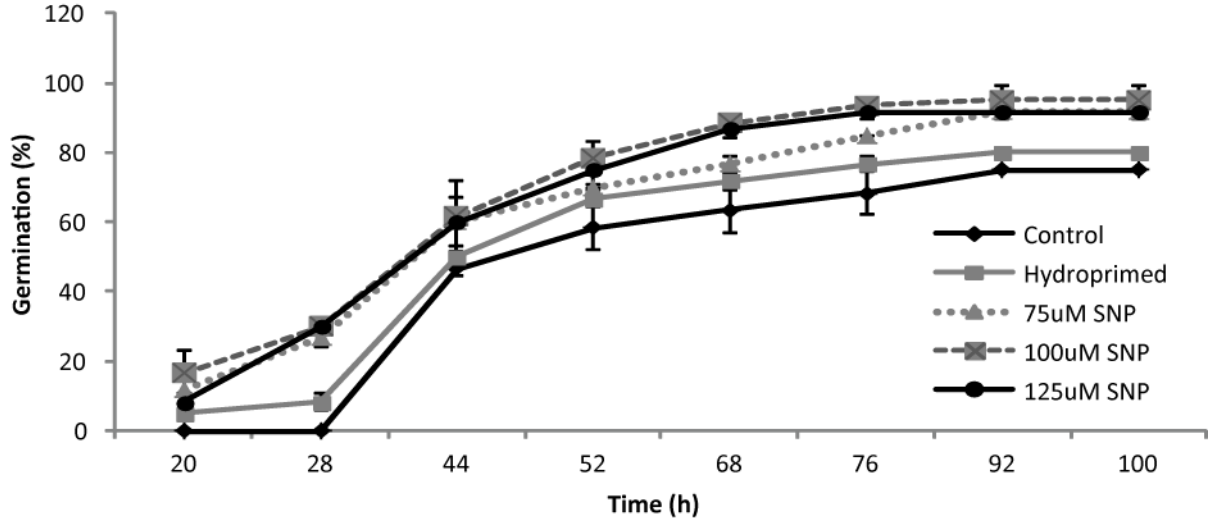


Fig. 3. Effect of sodium nitroprusside seed priming on wheat seed germination rate under water-deficit stress induced by PEG.

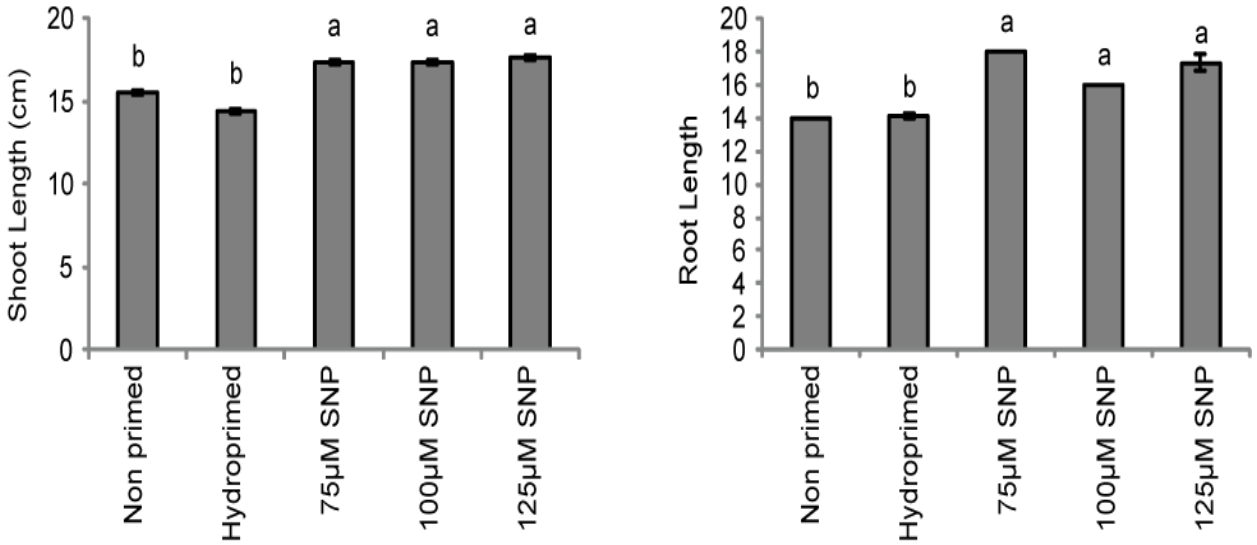


Fig. 4. Effect of sodium nitroprusside seed priming on shoot length and root length of wheat seedlings under water-deficit stress induced by PEG.

It has been reported that primed seeds showed better germination pattern and higher vigor level than non-primed seeds (Ruan *et al*., 2002). Similarly, better germination and seed vigor by SNP seed priming were also observed in present study. Nascimento and West (1998) indicated minimizing of seed coat adherence during emergence of muskmelon seeds. The improvement in germination and vigor was probably due to reserve mobilization of food material, activation and re-synthesis of some enzymes DNA and RNA synthesis started during osmotic priming (Sadeghi *et al*., 2011). The mean germination time was reduced under osmotic stress in present study by SNP seed priming. These positive effects are probably due to the stimulatory effects of priming on the early stages of germination process by mediation of cell division in germinating seeds (Hassanpouraghdam *et al*., 2009).

In conclusion, tested seed priming treatments not only improved the seed germination but also enhanced the wheat seedling growth under osmotic stress induced by PEG. Observed beneficial effects on germination and seedling vigor seemed to be due to induction of enzyme activities, rapid seed reserve mobilization and cell division.

However, these aspects related to SNP seed priming induced changes in seed biochemical process needs to be investigated.

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