

## WHEAT SEED ENHANCEMENT BY VITAMIN AND HORMONAL PRIMING

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### Abstract

Seed priming has proven beneficial in many important agricultural crops. The present study was conducted to explore the role (if any) of hormonal and vitamin seed priming to improve the germination, seedling emergence, early seedling establishment, electrolyte leakage and nutrients uptake in wheat seedlings. The wheat seeds were soaked for 48 hours in aerated solution of salicylic acid, ascorbic acid, kinetin and GA<sub>3</sub> with 20 ppm concentration of each solution, whereas untreated seeds were taken as control. Seed priming with ascorbic acid resulted in maximum final germination and emergence percentage (FGP and FEP), radicle and plumule length, root and shoot length, number of secondary roots, root shoot ratio, root dry weight, shoot dry weight and seedling dry weight compared to control (untreated seeds). Minimum mean germination and emergence time (MGT and MET) was recorded in seeds primed with kinetin and GA<sub>3</sub>. While in case of biochemical attributes, seedling potassium contents were decreased by hormonal seed priming while total soluble sugars were increased by salicylic acid and ascorbic acid seed priming. Hormonal seed priming had non-significant effect on phosphorus seedling contents. Untreated (Control) seeds showed maximum electrical conductivity at 0.5, 1, 2, 6, 12 and 24 h after imbibition than primed seeds against the minimum electrical conductivity that was recorded in seeds primed with salicylic acid and ascorbic acid. In conclusion, the wheat seeds primed with 20 ppm solution of ascorbic acid may be used for wheat seed invigoration.

### Introduction

Cotton-wheat and rice-wheat are the two most important cropping patterns followed in irrigated plains of Pakistan extending from the Peshawar Valley to the Indus Valley of the Punjab and Sindh provinces. More than 70% area is occupied by wheat crop in Rabi season in both the cropping patterns. Late maturity of basmati varieties of rice and delayed picking of cotton results in delayed sowing of wheat up to late December (Nayyar & Iqbal, 2001). From last week of October to mid of November is the optimum time for wheat sowing to attain its full potential. Any delay in sowing after mid November results in yield reduction by 50 kg ha<sup>-1</sup> per day (Khan, 2004). This yield reduction is primarily due to low plant population that resulted due to poor and erratic germination because of low temperature prevailing at that time. Due to photosensitive nature of crop, late planted wheat has to accomplish its all growth and developmental stages in lesser time as starch accumulation is terminated at same specific time and not depending on time of sowing (Khan *et al.*, 2010). Fast and homogeneous mixture provides both economical and environmental benefits in agriculture because it allows higher degree of automation, easier weed control, reduction of disease pressure in the field and aids to upcoming precision farm technologies.

Improved seed invigoration techniques are being used to reduce the germination time, to get synchronized germination, improve germination rate and better seedling stand in many horticultural crops (Rudrapal & Nakamura, 1998; Basra *et al.*, 2004; Ashraf *et al.*, 2008) and rice (Lee & Kim, 2000; Basra *et al.*, 2004). These invigoration techniques include hydropriming, osmoconditioning, osmohardening, hardening, hormonal and vitamin priming.

Seed priming is an important method associated with the process of seed germination and is widely used to synchronize the germination of individual seeds with improved germination or seedling growth (Heydecker *et al.*, 1973; Taylor & Harman, 1990). Seed priming enhances seed performance by rapid and uniform germination, normal and vigorous seedlings in different crops which have practical agronomic implications, notably under adverse germination conditions (McDonald, 2000; Cantliffe, 2003). It permits seedling development in a wide range of agro-climatic conditions and decreases sensitivity to external factors (Ashraf & Foolad, 2005; Welbaum *et al.*, 1998). Seeds performance of various crops can be improved by inclusion of plant growth regulators and hormones during priming and other pre-sowing

treatments (Lee *et al.*, 1998). Hormone like salicylic acid has also proved for alleviating salinity stress in wheat (Afzal *et al.*, 2005). During priming, the germination-related processes are initiated but the emergence of the radicle is prevented since hydration is followed by drying of seeds. The beneficial effects of seed priming with different priming agents have already been successfully expressed in many crop plants, for instance mustard (Srinivasan *et al.*, 1999), chickpea (Kaur *et al.*, 2002), maize (Nawaz & Ashraf, 2010), sunflower (Kaya *et al.*, 2006), wheat (Iqbal & Ashraf, 2007; Perveen *et al.*, 2010), cotton (Casenave & Toselli, 2007), rice (Habib *et al.*, 2010) and sugarcane (Patade *et al.*, 2009).

Therefore the present study was conducted to explore the role of vitamin and hormonal seed priming in improving emergence, early stand establishment and ion uptake in wheat seedlings.

### Materials and Methods

The present study was conducted at Agronomy Laboratory, University College of Agriculture, Bahauddin Zakariya University, Multan, and partially at Stress Physiology Laboratory, Department of Botany, University of Agriculture, Faisalabad. The experiments were laid out in a completely randomized design (CRD) with four replications. Wheat seed (*Triticum aestivum* L. cv. Ufaq) was obtained from Punjab Seed Corporation, Khanewal, Pakistan having 11.5% moisture contents and 98% germination percentage. A weighed quantity (250 g) of wheat seeds were soaked in aerated solution [having 1: 5 (w/v) seed to solution ratio] of salicylic acid (20 ppm), ascorbic acid (20 ppm), kinetin (20 ppm) and GA<sub>3</sub> (20 ppm) for 48 h, while untreated seeds were taken as control.

**Seed germination and seedling vigor evaluation:** Control (Untreated) and primed seeds were sown in Petri dishes between the layers of moist filter paper at 27°C in the incubator. Germination was observed daily according to the Association of Official Seeds Analyst (AOSA) method (AOSA, 1990). Mean emergence time (MET) was calculated according to the method described by Ellis and Roberts, (1981) as under:

$$MET = \frac{\sum Dn}{\sum n}$$

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

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Germination Index (GI) was calculated as described in the Association of Official Seeds Analyst (1983) as the following formulae:

$$GI = \frac{\text{No. of germinated seedlings}}{\text{Days of first count}} + \frac{\text{No. of germinated seedlings}}{\text{Days of final count}}$$

Final germination percentage (FGP) of seeds was obtained when experiment was terminated. It is the percentage of number of seeds germinated to total seeds planted. On 15<sup>th</sup> day after sowing, seedlings were evaluated for vigor. Radicle and plumule lengths of 10 randomly selected seedlings per replicate were measured and averaged to record radicle and plumule length. Root shoot ratio was obtained by dividing radicle length with plumule length. Fresh weight of 10 randomly selected seedlings was taken immediately after harvesting, dried at 70°C till constant weight and dry weight per seedling was recorded.

**Seed emergence and seedling vigor evaluation:** Control (Untreated) and primed seeds were sown in plastics pots having moist acid/water washed sand and placed in a growth chamber. Experiment was replicated four times with 25 seeds in each pot. Day and night length was kept 9 and 15 h with 25 and 22°C temperatures, respectively and 55% relative humidity. Numbers of emerged seeds were counted daily according to the seedling evaluation Handbook of Association of Official Seeds Analyst method (Anon., 1990) until constant count was obtained.

Mean emergence time (MET) was calculated according to the method described by Ellis & Roberts, (1981) as under:

$$MET = \frac{\sum Dn}{\sum n}$$

where n is the number of emerged seeds on day D, and D is the number of days counted from the beginning of emergence.

Emergence index (EI) was calculated according to method described by Association of Official Seeds Analyst (1983) by using formulae:

$$EI = \frac{\text{No. of emerged seedlings}}{\text{Days of first count}} + \frac{\text{No. of emerged seedlings}}{\text{Days of final count}}$$

Final emergence percentage (FEP) was recorded at the end of the experiment. It is the percentage of number of seedlings emerged to total seeds planted. On the 17<sup>th</sup> day after emergence, root and shoot lengths were measured of ten randomly selected seedlings from each replication and averaged to record root and shoot length. Root shoot ratio was obtained by dividing root length with shoot length. Root and shoot fresh weight was recorded immediately after harvesting by separating the roots from shoot for each replication, dried in an oven at 70°C until constant weight. Dry weight was recorded by using electrical balance and to get mean root and shoot dry weight per replication. Number of secondary roots of 10 seedlings from each replication was noted and averaged to record number of secondary roots per seedling.

After washing in distilled water, 5 g seeds were soaked in 50 ml distilled water at 25°C. Electrical conductivity of steep water was measured at 0.5, 1.0, 1.5, 2.0, 6.0, 12.0 and 24 h after soaking using conductivity meter (Model Twin Cond. B-173) and was expressed as  $\mu\text{S cm}^{-1} \text{ g}^{-1}$  (Ashraf *et al.*, 1999).

Seedling phosphorus content was quantified by spectrophotometer Hitachi-220. The extracted material (2 ml)

was dissolved in 2 ml of Barton reagent (Jackson, 1962) and total volume was made as 50 ml. The samples were kept for half an hour and values of phosphorus were calculated using standard curve. Seedling potassium was determined by feeding the digested samples into flame photometer, Jenway, PFP-7, (Allen *et al.*, 1986). Quantity of element was estimated in ppm by comparing the emission of flame photometer with that of standard curve, which was then converted into percentage by using the following formula:

$$\text{Total element \%} = \frac{C^* (\text{mg})}{10 \times \text{aliquot (ml)}} \times \frac{\text{Soln. Vol. (ml)}}{\text{Sample wt. (g)}}$$

$C^*$  = mg of each element obtained from calibration curve.

To record total soluble sugars, ground seedling sample (1 g) was mixed with 10 mL distilled water, kept at 25°C for 24 h and filtered through Whatman No. 42 filter paper (Lee & Kim, 2000). Sample solutions (100  $\mu\text{L}$ ) were taken in marked test tubes and added 900  $\mu\text{L}$  of distilled water and 1 mL of DNS reagent in a test tube. Reagent blank was also prepared by adding 1 mL of distilled water and 1 mL of DNS reagent in a test tube. Then test tubes were heated in boiling water for 10 min., allowed to cool and absorbance was read at 540 nm against a reagent blank. The absorbance values were converted to equivalent reducing sugars using a standard graph obtained by plotting standard glucose concentrations against absorbance values.

The data collected was analyzed by using the Fisher's analysis of variance technique under Completely Randomized Design (CRD) and the treatments means were compared by Least Significant Difference (LSD) test at 0.01 probability levels (Steel *et al.*, 1997).

## Results

Wheat seeds primed with kinetin and GA<sub>3</sub> showed statistically minimum mean germination time (MGT) against the maximum minimum mean germination time that was recorded in control and seeds primed with ascorbic acid (Table 1). Maximum germination index (GI) was recorded in ascorbic acid primed seeds but it was at par with control and kinetin primed seeds against the minimum GI that was recorded in GA<sub>3</sub> but it was also at par with all other treatments except seeds primed with ascorbic acid (Table 1). Maximum final germination percentage (FGP) was observed in control and ascorbic acid primed seeds (Table 1). Maximum radicle length was attained by salicylic acid treated seeds but it was at par with ascorbic acid and untreated (Control) seeds whereas maximum plumule length was recorded in ascorbic acid primed seeds but it was at par with salicylic acid and GA<sub>3</sub> priming technique. Minimum plumule length was recorded in control and kinetin primed seeds (Table 1). Salicylic acid primed seeds resulted in maximum root shoot ratio against the minimum root shoot ratio that was recorded in kinetin and GA<sub>3</sub> primed seeds (Table 1). Maximum seedling dry weight was recorded in seeds primed with ascorbic acid and salicylic acid against the minimum seedling dry weight that was recorded in GA<sub>3</sub> primed seeds (Table 1).

Mean emergence time (MET) was least in seeds primed with salicylic acid, kinetin and GA<sub>3</sub> against the maximum MET that was recorded in control and ascorbic acid primed seeds (Table 2). Untreated seeds (Control) resulted in maximum emergence index (EI) but it was at par with all other treatments except seeds primed with kinetin that resulted in minimum EI but it was also at par with all other priming treatments except control (Table 2). Maximum final

emergence percentage (FEP), root and shoot length was recorded in seeds primed with ascorbic acid against the minimum FEP, root and shoot length that was recorded in kinetin primed seeds but it was at par with control (Table 2). Seeds primed with ascorbic acid showed maximum root shoot ratio and number of secondary roots against the minimum root shoot ratio and number of secondary roots that were recorded in control (Table 2). Maximum root and shoot dry weight was recorded in seedlings whose seeds were primed with ascorbic acid against the minimum root and shoot dry weight that was recorded in control (Table 2).

Seed priming treatments resulted in lower electrical conductivity of seed leechates compared with control. After 0.5, 1, 2, 6, 12 and 24 h soaking of seeds, untreated seeds (Control) depicted maximum electrical conductivity of seed leechates against the minimum electrical conductivity of seed leechates that was recorded in seeds primed with salicylic acid and ascorbic acid (Table 3).

There was a non-significant effect of hormonal seed priming treatments on seedling phosphorus contents (Table 4). Maximum potassium (K) contents were found in untreated seeds against all primed seeds that resulted in minimum K contents (Table 4). While maximum total soluble sugars were recorded in seeds primed with ascorbic acid, and minimum total soluble sugars were noted in control and seeds primed with kinetin and GA<sub>3</sub> (Table 4).

## Discussion

The results of the study revealed that hormonal and vitamin seed priming enhanced invigoration of wheat seeds. Lesser MGT and MET was recorded in seeds treated with GA<sub>3</sub> and kinetin. Maximum GI was observed in seeds treated with GA<sub>3</sub> and salicylic acid. Lesser MGT and MET indicated earlier and rapid germination. The earlier and synchronized germination and emergence might be due to enhanced metabolic activities in primed seeds (Soon *et al.*, 2000). Maximum FGP, FEP, radicle length, root shoot ratio and shoot length was maximum in seed primed with salicylic and ascorbic acid. Whereas plumule length, seedling fresh and dry weight, root length, root shoot ratio, number of secondary roots, root fresh weight, shoot fresh and dry weight was maximum in seeds treated with ascorbic acid. These results support the findings of the earlier work on improved germination rate and percentage by hormonal treatment in wheat by Al-Hakimi and Hamada (2001). The higher germination percentage in primed seeds might be the consequence of breakdown of dormancy as fresh seeds were used during the investigations. Recently, Khan *et al.*, (2010) also reported that pre-sowing chilling treatments in wheat resulted in earlier and synchronized emergence and better early seedlings establishment.

**Table 1. Effect of hormonal and vitamin priming on germination and seedling vigor of wheat.**

Treatments	MGT (days)	GI	FGP (%)	Radicle length (mm)	Plumule length (mm)	Root shoot ratio	Seedling dry weight (mg)
Control	4.93 a	16.32 ab	94.98 a	104 ab	131.50 c	0.73 b	217.5 b
Salicylic acid	4.79 a	12.68 b	71.65 b	128.1 a	157.55 ab	0.85 a	245 ab
Ascorbic acid	3.68 b	19.29 a	90.02 a	97.3 ab	163.68 a	0.66 b	267.5 a
Kinetin	3.02 c	17.38 ab	76.66 b	59.02 c	143.60 bc	0.40 c	217.5 b
GA <sub>3</sub>	2.92 c	12.87 b	76.65 b	74.37 bc	151.58 ab	0.43 c	175 c
<b>LSD at 0.01</b>	<b>0.62</b>	<b>5.45</b>	<b>9.142</b>	<b>37.53</b>	<b>15.88</b>	<b>0.11</b>	<b>36.59</b>

**Table 2. Effect of hormonal and vitamin priming treatments on emergence and seedling vigor of wheat.**

Treatments	MET (days)	EI	FEP (%)	Root length (cm)	Shoot length (cm)	Root shoot ratio	Number of secondary roots	Root dry weight (g)	Shoot dry weight (g)
Control	6.26ab	23.92a	86b	11.26bc	17.88b	0.63b	3.1c	0.11c	0.12c
Salicylic acid	5.20c	19.01ab	97a	11.41bc	19.99a	0.56b	4.3b	0.12bc	0.15bc
Ascorbic acid	7.01a	17.49ab	93ab	16.36a	20.78 b	0.79a	5.3a	0.13ab	0.19a
Kinetin	5.10c	13.46b	85b	10.29c	16.82a	0.61b	4.3b	0.12b	0.16ab
GA <sub>3</sub>	5.77bc	19.07ab	85b	12.39b	20.09a	0.62b	4.9a	0.14a	0.15bc
<b>LSD at 0.01</b>	<b>1.00</b>	<b>6.53</b>	<b>9.13</b>	<b>1.80</b>	<b>1.65</b>	<b>0.09</b>	<b>0.53</b>	<b>0.01</b>	<b>0.02</b>

**Table 3. Effect of vitamin and hormonal priming on electrical conductivity ( $\mu\text{S cm}^{-1} \text{g}^{-1}$ ) of seed leechates of wheat.**

Treatments	0.5 Hrs	1 Hrs	2 Hrs	6 Hrs	12 Hrs	24 Hrs
Control	90.35 a	116.25 a	138.50 a	227.50 a	270.50 a	348.25 a
Salicylic acid	15.70 d	20.05 c	23.92 c	41.70 c	55.70 d	113.25 c
Ascorbic acid	16.58 cd	22.68 c	25.60 bc	43.40 c	65.00 c	109.75 c
Kinetin	21.15 bc	25.15 bc	29.75 bc	57.92 b	83.05 b	190.50 b
GA <sub>3</sub>	22.33 b	29.11 b	32.12 b	64.56 b	89.93 b	205.75 b
<b>LSD at 0.01</b>	<b>5.09</b>	<b>5.99</b>	<b>8.18</b>	<b>6.80</b>	<b>8.26</b>	<b>59.87</b>

**Table 4. Effect of vitamin and hormonal priming on biochemical attributes of wheat seedlings.**

Treatments	Phosphorus (%)	Potassium (%)	Total soluble sugars (mg g <sup>-1</sup> d. wt.)
Control	0.029	56.2 a	0.54 b
Salicylic acid	0.034	35 b	0.76 a
Ascorbic acid	0.033	36.2 b	0.77 a
Kinetin	0.032	25 b	0.56 b
GA <sub>3</sub>	0.032	37 b	0.56 b
<b>LSD at 0.01</b>	<b>NS</b>	<b>16.69</b>	<b>0.06</b>

The earlier and synchronized germination might be attributed to increased metabolic activities in the primed seeds (Soon *et al.*, 2000; Shakirova *et al.*, 2003; Iqbal & Ashraf, 2010). Hormonal and vitamin priming treatments not only improved the germination rate and time but also enhanced the seedling vigor as indicated by lower values of MET and higher FEP, root and shoot length and seedling fresh and dry weights (Table 1). Enhanced replication in root tips has also been reported by hormonal and vitamin priming (Shakirova *et al.*, 2003). Vigor enhancement by the incorporation of growth regulators in priming solution might be due to increased cell division within the apical meristem of seedling root, which caused an increase in plant growth. Moreover, hormonal treatments maintain the IAA and cytokinin levels in the plant tissues, which enhances the cell division (Sakhabutdinova *et al.*, 2003).

The current study revealed that hormonal and vitamin priming treatments could significantly lower the EC of seed leachates. Minimum electrical conductivity was observed in seeds primed with salicylic acid and ascorbic acid. The lower EC of steep water for the pre-sowing seed treatments is an indication of better membrane repair during controlled hydration. Greater membrane integrity in treated seeds of eggplant and radish (Rudrapal & Nakamura, 1988) and rice (Basra *et al.*, 2005) has been reported earlier. The lower EC value indicates that hormonal and vitamin priming did not damage the seed structure rather it allowed the better membrane repair. Lower EC induced by seed priming was accompanied with lower MGT, and higher GI and GE in present studies. This suggests successful membrane and genetic repair and trigger of metabolic activities promised by seed priming.

## Conclusion

Seeds primed with ascorbic acid resulted in maximum FGP, FEP, radicle and plumule length, root and shoot length, number of secondary roots, root shoot ratio, root and shoot dry weight and seedling dry weight compared control (untreated seeds). Minimum MGT and MET was recorded in seeds primed with kinetin and GA<sub>3</sub> compared with control. Seedling potassium contents were decreased by hormonal seed priming while total soluble sugars were increased by salicylic acid and ascorbic acid seed priming. Seeds primed with ascorbic acid and salicylic acid resulted in minimum electrical conductivity at 0.5, 1, 2, 6, 12 and 24 h after imbibitions than unprimed seeds. Therefore, wheat seeds primed with 20 ppm solution of ascorbic acid may be used for wheat seed invigoration.

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