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INFLUENCE OF PRE-SOWING SEED TREATMENTS ON GERMINATION PROPERTIES AND SEEDLING VIGOR OF WHEAT

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Abstract. Healthy seed germination and vigorous seedlings are important in all crops for obtaining bumper yield under harsh environment. The objective of this study was to assess the potential of pre-sowing seed treatments in improving germination and early seedling growth of wheat cultivars. Laboratory experiments were conducted to examine the efficacy of different pre-sowing treatments on seed germination behavior and vigor attributes of four wheat cultivars, Pirsabak-2013, Shahkar-2013, Hashim and Lalma at Agronomy research laboratory, University of Agriculture, Peshawar.Studied treatments include hydropriming (distilled water) and halopriming with Calcium Ammonium Nitrate (2%) for 24 hours. For seed hardening, seed were soaked in CAN solution (2%) for 24 hours and drying back to original moisture content at room temperature and this cycle was repeated twice for 12 hours. Dry/non primed seeds were used as control. Results revealed that halopriming and seed hardening with CAN solution (2%) improved the rate of germination in all wheat cultivars and reduced the mean germination time compared to hydropriming and control treatment. Among vigor evaluation tests, halopriming, seed hardening and hydropriming treatment were found effective as expressed by final emergence percentage, root and shoot length and their fresh and dry weight,. Control/non primed seeds gave minimum final emergence percentage, seedling dry weight and minimum fresh weight of seedling, root and shoot length. Pirsabak-2013 performed better in all the studied parameters over other cultivars.

Keywords: *hydropriming, seed hardening, halopriming, germination, seedling vigor, wheat.*

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1. Introduction

Wheat (*Triticumaestivum* L.) is an important food crop of Pakistan and mostly cultivated in rainfed areas, where rainfall is scarce. Extreme temperatures, soil crusting, low rainfall and salinity may individually or in combination adversely affect the germination and stand establishment of the crop and reduces grain yield (Amin *et al*., 2016: Basra *et al*., 2002). Rapid seed germination and stand establishment are critical factors for crop production under stress conditions. In many crop species, seed germination and early seedling growth are the most sensitive stages to stresses. Constraints to good cost abolishment include improper seedbed preparation (Joseph and Nair, 1989), low quality seed, untimely sowing (Khan, 1992), inadequate soil moisture (Harris *et al*, 1999) and adverse soil conditions (Lee *et al*, 1998). It has been reported that seed priming is one of the most important developments to help rapid and uniform germination and emergence of seeds and to boost seed tolerance to adverse environmental conditions. Efforts made to exploit yield potential were hampered by the adverse effect of abiotic stress such as drought and salinity. These effects cause huge losses due to low yield and failure of the crop to establish in dryland areas (Khan, 1992). Pre-sowing seed treatment techniques has been used as an alternate approach to overcome the ailing effects of abiotic stresses in agricultural production because of its low cost and risk (Khan *et al*, 2016). Pre-sowing seed treatment technology is a control seed hydration in solution containing organic or inorganic solutes followed by redrying that allows pre-germinative metabolic activities but prevent radical emergence (Khalil *et al*., 2015: Basra *et al.*, 2005). The primed/hardened treatments proved to be better for vigor improvement than traditional soaking (Manjunath and Dhanoji, 2011). Seed priming/hardening treatments enhances seeds vigor by protecting structure of the plasma membrane against injury during stress environment (JunMin *et al*., 2000). Pre-soaking seeds with optimal concentration of phyto-hormones enhance their germination, growth and yield under stress condition by increasing nutrient reserves mobilization through increased physiological activities and root proliferation (Khan *et al*., 2016). Previous studies have also shown that pre-sowing seed treatment in various concentrations of Indole acetic acid, Gibberellic acid (Shah 2007) and Indole Butyric acid (Strader *et al*., 2011) may promote or inhibit seedling growth. Therefore, the aim of this study was to evaluate the potential of pre sowing seed techniques in improving germination and early seedling growth of the newly released wheat cultivars.

2. Materials and methods

The experiment was conducted to study the influence of different seed priming techniques on germination and vigor attributes of the new released wheat cultivars in the seed laboratory, Department of Agronomy, University of Agriculture, Peshawar during the year 2015. Seeds of wheat cultivars, Pirsabak-2013, Shahkar-2013, were obtained from Cereal Crop Research Institute (CCRI), Nowshera, whereas Hashim and Lalma were obtained from the Nuclear Institute for Food and Agriculture, (NIFA), Peshawar. Before the start of experiment, seeds of all cultivars were surface sterilized in 10% sodium hypochlorite solution for 10 minutes, then rinsed with sterilized water and air-dried at room temperature closely to original moisture level. The following pre-sowing seed treatments were included

T1 = Control
T2 = Hydropriming for 24 h
T3 = Halopriming with 2% CAN for 24 h
T4 = Hardening with CAN (2%) for 24 h (One cycle)

A weighed quantity (250 g) of wheat seeds from each cultivar was put in glass beakers containing 500 mL of respective solution for 24 hours for hydropriming and halopriming. Seed hardening was carried out with CAN (2%) for 24 h, dried back and this cycle was repeated twice for 12 hours (Shah and Khan*.*, 2016). These seeds were sealed in polythene bags and stored in a refrigerator at $7^{o}C$ for further studies. The treated seeds were compared with control ones for vigor by germination, emergence and electrical conductivity tests.

Vigor evaluation

Germination test: Germination potential of controlled and treated seeds was estimated in accordance with (ISTA, 2007). To test seed germination, four replicates of 25 seeds were germinated in 12 cm diameter petridishes. The petridishes were covered with lids and placed in incubator at 25°C. A seed was considered to have germinated when coleoptile and radical lengths have reached 2 mm. Counts of germinating seeds were made daily, starting on the first day of imbibition and terminated when maximum germination was achieved. The final germination percentage, days to reach 50% germination (T_{50}) and mean germination time (MGT) (days) were recorded up to 4 days after sowing. The final germination was calculated according to the following formula of Coolbear *et al*. (1984)

$$T_{50} = ti + \frac{[N/2 - ni](tj - ti)}{nj - ni},$$

where N is the final number of germination and ni, nj cumulative number of seeds germinated by adjacent counts at time ti and tj when ni< N/2 <nj.

Emergence test: The experiment was conducted in pots filled with sand. 25 seeds were sown per pot at the depth of 4cm and the experiment was replicated thrice. The pots were placed in the wire house of New development Farm of Agricultural University Peshawar. The data regarding the final emergence percentage (%), days to 50% emergence (E_{50}) (days) and mean emergence time (MET) (days) were recorded after the start of experiment. The data on the shoot length (cm), root length (cm), fresh weight of seedling (g) and dry weight of seedling (g) was recorded after 10 days of sowing.

Electrical conductivity test: Four replicates of two-gram seeds were drawn randomly from each sample. They were placed into 100 ml glass bottles containing 50 ml de-ionized water. Each bottle was gently swirled to ensure complete immersion of all seeds and was covered with aluminium foil and then kept at 25°C for 12 and 24 hours. At the end of the soaking period, the bottles were swirled, the aluminium foil removed and the conductivity was determined by immersing a dip-type EC meter cell (model – HI 8820 N) into the seed water solution with due care to avoid direct placement of the cell on the seed. After testing the dip-cell was rinsed twice using de-ionized water prior to testing the next sample. The electrical conductivity per gram of seed weight for each sub-sample was calculated as:

$$\textbf{Electrical conductivity (mscm}^{-1}\textbf{g}^{-1}\textbf{)} = \frac{\text{Conductivity of each flask}}{\text{Weight of seed sample (g)}}$$

Data on different parameters were subjected to analysis of variance (ANOVA) according to the methods described for completely randomized design (CRD) and means between treatments were compared using LSD test at 5% probability level (Steel &Torrie, 1997).

3. Results and discussion

Germination (%)

Seed germination and vigorous seedlings are important characteristics for wheat which could provide advantages for crop establishment. The data revealed that all pre-sowing treatments significantly affected the germination percentage and its related attributes in all wheat cultivars. The interaction between C x P was found non-significant. In germination test, seed hardening with CAN (2%) and halopriming gave faster and earlier germination (89.1%) as compared to hydroprimed seed (Fig.1).

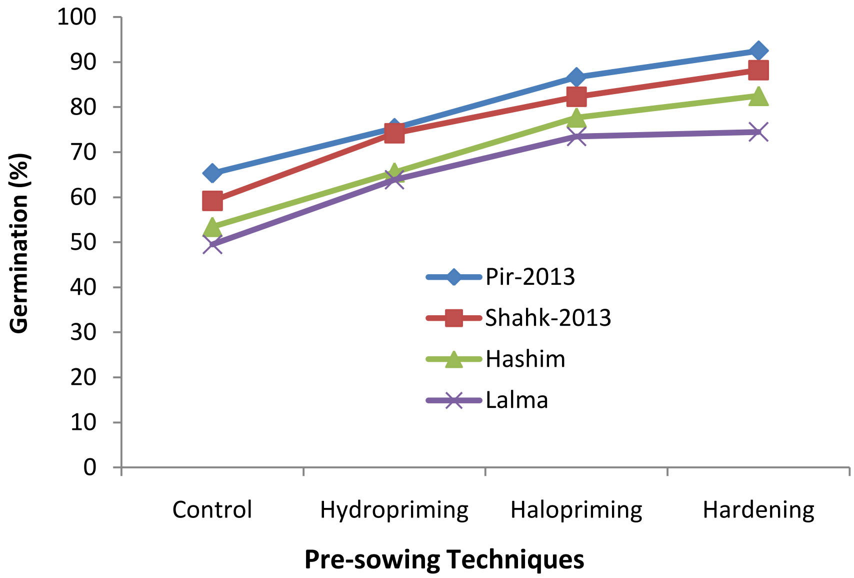

Fig.1. Germination % of wheat cultivars as affected by different pre-sowing techniques.

Germination Index also showed similar trend of improvement (37.5a) in halopriming and seed hardening with CAN (2%). A steady decrease in germination % and germination index took place, when seed were soaked in water for 24 hours. Non-primed seeds/control gave minimum germination % and germination index. The improvement in germination % and germination index by seed hardening and halopriming with CAN (2%) may be attributed to stimulation of hydrolytic enzyme activity known to be induced by these pre-sowing chemical agents. The reason for higher germination % and germination index may be due to greater hydration of colloids, higher viscosity and elasticity of protoplasm, offer an increase in bound water content, lower water deficit and increased metabolic activity (Ghassimi *et al.* 2008; Harris., 2002). These findings support the earlier work on wheat [*Triticum aestivum*] (Amin *et al., 2016*), canola [*Brassica compestris*] (Zheng *et al.*, 1994), and rice [*Oryza sativa*] (Lee and Kim 2000; Basra *et al.*, 2006) who reported improved germination rate and percentage in seeds subjected to seed hardening and hydropriming for 24 h. Among cultivars, Pirsabak-2013 had the highest germination percentage of (83.6a) as compared to Shahkar-2013 and Hashim cultivars. Lalma gave minimum germination percentage in all pre-sowing treatments. Statically similar trend of increase in germination index was observed in cultivar Pirsabak-2013 and Shahkar-2013, whereas a non significant was found between Hashim and Lalma genptypes. The variation among cultivars with respect to germination and its related attributes may be due to genetic potentiality of these newly released wheat cultivars and their positive response to different priming medias and durations. The findings of this study support the earlier work on wheat [*Triticum aestivum*] (Khalil *et al., 2015*), who stated that osmo-priming with PEG 20% for 24 hours may be used as tool to improve germination and seedling growth of wheat under drought condition.

Seedling Vigor

Data regarding seedling vigor evaluation are reported in tables and figures. The results indicate that halopriming and seed hardening treatments significantly reduced the germination time (T_{50}) and improved the emergence rate and seedling vigor despite the seed lot selected was of high vigor (Table 1).

Table 1. Effect of different pre-sowing seed treatments on germination attributes of wheat cultivars.

Pre-sowing Treatments	T_{50} (days)	MET (days)	Germ. Index	S.L (cm)	S.f.wt (mg)	S.d.wt (mg)
Control	2.4a	4.8a	18.7c	1.4c	6.7c	5.9d
Hydropriming	1.8b	3.0b	27.3b	1.4b	10.5b	9.7b
Seed Hardening	1.4bc	2.5bc	37.5a	1.8a	13.1a	11.9a
Halopriming	1.4c	2.6c	36.9a	2.4a	13.2a	11.2a
Wheat Cultivars						
Pirsabak-2013	1.5b	2.8c	31.1ab	1.5b	11.7a	9.7b
Shahkar-2013	1.4b	3.0bc	31.7a	1.4b	10.4b	10.1a
Hashim	2.0a	3.4ab	28.5c	2.0a	10.3b	10.3b
Lalma	2.1a	3.6a	29.1bc	2.1a	10.4b	9.3b
Interaction						
C x P	Ns	ns	ns	ns	ns	ns

*Means of the same category followed by different letters are significantly different at 0.05 level of probability using LSD test

It demonstrate that seed hardening and halopriming with CAN (2 %) not only improve the performance of low vigor seeds but also invigorate and induce early, synchronized and healthier crop stand. Significantly maximum time to E_{50} was recorded in non-primed seed/control treatment, whereas minimum day to E_{50} was observed in seed hardening and halopriming followed by hydropriming. Maximum shoot length (2.4 cm) was observed in seeds subjected to halopriming and seed hardening as compared to hydroprimed seeds.Hashim and Lalma cultivars gave maximum shoot length (2.0 & 2.1cm) than Pirsabak-2013 and

Shahkar-2013. Longest roots were measured in seed subjected to halopriming followed by seed hardening and hydroprimed seed (Fig. 2). The minimum root length was recorded in non-primed seeds/control treatment. Wheat cultivar Pirsabak-2013Shahkar-2013 gave maximum root length (9.4cm) followed by Shahkar-2013. Root length of Lalma cultivars increased linearly in hydro and halopriming and become plateau in seed hardening treatment.

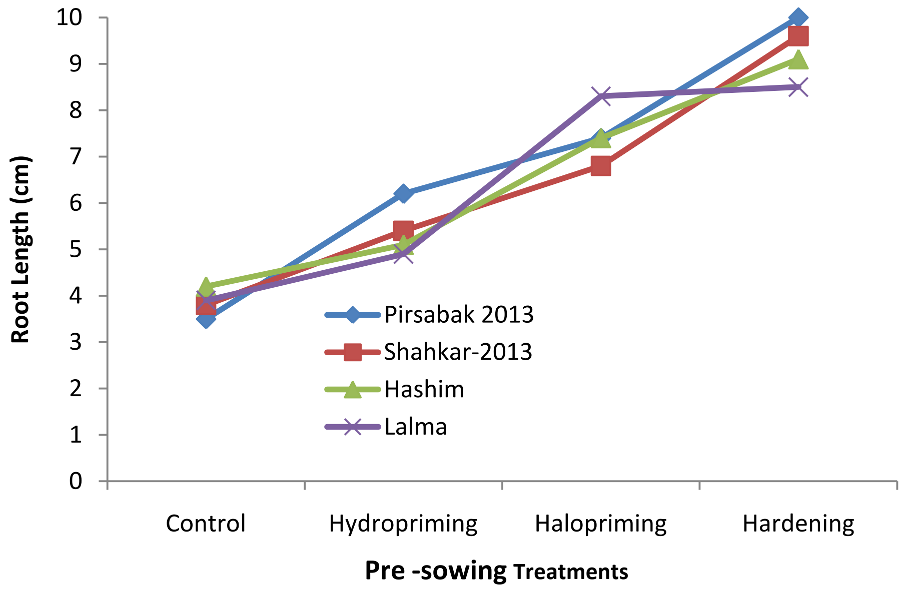

Fig.2 Influence of different Pre-Sowing Treatments on Root Length of Wheat Cultivars**.**

Maximum seedling fresh and dry weight was obtained from seed hardening and halopriming followed by hydropriming as compared to non-primed seed/control treatment (Table-1). Maximum fresh and dry seedling weight from pre-sowing treatment may be attributed to more synchronize germination, which resulted in early stand establishment. These finding are in agreement with those of Khan *et al*., (2016) started that both hydro-priming and osmo-priming with Calcium Ammonium Nitrate (CAN) at 0.5% for 24 hours are practical approaches for improving growth and vigor of maize crop under harsh environment. The overall results indicate that the halopriming and hardening of wheat seeds by CAN (2 %) could invigorate the wheat seeds by improving the vigor and germination percentage (Pawar *et al*., 2003). Non-viable or deteriorated seeds have been reported to leak more solutes when placed in water than viable or vigorous seeds. The effect of different pre-sowing seed treatments on solute leakage of wheat seeds has been reported in (Fig. 3).

Generally maximum EC was noted in 24 hour duration than 12 hours period. Halopriming and hardening treatments showed minimum electrical conductivity on 12 and 24 hours measuring period than all other treatments

(Khan, 2001). Maximum EC of 0.12 micro-siemen $cm^{-1}g^{-1}$ was recorded in non-primed/dry seed followed by hydropriming. Overall results of EC test showed that EC of seed leachates decreased by most of pre-sowing treatments. Minimum solute leakage from pre-sowing treatments was recorded as compared to control/dry seed treatment. The minimum electrical conductivity from pre-sowing treatment may be due to better membrane repair during hydration. Greater membrane integrity in treated seeds of sunflower and wheat were reported by Kaya, *et al*; 2006 and Afzal *et al.* (2002) for hybrid maize. All cultivars at 24 hours soaking period exhibited higher EC than 12 hours duration. Increased seed leachate conductivity from control/dry seed was probably due to the loss of ability to recognize cellular membranes rapidly and completely.

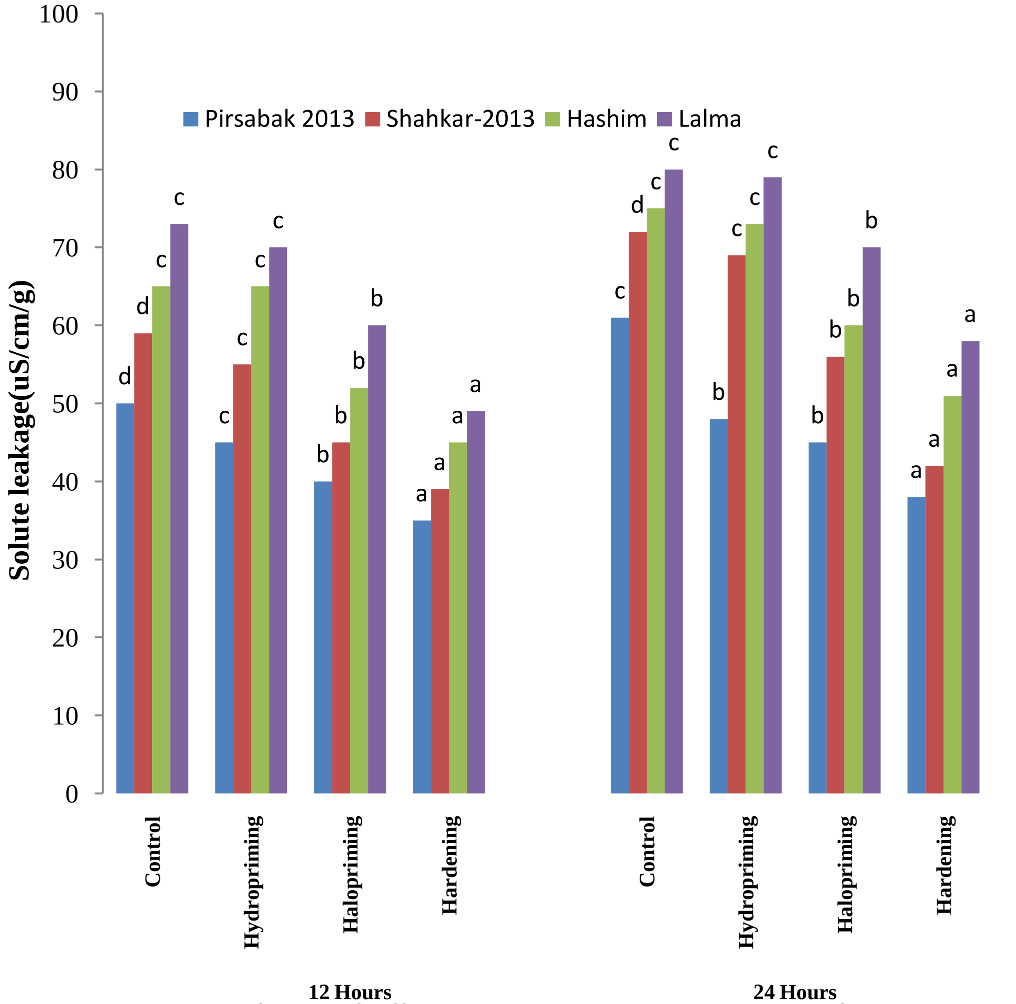

Fig.3. Influence of different soaking period on solute leakage of wheat cultivars as affected by pre-sowing treatments.

4. Conclusion

It is concluded from this experiment that all newly released wheat cultivars responded positively to different pre-sowing seed treatment. Seed hardening and halopriming with CAN (2%) for 24 hours are effective tool for seed invigoration in these cultivars. There is a close association between the germination and seedling vigour. The feasible basis of seed invigoration by these seed treatments are increased membrane repair and enzymatic activities.

Authors' contribution

Conceived and designed the experiments: A.Z. Khan, Performed the experiments: Tariq Shah, Analyzed the data: T.Shah, A.Rehman, Contributed reagents/ materials/ analysis tools: H Akbar & A Muhammad, Wrote the paper: A.Z. Khan & S.K.Khalil.

Competing Interests: Authors declare that they have no competing interests.

References

1. Afzal I., Basra S.M.A., Ahmad N., Cheema M.A., Warraich E.A., Khaliq A., (2002) Effect of priming and growth regulator treatment on emergence and seedling growth of hybrid maize (*Zea mays*), *Intern. J. Agric. and Biol*. 4(3), 303-306.
2. Amin R., Khan A.Z., Muhammad A., Khalil S.K., Gul H., Daraz G., Akbar H., (2016) Influence of Seed Hardening Techniques on Vigor, Growth and Yield of Wheat under Drought Conditions, *J.Agric. Studies*, 4(3), 121-130.
3. Basra S.M.A., Farooq M., Tabassum R., (2005). Physiological and biochemical aspects of seed vigor enhancement treatments in fine rice (Oryza sativa L.), *Seed Sci. & Technology*, 3, 29-33.
4. Basra S.M.A., Zia M.N., Mahmood T., Afzal I., Khaliq A., (2002) Comparison of different invigoration techniques in wheat (TriticumaestivumL.), *Pak. J. Arid. Agric.*, 5(2), 325-329.
5. Basra S.M.A., Afzal I., Anwar S., Shafq M., Majeed K. (2006) Alleviation of salinity stress by seed invigoration techniques in wheat, *Seed Technol*., 28, 36-46.
6. Coolbear P., Francis A., Grierson D., (1984) The effect of low temperature pre-sowing treatment under the germination performance and membrane integrity of artificially aged tomato seeds, *J. Exp. Bot*., 35, 1609–1617.
7. Ghassemi G., Alito A.A., Valizadahe M., Moghaddam M., (2008) Effect of hydro and osmo priming on germination and field emergence of lentil, *Hort. Agrobot (Online)*, 36 (1), 29-33.
8. Harris D., (2002) On-farm seed priming to increase yield of crops and reduce risk of crop failure in marginal areas of developing countries, Second International Agronomy Congress on Balancing Food and Environmental Security a Continuing Challenge, New Delhi 2002, Indian Society of Agronomy, Indian Council of Agricultural Research, 1509-1511.
9. Harris D., Joshi A., Khan P., Gothakar A., Sodhi P. S., (1999) On farm seed priming in semi-arid agriculture: Development and evaluation in corn, rice and chickpea in India using participatory methods, *Exp. Agric*., 35, 15-29.
10. ISTA (2007) International Rules for Seed Testing: ISTA, Bassersdorf, Switzerland.

11. Joseph K., Nair P., (1989) Effect of seed hardening on germination and seedling vigor in paddy, *Seed Res*., 17, 183-190.
12. JunMin H., Xiaoping S., Jian Z., (2000) Mitigative effects of hydration-dehydration
13. treatments on salt stress induced injury to tomato seed germination, *Deta.Hort.Sinica*, 27, 123-126.
14. Kaya M.D., Okcu G., Atak M., Cikili Y., Kolsaric O., (2006) Seed treatments to overcome salt and drought stress during germination in sunflower, *Europ. J. Agronomy*, 24, 291-295.
15. Khalil S.K., Rahman S., Rehman A., Wahab S., Khattak M.K., . Khalil I.H., Khan A.Z., Amanullah, (2015) Osmoconditioning improve germination, assimilate partitioning and harvest index of wheat under drought, *International Journal of Biosciences*., 6 (7), 44-57.
16. Khan A. A., (1992), Pre-plant physiological seed conditioning, *Annual Review of Horticultural Sciences*, 132–179.
17. Khan A.Z., Imran A., Muhammad A., Khalil A., Gul H., Wahab S., Akbar H., (2016) Impact of fertilizer priming on seed germination behavior and vigor of maize, *Pure & Appl. Bio*., 5(4), 744-751.
18. Khan A.Z., (2001) Influence of planting dates and plant density on canopy temperature, seed development, seed quality and yield of Soybean. Ph.D dissertation, Department of Agronomy, University of Agriculture, Faisalabad.
19. Lee S.S., Kim J.H., (2000) Total sugars, α-amylase activity and germination after priming of normal and aged rice seeds, *Korean Journal of Crop Sciences*, 45, 108–111.
20. Lee S.S., Kim J.H., Hong S.B., Yun S.H., (1998) Effect of humidification and hardening treatment on seed germination of rice, *Korean J. Crop Sci*., 43, 157-160.
21. Manjunath B.L., Dhanoji M.M., (2011) Effect of seed hardening with chemicals on drought tolerance traits and yield in chickpea, *J. Agric. Sci*., 3(3), 186-189.
22. Pawar K N., Sajjan A.S., Prakash B.G., (2003) Influence of seed hardening on growth and yield of sunflower, *Karnataka J. Agric. Sci*., 16(4), 539-541.
23. Shah S.H., (2007) Physiological effects of presowing seed treatment with gibberellic acid on Nigella sativa Linn, *Acta Bot. Croat*., 66(1), 67-73.
24. Strader LC., Wheeler D.L., Christensen S.E., Berens J.C., Bartel B., (2011) Multiple facets of Arabidopsis seedling development require indole-3-butyric acid derived auxin, *Plant Cell*, 23, 984–999.
25. Steel R.G.D., Torrie J.H., (1997) Principles and Procedure of Statistics McGraw Hill, Book Co., Inc. Singapore, 173–177.
26. Zheng G.H., Wilen R.W., Slinkard A.E., Gusta L.V., (1994) Enhancement of canola seed germination and seedling emergence at low temperature by priming, *Crop Science*, 34, 1589–1593.