

Effect of seed hardening with nitrate salts on physiological attributes at ear head emergence stage and yield of wheat (*Triticum aestivum* L.)

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ABSTRACT

A pot experiment was conducted to investigate the effect of seed hardening treatments with distilled water (DW) and nitrate (15mM) salts viz. $Mg(NO_3)_2$ and KNO_3 on some physiological attributes at ear head emergence stage and yield of two wheat varieties (HUW-234 and HUW-468). The use of nitrate salt KNO_3 is effective to improve physiological and morphological attributes like number of tillers, plant height, number of leaves, leaf area, fresh and dry weight of shoot at ear head emergence stage in comparison to $Mg(NO_3)_2$ and followed by DW and control whereas the fresh and dry weight of root, total chlorophyll and nitrogen contents were found to increase with $Mg(NO_3)_2$ hardened sets in respect to other treatments. Nitrate reductase (NRase) activity in flag leaf was also found maximum in KNO_3 hardened sets. The application of KNO_3 showed significant effect at harvest stage. Plants hardened with nitrate salts produced higher dry matter, number of ear head, number of grains and weight of grains per plant in comparison to DW and control sets. $Mg(NO_3)_2$ hardened plants were found with maximum test weight and superior over rest of seed hardening treatments.

Key words : Hardening, Nitrate salts, Wheat, Ear head emergence stage, Yield

INTRODUCTION

A major challenge for the first two to three decades of 21st century is going to food and nutrition security for specially the families living below poverty line. Wheat is the world's leading cereal crop cultivated over an area of about 226.45 million h. In India wheat is cultivated over an area of 26.6 million h with a total production of 72.1 mt. with average productivity of 27.01 qt.h⁻¹ during 2003-2004. In Uttar Pradesh wheat is often sown late due to late harvest of rice or stagnation of water after flooding in diara areas of Indo-gangatic plains of India. Late sown wheat seeds suffer from slow rate of seedling emergence due to very low temperature, which causes a delay in vegetative growth as a result hot desiccating winds cause premature ripening of spike of this crop. Consequently it reduces the yield and productivity which make it unprofitable. Therefore, early seedling emergence in the field followed by rapid vegetative growth and early flowering are desired attributes for wheat cultivators. The expected demand of wheat accounts 900 mt. by the year 2020 for exponential increase in the world's population. Indian point of view the projected demand of wheat is 109 mt. by the year 2020, which is a challenging task for Indian as well as world wide wheat planners.

From the study of literature it is realized that number of seed treatments like pre-sowing soaking, hardening etc. by various salts and plant growth regulators (PGR)

improve germination/emergence in field and increase further vegetative growth as well as yield of plants (Pfahler *et al.*, 1991; Bose *et al.*, 1992; Bose, 1997; and Bose and Mishra, 2001). Seed hardening with distilled water and nitrate salts are found to improve seed germination, seedling emergence and vegetative growth of wheat (Sharma and Bose, 2006). Hence, a study has been carried out on wheat by hardening its seeds with nitrate salts { $Mg(NO_3)_2$ and KNO_3 } in respect to physiological attributes at ear head emergence stage (75 days after sowing) and at harvest stage of two wheat varieties (HUW-234 and HUW-468).

MATERIALS AND METHODS

Treatments description and sampling:

Surface sterilized (0.1% $HgCl_2$) wheat (var. HUW-234 and HUW-468) seeds were hardened with DW, nitrate (15mM conc.) salts ($Mg(NO_3)_2$ and KNO_3). In the hardening treatments seeds were soaked either in DW or in the salt solution for 16 h in normal light condition at an average temperature of 20 ± 2 °C and then seeds were dried back to their original weight at room temperature by placing them under a blower. These seeds were stored in paper bags for two months for further studies. Hardened and non-hardened (control) seeds were sown in earthen pots of 30 cm diameter and 40 cm height filled with garden soil for the study of plant growth attributes at ear head

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emergence stage representing 75 days after sowing (DAS) and yield attributes at harvest. NRase activity in leaves, Total chlorophyll content and total nitrogen in dried samples estimated by employing the methods Srivastva (1974), Strain and Svec (1966) and modified micro kjeldahl (Lang, 1958), respectively.

Statistical analyses:

All the experiments were repeated twice with three replicates and the data were statistically analyzed by analysis of variance as described by Cochran and Cox (1963) for randomized block design.

RESULTS AND DISCUSSION

The data pertaining to number of tillers and plant height (cm) at ear head emergence stage were found significantly higher for both the varieties hardened with KNO_3 and found equally effective with $\text{Mg}(\text{NO}_3)_2$ treatment and superior over other treatments like DW and control. The results follow same trend for the parameters like leaf area, fresh and dry weights of shoot but the fresh and dry weights of roots found to be more in sets hardened with $\text{Mg}(\text{NO}_3)_2$ (Table 1). Paul and Choudhury (1993) reported similar results by using potassium containing salts viz., KCl, KH_2PO_4 and K_2HPO_4 for wheat seed hardening. They noticed that seed germination, seedling vigor and shoot and root lengths were higher with all these salts having concentration 0.5

and 1.0 % only and 18 h soaking treatment. Greef and Kullmann (1992) reported that while wheat plants were grown in controlled environment with application of nitrate as nutrient solution showed a considerable increase in shoot and root dry matter and N uptake.

In present investigation the study related to biochemical aspects showed that chlorophyll concentration (mg g^{-1} fresh wt. of leaves) in leaves found to increase in $\text{Mg}(\text{NO}_3)_2$ hardened sets i.e. 1.76 which was significantly superior over other treatments i.e. KNO_3 , DW and control and the values were 1.46, 1.41 and 1.61, respectively. Same trend was recorded for total N content for shoots. However, the NRase activities were almost same in KNO_3 and $\text{Mg}(\text{NO}_3)_2$ hardened sets and also found significantly higher in respect to DW and control. These findings are in close agreement with the results of Bose and Tandon (1992) and Verma and Srivastava (1998).

Study related to yield attributing parameters showed that KNO_3 treatment was significantly superior over other treatments. Number of ear head plant⁻¹, length of ear head (cm.), number of effective ear head plant⁻¹, total weight of ear heads plant⁻¹(g), total dry matter plant⁻¹(g), number of grains plant⁻¹ and weight of grains plant⁻¹(g) were found maximum in KNO_3 hardened plants whereas, in $\text{Mg}(\text{NO}_3)_2$ treated sets the test weight was more (Table 2). Podlaski and Wyszowska (1994) reported that field emergence, seedling weight (at 60 DAS), plant survival during winter, harvest index, test weight and density all these parameters improved more with seed hardening with

Table 1 : Influence of seed hardening with DW and nitrate (15mM) salts on some physiological and biochemical parameters at the ear head emergence stage of wheat (*Triticum aestivum* L.) varieties

Parameters	Treatments						Varieties			
	Control (T ₁)	DW (T ₂)	KNO_3 (T ₃)	$\text{Mg}(\text{NO}_3)_2$ (T ₄)	S.E.±	CD (P=0.05)	HUW- 234 (V ₁)	HUW-468 (V ₂)	S.E.±	CD (P=0.05)
No. of tiller plant ⁻¹	3.50	3.50	5.00	4.50	0.08	0.24	4.25	4.00	0.05	0.16
Plant height (cm.) plant ⁻¹	53.83	64.19	66.67	65.08	0.15	0.54	65.09	59.79	0.10	0.30
No. of leaves plant ⁻¹	15.00	15.67	18.33	17.17	0.14	0.42	15.75	17.33	0.09	0.28
Leaf area plant ⁻¹ (cm ²)	119.33	259.50	299.97	294.50	3.03	9.18	237.19	288.98	6.12	20.02
Fresh wt. of shoot (g plant ⁻¹)	14.72	17.93	21.35	18.85	0.31	0.94	21.16	15.28	0.21	0.63
Dry wt. of shoot (g plant ⁻¹)	2.48	2.83	3.39	3.24	0.05	0.15	2.83	3.14	0.03	0.1
Fresh wt. of root (g plant ⁻¹)	0.50	0.62	0.82	0.94	0.02	0.05	0.74	0.71	0.1	0.4
Dry wt. of root (g plant ⁻¹)	0.093	0.109	0.161	0.242	0.005	0.014	0.176	0.127	0.003	0.009
Chlorophyll concentration (mg g^{-1} fresh wt. of leaves)	1.61	1.41	1.46	1.76	0.02	0.06	1.50	1.75	0.01	0.04
Nitrogen content (mg g^{-1} dry wt. of shoot)	28.58	31.21	35.93	37.61	0.30	0.91	29.81	36.85	0.20	0.61
NRase activity (n mol NO_2^- $\text{h}^{-1} \text{g}^{-1}$ leaf fresh wt.) in flage leaf	517.33	683.67	828.00	822.00	14.42	43.75	593.17	832.33	9.62	29.16

Table 2 : Influence of seed hardening with DW and Nitrate (15mM) salts on some yield attributes at the harvest stage of wheat (*Triticum aestivum* L.) varieties

Parameters	Treatments				Varieties					
	Control (T ₁)	DW (T ₂)	KNO ₃ (T ₃)	Mg(NO ₃) ₂ (T ₄)	S.E.±	CD (P=0.05)	HUW-234 (V ₁)	HUW-468 (V ₂)	S.E.±	CD (P=0.05)
No. of earhead plant ⁻¹	2.17	3.00	3.83	3.00	0.06	0.18	3.08	2.92	0.04	0.12
Length of earhead (cm)	6.72	7.93	9.14	8.93	0.03	0.09	7.72	0.64	0.02	0.06
No. of effective earhead plant ⁻¹	4.21	4.88	5.75	5.42	0.03	0.09	5.17	4.96	0.02	0.06
Total weight of earhead (g plant ⁻¹)	5.49	5.62	7.04	6.81	0.06	0.19	5.58	6.90	0.04	0.13
Total dry matter (g plant ⁻¹)	9.85	9.49	13.07	11.11	0.07	0.21	10.48	11.29	0.05	0.14
No. of grain plant ⁻¹	144.88	148.63	187.23	151.88	1.24	3.77	109.50	206.80	0.83	2.51
Total weight of grain (g plant ⁻¹)	3.61	3.46	4.89	4.70	0.04	0.14	3.80	4.53	0.03	0.09
Test weight (g plant ⁻¹)	26.73	26.03	28.95	35.80	0.33	2.70	35.01	23.74	0.22	0.67

water. Findings of Mandal and Basu (1993) were also in accordance to the present study; they investigated that hardening treatment to wheat seeds of cv. Sonalika with water and 10⁻⁴ M sodium phosphate for 2 h improve the germination percentage, field performance and grain yields over untreated control.

Disa *et al.* (1985) suggested that induction of NRase in embryos of wheat required 20 h incubation with KNO₃ at 25°C during the early stages of seed germination under continuous white light. They also concluded that the development of NR activity at lag phase was not due to non-availability of nitrate, presence of NR inactivating factor or synthesis of inactive NR, as at the onset of the inducible phase NR was synthesized de novo independent of fresh m-RNA synthesis, and protein synthesis during the lag phase was essential for subsequent induction of NR in the inducible phase. Therefore, this type of induction may be carried out in the vegetative phase as found in present investigation. Further it has been also observed that during hydration (first phase of hardening) of seeds in the salts of nitrate, cation (Mg⁺² / K⁺¹) and anion (NO₃⁻) both entered in the seed along with water (Bose and Mishra, 1999 and Bose and Pandey, 2003). However, it may be hypothesized on the basis of this that the NO formation may occur from this path way which eventually transduces the signaling activity of the system towards adverse situation by improving its growth rates as was observed by Sharma and Bose (2006) in their study with wheat. Magalhaes *et al.* (2002) also reported that the constitutive NR has the capacity to generate NO which is a very important molecule for signal transduction; hence, in the present study the effect of nitrate seed hardening treatment may be carried over up to the yield via improving the vegetative growth.

Conclusion:

Thus, it may be suggested that this type of short time treatment for hardening of wheat seeds with nitrate salts [Mg(NO₃)₂; 7.5 mM and KNO₃ 15 mM con.] may over come the stressful situation for plant growth like temperature, water, salinity stresses. These treatments are very economic too.

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REFERENCES

- Bose, B. (1997).** The influence of pre-sowing soaking treatment of seed with different nitrates on growth, nitrogen content and nitrate reductase activity in maize. *Physiol. Molecular Bio. Plants*, **3**: 81- 84.
- Bose, B. and Mishra, T. (1999).** Influence of pre-sowing soaking treatment in *Brassica juncea* seeds with Mg-salts on growth, nitrate reductase activity, total protein content and yield responses. *Physiol. Molecular Bio. Plants*, **5**: 83- 88.
- Bose, B. and Mishra, T. (2001).** Effect of seed treatment with magnesium salts on growth and biochemical attributes of mustard. *Indian J. Plant Physiol.*, **6**(4): 431- 434.
- Bose, B. and Pandey, M.K. (2003).** Effect of nitrate presoaking of okra (*Abelmoschus esculentus* L.) seeds on growth and nitrate assimilation of seedling. *Physiol. Molecular Bio. Plants*, **9** (2): 287- 290.

- Bose, B. and Tondon, A. (1992).** Effect of magnesium nitrate on nitrogen metabolism in germinating maize seeds. *Indian J. Plant Physiol.*, **34** : 69 – 17.
- Bose, B., Tyagi, B. and Devi, T.M. (1992).** Influence of seed soaking with $\text{Ca}(\text{NO}_3)_2$ and GA_3 on the growth of seedling of *Zea mays* L. (Var. Tin Pakhia). *Indian Biologist*, **24** (1) : 64-71.
- Cochran, W.G. and Cox, G.M. (1963).** *Experimental designs*. Asia Publishing House, New Delhi, 150-154.
- Disa, S., Gupta, A., Guha, Mukherjee, S. and Sopory, S.K. (1985).** Requirement for a long lag period for the induction of nitrate reductase in wheat (*Triticum aestivum*) embryos during germination. *New Phytologist*, **99** (1) : 71-80.
- Greef, J.M. and Kullmann, A. (1992).** Effect of nitrate application on shoot and root development of wheat seedlings (*Triticum aestivum* L.). *J. Agron. Crop Sci.*, **169**(1-2): 104-113.
- Lang, C.A. (1958).** Simple micro determination of kjeldahl nitrogen in biological material. *Analytical Chemistry*, **30**: 1692-1694.
- Magalhaes, J.R., Filomena, Silva, L.I.M., Salgado, I., Ferraresefilho, O., Rockel, P. and Kaiser, W.M. (2002).** Nitric oxide and nitrate reductase in higher plants. *Physiol. Molecular Bio.Plants*, **8** : 11-17.
- Mandal, A.K. and Basu, R.N. (1983).** Maintenance of vigour, Viability and yield potential of stored wheat seed. *Indian J. Agric. Sci.*, **53** (10) : 905-912.
- Paul, S.R. and Choudhury, A.K. (1993).** Effect of seed hardening with potassium salts at different concentration and soaking duration on germination and seedling vigour of wheat. *Ann. Agric. Res.*, **14** (3) : 357-359.
- Pfahler, P.L., Barnett, R.D. and Soffes, A.R. (1991).** Effect of gibberellic acid and potassium nitrate seed treatments on early seedling growth in two wheat cultivars. *Proceeding Soil and Crop Society of Florida*, **50** : 17-21.
- Podlaski, S. and Wyszowska, Z. (1994).** Technical and physiological ways of improving seed vigour and increasing grain yield of winter wheat cv. Liwilla. II. Results of field experiments. *Roczniki Nauk Rolniczych (Seria)*, **110** (3-4): 21-32.
- Sharma, M.K. and Bose, B. (2006).** Effect of seed hardening with nitrate salts on seedling emergence, plant growth and nitrate assimilation of wheat (*Triticum aestivum* L.). *Physiol. and Molecular Biol. Plants*, **12** (2) : 173-176.
- Srivastava, H.S. (1974).** *In vivo* activity of nitrate reductase in maize seedling. *Indian J. Biochem. and Biophysics*, **11**: 230-232.
- Verma, J. and Srivastava, A.K. (1998).** Physiological basis of salts stress resistance in pigeon pea (*Cajanus cajan* L.)-II. Pre-sowing seed treatment in regulatory early seedling metabolism during seed germination. *Plant Physiol. Biochem.*, **25** (2) : 89-94.

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