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WHEAT SEED INVIGORATION BY PRE-SOWING CHILLING TREATMENTS

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Abstract

This study was conducted to explore the potential of pre-sowing chilling treatments to improve the germination, seedling emergence, early seedling growth, electrolyte leakage and ion uptake in wheat seedlings. The wheat seeds were incubated at -19°C for 24, 48 and 96 h; untreated dry seeds were taken as control. Chilling treatment for 96 h reduced the mean germination time (MGT), mean emergence time (MET) and increased FGP compared with control and chilling for 24 h. GI, EI and FEP were not significantly affected by chilling treatments. Chilling treatment with 24 and 96 h increased the plumule length, root length, shoot length, number of secondary roots, root dry weight and shoot dry weight as compared with control. Regarding biochemical attributes, seedling potassium contents were decreased while total soluble sugars were increased in wheat seedlings by chilling treatments particularly by chilling for 96 h. Phosphorous contents in the wheat seedling remained unaffected by chilling treatments. Chilling for 96 h showed maximum electrical conductivity than other treatments including control at 1, 2, 6 and 12 h after imbibition. While, minimum electrical conductivity of seed leachates was recorded in 24 h chilling. Pre-sowing chilling treatment for 24 h may be opted for wheat seed invigoration.

Introduction

Wheat is the major staple food at global level and increasing demographic pressure has presented a consistent challenge to increase its productivity. Among the chief yield limiting factors, late planting of wheat is the major agronomic problem in rice-wheat and cotton-wheat cropping systems of South Asia including Pakistan (Fugisaka *et al*., 1994). In aforementioned cropping systems 70% wheat is sown after the harvest of cotton and rice crops, which results in delayed wheat sowing by 45-60 days. Mid November is the optimum time for wheat planting; any further delay results in yield reduction by 50 kg ha^{-1} per day (Khan, 2004). This reduction in yield is primarily attributed to poor and erratic germination because of low ambient temperature that prevail at that time. Moreover, late planted wheat plants have to complete all growth and developmental stages in lesser time as starch accumulation process is terminated at the same specific temperature irrespective of time of sowing because of photosensitive nature of wheat.

Seed invigoration techniques may be employed to improve stand establishment under sub-optimal conditions. Various seed invigoration techniques have been listed elsewhere (Ashraf & Foolad, 2005; Ashraf *et al*., 2008).

Pre-sowing chilling treatments are being effectively used alone or in integration with other invigoration techniques in order to shorten the time between planting and emergence, and to protect seeds from abiotic and biotic stresses during critical phase of seedling establishment (Basra *et al*., 2002; Ashraf & Foolad, 2005). Pre-sowing chilling treatments are being effectively used separated or in integration with other invigoration techniques in order to shorten the gap between the planting and emergence, and to protect seeds from a biotic and biotic stresses during critical phase of seedling establishment (Basra *et al*., 2002).

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Furthermore, the invigoration persists under less than optimum field conditions, such as high and low temperature, and high and low soil moisture contents (Raun *et al*., 2002).

The present study was therefore planned to investigate the role of pre-sowing chilling treatments on emergence, early stand establishment and ion uptake in wheat seedlings.

Materials and Methods

This study was conducted in Agronomy Laboratory, University College of Agriculture, Bahauddin Zakariya University, Multan and partially at Stress Physiology Laboratory, Department of Botany, University of Agriculture, Faisalabad. The experiment was laid out in a completely randomized design (CRD) with four replications. Seed of wheat (*Triticum aestivum* L. cv. Ufaq) was obtained from Punjab Seed Corporation, Khanewal, Pakistan and initial moisture content of 11.5% with germination percentage of 98% respectively. For all treatments, selected healthy seeds of uniform size were used in same numbers.

A weighed quantity (250 g) of wheat seeds were sealed in polythene bags and placed in refrigerator (Model National NR 245 TES) at -19±2^{o}C for 24 h (C_2), 48 h (C_3) and 96 h (C_4), while untreated seeds were taken as control. Treated seeds were then sown immediately.

Seed germination and seedling vigor evaluation*:* Control and treated seeds were sown in Petri dishes between the layers of moist filter paper at 27^{o}C in the incubator. Germination was observed daily according to the Association of Official Seeds Analysts (AOSA) method (Anon., 1990).

Mean germination time (MGT) was calculated according to the equation of Ellis & Roberts, (1981) as under:

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

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.

Germination index (GI) was calculated as described in the Association of Official Seed Analysts (1983) as the following formulae:

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

Energy of germination was recorded 4th day after planting. It is the percentage of germinating seeds 4 days after planting relative to the total number of seeds tested (Ruan *et al*., 2002). 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 for observing germination.

On 15th day after sowing, the seedlings were evaluated for vigor. Radical and plumule lengths were recorded of 5 randomly selected seedlings per replicate and averaged. Seedling fresh weight was determined immediately after harvest while dry weight was taken after drying at 70^{o}C for 7 days.

Seed emergence and seedling vigor evaluation: Control and treated seeds were sown in 5 kg plastic pots (25 in each) containing moist acid/water washed sand and placed in a growth chamber (Vindon, England) with a 15 h light period, 25/22°C day/night temperatures and 55% relative humidity. Number of emerged seeds was recorded daily according to the seedling evaluation Handbook of Association of Official Seed Analysts, (Anon., 1990) until a constant count achieved.

Mean emergence time (MET) was calculated according to the equation of Ellis & Roberts (1981) as under:

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

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

Emergence index (EI) was calculated as described in the Association of Official Seed Analysts (Anon., 1983) as the following formulae:

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

Energy of emergence was recorded 4th day after planting. It is the percentage of germinating seeds 4 days after planting relative to the total number of seeds tested (Ruan *et al*., 2002). On 17th day after emergence, the seedlings were tested for vigor after carefully removing from the sand. Number of secondary roots and seedling shoot and root length were recorded of 10 randomly selected seedlings per replicate and averaged. Seedling fresh weight was determined immediately after harvest while dry weight was taken after drying at 70°C for 7 days.

After washing in distilled water, 5 g seeds were soaked in 50 mL distilled water at 25ºC. Electrical conductivity of steep water was measured at 0.5, 1.0, 1.5, 2.0, 6.0, 12.0 and 24.0 h after soaking using conductivity meter (Model Twin Cod B-173) and expressed as $\mu S\ cm^{-1}\ g^{-1}$.

Seedling nitrogen content was determined by microKjeldahl method. For the estimation of seedling K^+, nitric acid: perchloric acid (3:1 ratio) digested samples were analyzed using flame-photometer (Jenway, PFP7, Essex, UK). For the determination of total soluble sugars, ground seed sample (1.0 g) was mixed with distilled water (10 mL) and left for 24 h at 25°C, filtered through a Whatman No. 42 filter paper (Lee & Kim, 2000). Sample solution (100 µL) of was taken in the marked test tubes, 900 µL of distilled water and 1 mL of DNS reagent was added in each test tube. Reagent blank was prepared by adding 1 mL distilled water and 1 mL DNS reagent in a test tube. These test tubes were heated in boiling water for 10 min, allowed to cool and then absorbance was read at 540 nm against a reagent blank. The absorbance values were then translated to equivalent reducing sugar using a standard graph obtained by plotting standard glucose concentration against absorbance.

The data collected were analyzed by using the Fishers’s analysis of variance technique under completely randomized block design (CRD) and the treatments means were compared by Least Significant Difference (LSD) test at 0.01 probability level (Steel & Torrie, 1984).

Results

Wheat seeds treated at -19°C for 96 h (C_4) showed statistically minimum mean germination time (MGT), which was similar to all other chilling treatments except control showing maximum MGT, however it was statistically at par with at -19°C for 24 h (C_2) (Table 1). Pre-sowing chilling treatments did not affect the germination index (GI). Maximum FGP was observed in untreated seeds (C_1) against the minimum FGP found in 24 h (C_2) chilling treatment but it was at par with all other treatments except control (Table 1). Maximum and minimum values of radicle length were recorded in control (C_1) and chilling for 48 h (C_3), respectively. However, maximum and minimum plumule lengths were recorded in chilling for 96 h (C_4) and untreated seeds (C_1), respectively (Table 1). Likewise, maximum and minimum seedling fresh weights were recorded in chilling at for 96 h (C_4) and in control, respectively. In contrast, maximum seedling dry weight were recorded in control (C_1) and chilling for 24 h, while minimum seedling dry weight was recorded in chilling for 96 h (Table 1).

Wheat seeds treated at -19°C for 96 h (C_4) showed minimum mean emergence time (MET), which was at par with chilling for 48 h, however maximum MET was recorded from chilling for 24 h, which was similar with untreated seeds (Table 2). While, pre-sowing chilling treatments did not affect the emergence index (EI) and final emergence percentage (FEP; Table 2). Maximum root length was recorded from chilling for 24 h and minimum from chilling for 96 h. Likewise maximum shoot length was recorded from chilling for 24 h, which was similar with all other treatments except control (Table 2). Maximum number of secondary roots was observed in chilling for 24 h, which was at par with chilling for 96 h, while, minimum number of secondary roots were observed in control (Table 2). Maximum root dry weight was recorded from chilling for 24 h, which was similar with all other treatments except control (Table 2). Likewise, maximum shoot dry weight was recorded from chilling for 24 h, which was similar to chilling for 96 h, while minimum number of secondary roots was observed in control (Table 2).

There was no significant difference in seedling phosphorus contents (Table 3), while maximum potassium (K) contents were recorded in untreated seeds, however minimum K was recorded in chilling treatment for 48 h (Table 3). While maximum total soluble sugars were noted from chilling for 96 h, and the minimum total soluble sugars in control (Table 3).

Pre-sowing chilling treatments resulted in lower electrical conductivity of seed leachates. After a half an hour period of soaking, treated seeds depicted lower electrical conductivity than untreated seeds (Fig. 1). While, 1, 2, 6 and 12 h after soaking, chilling for 96 h resulted in maximum electrical conductivity than other treatments including control, while chilling for 24 h demonstrated minimum electrical conductivity of seed leachates (Fig. 1).

Table 1. Effect of pre-sowing chilling treatments on germination and seedling vigor of wheat.

Treatments	MGT (days)	GI	FGP (%)	Radicle length (mm)	Plumule length (mm)	Seedling fresh weight (mg)	Seedling dry weight (mg)
Control	4.93 a	16.32	94.9 a	134.8 a	159.6 c	2325 c	227.5 a
Chilling at -19°C for 24 h	4.42 ab	14.94	86.7 b	83.8 d	164.5 b	2550 b	227.5 a
Chilling at -19°C for 48 h	3.66 b	16.20	88.4 b	111.7 c	166.1 b	2660 b	170 b
Chilling at -19°C for 96 h	3.63 b	16.97	88.4 b	123.6 b	173.0 a	3037 a	145 c
LSD at 0.01	0.91	NS	6.20	5.32	3.66	128.7	22.04

Means sharing the same letters in a column do not differ significantly at p 0.01

MGT= Mean germination time, GI= Germination index, FGP= Final germination percentage, NS= Non-significant

Table 2. Effect of pre-sowing chilling treatments on seedling vigor of wheat.

Treatments	MET (days)	EI	FEP (%)	Root length (cm)	Shoot length (cm)	No. of Secondary roots	Root dry weight (g)	Shoot dry weight (g)
Control	6.14 a	23.93	86	12.33 c	17.80 b	3.15 c	0.11 b	0.12 c
Chilling at -19^{o}C for 24 h	6.34 a	18.86	88	16.02 a	21.25 a	4.50 a	0.16 a	0.15 ab
Chilling at -19^{o}C for 48 h	5.41 b	18.72	90	12.90 b	20.50 a	3.97 b	0.15 a	0.14 bc
Chilling at -19^{o}C for 96 h	4.96 b	17.40	90	9.25 d	20.35 a	4.22 ab	0.15 a	0.17 a
LSD at 0.01	0.55	NS	NS	0.54	1.22	0.33	0.03	0.01

Means sharing the same letters in a column do not differ significantly at p 0.01
MET= Mean emergence time, EI= Emergence index, FEP= Final emergence percentage, NS= Non-significant

Table 3. Effect of pre-sowing chilling treatments on biochemical attributes in wheat seedlings.

Treatments	Phosphorus	Potassium (%)	Total soluble sugars
Control	0.029	55.5 a	0.59 d
Chilling at -19^{o}C for 24 h	0.030	31.2 c	0.77 b
Chilling at -19^{o}C for 48 h	0.033	26.0 d	0.62 c
Chilling at -19^{o}C for 96 h	0.030	39.2 b	0.82 a
LSD at 0.01	NS	2.53	0.01

Means sharing the same letters in a column do not differ significantly at p 0.01
NS = Non-significant

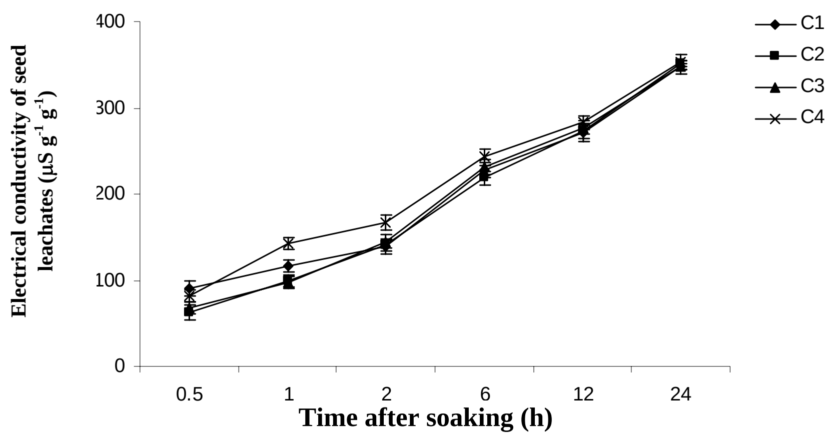

Fig. 1. Effect of pre-sowing chilling treatment on electrical conductivity of seed leachates of wheat seeds. C_1= Control, C_2= Pre-sowing chilling at -19^{o}C for 24 h, C_3= Pre-sowing chilling at -19^{o}C for 48 h and C_4= Pre-sowing chilling at -19^{o}C for 96 h

Discussion

Pre-sowing chilling treatments improved the MGT, MET, FGP but failed to improve GI, EI and FEP of wheat seeds. While, Watkinson & Pill (1998) reported that pre-sowing chilling increased the emergence by 34% in Indian grass. The earlier and synchronized germination and emergence may be attributed to increased metabolic activities in the primed seeds (Soon *et al*., 2000). Radicle length was decreased with chilling treatments; however, plumule length, seedling dry weight, root length, shoot length and numbers of secondary roots were significantly improved by chilling for 24 h. Shoot length and root dry weight were the maximum in all pre-sowing chilling treatments than untreated seeds. Sharma & Kumar (1999) reported that pre-sowing chilling treatments to Indian mustard for 5, 10 and 15 d enhanced the seedling dry weight under normal and NaCl stress conditions. Nonetheless, pre-sowing chilling treatment did not affect seedling phosphorus content, and maximum K contents were recorded from untreated seeds (Table 3). While, total soluble sugars were maximum in chilling for 96 h. EC of seed leachates was significantly lower in seeds chilled for 24 h which might be result of better membrane repair, which resulted in lower rate of seed leachates.

Conclusion

Chilling treatment 24 and 96 h resulted in improved FGP, root length, shoot length, number of secondary roots, root dry weight and shoot dry weight compared with control. In addition, seedling potassium contents were decreased while total soluble sugars were increased in wheat seedlings by chilling treatments. Minimum EC was recorded in 24 h chilled seed leachates at 0.5, 1, 2, 6 and 12 h after imbibition. Wheat seeds treated at -19°C for 24 h was the best treatment for vigor enhancement in wheat.

References

Anonymous. 1983. Association of Official Seed Analysis (AOSA). Seed vigor Testing Handbook. Contribution No. 32 to the handbook on Seed Testing. Association of Official Seed Analysis. Springfield, IL.

Anonymous. 1990. Rules for testing seeds. *J. Seed Technol.*, 12: 1-112.

Ashraf, M. and M.R. Foolad. 2005. Pre-sowing seed treatment – a shotgun approach to improve germination, plant growth and crop yield under saline and non-saline conditions. *Adv. Agron*., 88: 223-271.

Ashraf, M., H.R. Athar, P.J.C. Harris, and T.R. Kwon. 2008. Some prospective strategies for improving crop salt tolerance. *Adv. Agron*., 97: 45-110.

Basra, S.M.A., M.N. Zia, T. Mahmood, I. Afzal, and A. Khaliq. 2002. Comparasion of different invigoration techniques in wheat (*Triticum aestivum* L.) seeds. *Pak. J. Arid Agric*., 5: 11-16.

Ellis, R.A. and E.H. Roberts 1981. The quantification of ageing and survival in orthodox seeds. *Seed Sci. Technol*., 9: 373-409.

Fujisaka, S., L.W. Harrington and P.R. Hobbs. 1994. Rice-wheat in South Asia: Cropping systems and long term priorities established through diagnostic research. *Agricul. Systems*, 46: 169-187.

Khan, M.A. 2004. Wheat crop management for yield maximization. Annual Research program, Arid Zone Research Institute, Bhakkar, Pakistan.

Lee, S.S. and J.H. Kim. 2000. Total sugars, amylase activity and germination after priming of normal and aged rice seeds. *Kor. J. Crop Sci.,* 45: 108-111.

Raun, S., Q. Xue, and R. Tylkowska. 2002. Effects of seed priming on germination and health of rice (*Oryza sativa* L.) seeds. *Seed Sci. Technol*., 30: 451-458.

Sharma P.C. and P. Kumar 1999. Alleviation of salinity stress during germination in *Brassica juncea* by pre-sowing chilling treatments to seeds. *Biol. Plant*., 42: 451-455.

Soon, K.J., C.Y. Whan, S.B. GU, A.C. Kil and C.J. Lai. 2000. Effect of hydropriming to enhance the germination of gourd seed. *J. Kor. Soc. Hort. Sci*., 41: 559-564.

Steel, R.G.D. and J.H. Torrie. 1984. *Principles and Procedures of Statistics. A Bionetrical Approach*. 2nd Ed. McGraw Hill Book Co. Inc., Singapoe. pp. 172-177.

Watkinson, J.I. and W.G. Pill. 1998. Gibberellic acid and presowing chilling increase seed germination of Indian grass (*Sorghastrum nutans* (L.) Nash.). *Hort. Sci*., 33: 849-851.

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