



Enhanced seed quality of late sown wheat (*Triticum aestivum*) at stress temperature via safe limits of seed hydro-priming

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ABSTRACT

Seed priming is an important physiological seed quality enhancement method. It provides a low-cost practical solution to seed germination under stress conditions. Abiotic stresses like low temperature and inadequate moisture during sowing season, many a times result in poor germination and poor plant stand leading to drop in crop yield. To harvest the full genetic potential, seeds must germinate and seedlings emerge quickly and uniformly throughout the field so that light, water and other soil nutrients may be utilized with maximum efficiency. However, this does not always happen in nature. In Punjab during sowing, prevailing low temperature, closure of canals or scanty rains result in poor germination and poor crop stand of late sown wheat. Therefore, keeping in view the prospects of this technique, the present investigations were undertaken to standardize the safe time limit of seed hydration and to register its effect on different physiological and molecular parameters in late sown wheat (*Triticum aestivum* L.) variety PBW 509. Different hydration durations applied were 8 to 10hr, 12 to 14hr, 14 to 16 hr and 16 to 18 hr. The results revealed that hydration for 16 to 18hr enhanced the seed quality parameters in terms of seed germination, emergence and seed vigour, Activity of hydrolyzing enzymes was elevated during initial 48hr of its germination. It is due to the efficient production and utilization of germination metabolites and better genetic repair.

Key words: Hydrolysing enzymes, Seed priming, Seed quality, Seed vigour, Stress tolerance, *Triticum aestivum*

Yield of late sown wheat (*Triticum aestivum* L.) varieties per unit area is less as compared to the early sown wheat varieties. This decline in yield is due to the abiotic stress like low temperature and inadequate moisture during cropping season that results in poor germination and poor plant stand ultimately leading to the poor yield and crop failure (Livingston and de Jong 1990). Sub-optimal field conditions (temp ranges 5 to 12°C) prevailing during sowing (December) lead to non-uniform crop stand which makes sometimes re-sowing necessary and this increases the financial burden on the farmers. Thus, rapid germination and emergence are essential for the successful crop establishment, for which seed priming has been reported to help rapid and uniform germination of seeds and increase seed tolerance to adverse environmental conditions (Harris *et al.* 1999).

Traditionally seed soaking is practised by farmers in which seeds are soaked before sowing to raise the successful crop sown during stress conditions (Heydecker *et al.* 1973). But the practice is being followed without the knowledge of safe limits of seed soaking duration (Harris 1996). The present experiment was therefore undertaken with the objective to standardize the optimum seed priming duration

for late sown wheat and registering its effect at molecular level.

MATERIALS AND METHODS

Seeds of late sown wheat variety PBW 509 having germination marginally less than Indian Minimum Seed Certification Standards (IMSCS) which is 85% in wheat, were used for the study which was carried out at the Seed Technology Centre, Punjab Agricultural University Ludhiana during 2009, 2010 and 2011. The pooled data of three years has been provided. The seeds were subjected to slow hydration treatment at 25°C ±1°C for 8 to 10 hr (T₁), 12 to 14hr (T₂), 14 to 16 hr (T₃) and 16 to 18 hr (T₄) along with untreated control (T₀) in triplicate. Hydration was performed as slow hydration using moist blotter papers. Treated seeds were surface dried at room temperature. Germination test was conducted in four replications of 100 seeds each by paper towel method. Daily radicle emergence was recorded for eight days, for the calculation of speed of emergence. Seedling vigor index I and vigor index II were calculated by using method suggested by Abdul-Baki and Anderson (1973). Fifty seeds of each sample were sown in two replications for the estimation of field emergence percentage. For calculation of electrical conductivity 50 sterilized seeds from each sample were dipped in double distilled water in four replicates in sterilized beakers

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Table 1 Seed Quality parameters as affected by different seed priming treatments in late sown wheat (Mean of three years)

Treatment	Germination (%)	Speed of emergence	Field emergence (%)	Vigor index I	Vigor index II	Electrical conductivity (mmhos/g)
T0	75	4.8	57	1921.79	10.59	0.030
T1	80.5	6.1	63	2554.33	15.88	0.027
T2	85.3	7.5	75	3002.09	19.67	0.026
T3	86.5	9.0	77	3066.90	23.59	0.018
T4	89.5	11.3	80.2	3460.99	26.45	0.015
CD (P=0.05)	5.39	3.14	12.34	729.37	7.78	0.09

incubated at 25°C ±1°C in an incubator for 24 hr. Electrical conductance was measured from the leachates obtained. Seed samples from hydro-primed seeds incubated at ±25°C were drawn at different intervals, i.e. 0hr, 12hr, 24hr and 48h after putting for germination. These seed samples were subjected to estimation of activity of hydrolyzing enzymes as per Duffus and Rosie (1973). Amylase activity was expressed as µg of starch hydrolysed min/g tissue.

RESULTS AND DISCUSSION

Primed seeds showed overall superiority in terms of germination percent as compared to un-treated seeds. The lowest percent was recorded in control, i.e. untreated seeds (75%) and highest was recorded in T4 (89.5%) when the seeds were hydro-primed for 16 to 18 hr. Primed seeds exhibited higher germination percent than that in non-primed seeds (Table 1). This result corroborates findings of Mandal *et al.* (1999) who observed higher germination percent in primed seeds of wheat. Accelerated germination in primed seeds is due to the increased activity of the degrading enzymes, such as α-amylase, synthesis of RNA and DNA, the amount of ATP and the number of mitochondria (Afzal *et al.* 2002). The difference in germination percent was attributed to the altered physiological condition of the embryo and due to the liberation of enzymes which rapidly increase the production of soluble food nutrients (Kattimani *et al.* 1999). The speed of emergence increased in a regular fashion from control to 16 to 18 hr and the maximum value was recorded in seeds hydro-primed for 16 to 18 hr (11.3) and it was (4.8) in control. Nagar *et al.* (1998) observed similar findings in maize. Hydro-priming of seeds for 16 to 18 hr was found significantly superior for other seed quality parameters, viz. seedling length, seedling dry weight, vigor index I, vigor index II and field emergence percent (38.68cm, 0.304g, 3460.99, 26.45 and 80.2%, respectively). However, the minimum values were recorded in untreated seeds for the same parameters (24.32 cm, 0.134g, 1921.79, 10.59 and 57%) respectively. The probable reason for early and higher germination of seeds soaked for 16 to 18 hr is the completion of pre-germinative metabolic activities, making the seed ready for radicle protrusion. Emergence enhancement also attributed to metabolic repair processes, a build of germination metabolites during 16 to 18 hr duration of hydro-priming (Afzal *et al.* 2002). The electrical conductivity from seed leachates was recorded minimum in seeds hydro-primed for 16 to 18 hr and maximum in un-

primed control seeds. Similar observations were reported by Afzal *et al.* (2002) who observed low electrical conductivity in hydro-primed seeds of maize. This may be attributed to the reason that hydro-priming followed by re-drying increases membrane stability.

Amylases are key enzymes that play a vital role in hydrolyzing the starch reserve of seeds, thereby supplying sugars to the developing embryo. Effect of hydropriming on water potential, i.e. driving force for water uptake during imbibition and the activity of α-amylase were examined in wheat and rice kernels by Andoh and Kobata (2002). In seeds of both wheat and barley, the activity of α-amylase after priming of 12 hr was found to be 2.7 and 2.8 times, respectively greater than that in non-primed seeds (Ashraf and Foolad 2005). Data pertaining to the activity of alpha amylase (Table 2) revealed that during germination durations it showed significant difference. In un-germinated seeds activity in terms of moles/min/g of alpha amylase ranged from 2.50 (T₀), 3.75 (T₁), 5.41 (T₂), 7.90 (T₃) and 10.83 (T₄). Seeds after 12hr of incubation for germination indicated 2.90 (T₀), 4.16 (T₁), 6.66 (T₂), 8.75 (T₃) and 11.25 (T₄) moles/min/g of alpha amylase activity. In seeds after 24 hr germination, its activity was 4.16 (T₀), 6.25 (T₁), 7.91 (T₂), 9.58 (T₃) and 12.08 (T₄) and in seeds after 48 hr it was 5.00 (T₀), 7.08 (T₁), 9.16 (T₂), 11.25 (T₃) and 13.75 moles/min/g of alpha amylase (T₄). Dell-Aquila and Tritto (1991) reported that primed seeds emerged 12 hr earlier than non-primed seeds which is due to increase in activity of enzymes such as amylase, protease and lipase which have great role in breakdown of macromolecules for growth and development of embryo that ultimately resulted in early and higher seedling emergence.

Table 2 Activity of alpha amylase as affected by different seed priming treatments among different germination durations (moles/min/g)

Treatment	Germination durations			
	0hr	12hr	24hr	48hr
T0	2.50	2.90	4.16	5.00
T1	3.75	4.16	6.25	7.08
T2	5.41	6.66	7.91	9.16
T3	7.90	8.75	9.58	11.25
T4	10.83	11.25	12.08	13.75
CD (P=0.05)	4.14	4.20	3.77	4.26

Table 3 Activity of β -amylase as affected by different seed priming treatments among different germination durations (moles/min/g).

Treatment	Germination durations			
	0h	12h	24h	48h
T0	3.33	5.40	7.10	7.88
T1	3.75	6.56	7.57	8.68
T2	7.90	8.68	11.60	12.09
T3	9.13	10.43	12.51	13.32
T4	13.50	12.50	13.75	15.01
CD (P=0.05)	5.20	3.56	3.72	3.78

The activity of β -amylase (Table 3) in un-germinated seeds in terms of moles/min/g ranged from 3.33 (T0), 3.75 (T1), 7.90 (T2), 9.13 (T3) to 13.50 (T4). While seeds after 12 hr of germination duration indicated the activity of β -amylase 5.40 (T0), 6.56 (T1), 8.68 (T2), 10.43 (T3) and 12.50 (T4). When quantified after 24 hr of germination it ranged from 7.10 (T0), 7.57 (T1), 11.60 (T2), 12.51 (T3) to 13.75 (T4) and after 48 hr of germination 7.88 in control, 8.68 (T1), 12.09 (T2), 13.32 (T3) and 15.01 moles/min/g in T4. It indicated maximum activity of amylases during this period, i.e. 48hr after putting wheat seed hydro-primed for 16-18 hr and kept for germination.

These studies indicated that out of different durations of seed priming treatments applied to late sown wheat variety PBW 509, pre-sowing seed hydration for 16 to 18 h was found to be the best as this duration significantly invigorated seed in terms of improved germination per cent, speed of germination, emergence per cent, vigor index I, vigor index II and reduced electrical conductivity and increased amylase activity.

It is therefore inferred that pre-sowing seed hydration for 16 to 18h is the safe limit of seed priming duration and it can be applied as pre-sowing seed treatment for invigorating the seed quality in late sown wheat seed.

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