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Short Communication

# Germination, α-amylase Activity and Protein Profiling of Calcium Salt Hardened Wheat (*Triticum aestivum* L.) Seeds during Low Temperature Stress

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***Key words*:** Wheat, Calcium salt, Seed hardening, Low temperature, Protein profiling

Late planted wheat, low temperature prevailing during germination substantially affects the germination and seedling emergence. In wheat uniform stand establishment and early vigor are the principal determinant of crop performance [1]. Seed germination and early seedling growth of wheat are the most sensitive stages to low temperature stress. Low temperature may delay the onset, reduce the rate, and increase the dispersion of germination events, leading to reductions in plant growth and final crop yield. Seed hardening with calcium salt is one the process by which thermotolerance can be induced in seed for better germination and establishment. The promising morpho-physiological and biochemical traits conferring thermotolerance in this crop is yet to be identified. $Ca^{2+}$ has been found to be involved in the regulation of responses of plants to environmental stresses [2-4]. Free $Ca^{2+}$ levels in plant cells often significantly increased under various stresses. The second-messenger $Ca^{2+}$ was found to be involved in regulation of many responses of plants to environmental signals. $Ca^{2+}$ level often shows significant changes in plant cells under the influence of various stress signals such as cold shock or mechanical stimulation [5-6]. Calmodulin (CaM) is a multifunctional receptor for intracellular $Ca^{2+}$ signal. It regulates a number of intracellular physiological processes. There are various signal transduction molecules related to stress responsive gene activation depending upon plant type, types of stresses. Some broad group of those are the Ca-dependent protein kinases (CDPKs), mitogen-activated protein kinase (MAPK/MPKs), Nitric oxide, sugar (as signaling molecule), phytohormones [7]. Low temperature is responsible for the up-regulation of several low temperature inducible genes, commonly referred as "heat shock genes" (HSGs) which encode HSPs and these active products are very much necessary for plant's survival under low temperature.

Wheat genotypes used for study were HD2329 (Thermosensitive) and HD2643 (Thermotolerant). Calcium salts were selected for hardening on the basis of the role of calcium in stress condition. $CaCl_2$ and $Ca(NO_3)_2$. Two concentrations of calcium salts were selected for seed hardening treatments i.e., 15mM and 20 mM and for control seeds were hardened with water. Temperature regimes (15 $^oC$ and 10 $^oC$) were selected on the basis of previous five-year meteorological data during the germination period of wheat crop during normal and late sowing. Seed of both the genotypes HD2329 and HD2643 were hardened with $CaCl_2$ and $Ca(NO_3)_2$ . Seeds were first sterilized with $HgCl_2$ 0.05% for 5 min and rinsed with distilled water 2-3 times and then soaked in respective concentrations of calcium salts and control (in $H_2O$) for 24 hours and then re-dried at room temperature for 72 hours and constant moisture content. Seed were stored in cool and dry place. Two sets of sterilized Petri plates were prepared with hardened and seeds placed in BOD at two temperature 10 $^oC$ and 15 $^oC$ in four replications of each concentration. Total soluble protein content was determined by Bradford [8] method. Solubilized protein were separated by SDS-PAGE [9]. Vertical slab gel electrophoresis system of Genei was used.

The profiling of proteins from the endosperm of wheat seeds at (15$^oC$) after the 7 days of germination has been presented in (Plate 1). Major bands of size ~45 kDa, and ~116 kDa are found to be prominent in 15 mM $CaCl_2$ hardened seeds of both the genotype (HD2329 and HD2643), whereas in $Ca(NO_3)_2$ hardened seeds of both genotypes, major bands are present in only at ~45 kDa protein size. In case of hydro hardened of both genotypes no major bands of equivalent sizes were observed. Minor bands of size ~100 kDa, ~37 kDa and~32 kDa are found to be present in $CaCl_2$ hardened seeds of both the genotypes but protein bands size of ~100 kDa is not present in $Ca(NO_3)_2$ hardened seeds. Among genotypes HD2329 and HD2643 hardened with 15 mM $CaCl_2$ a major bands of protein size ~97 kDa is found to be present in HD2329 and absent in HD2643. In $Ca(NO_3)_2$ seeds of the genotypes, minor bands of sizes ~37 kDa is observed to be present. In hydro hardened of both the genotypes any such bands were found to be not present.

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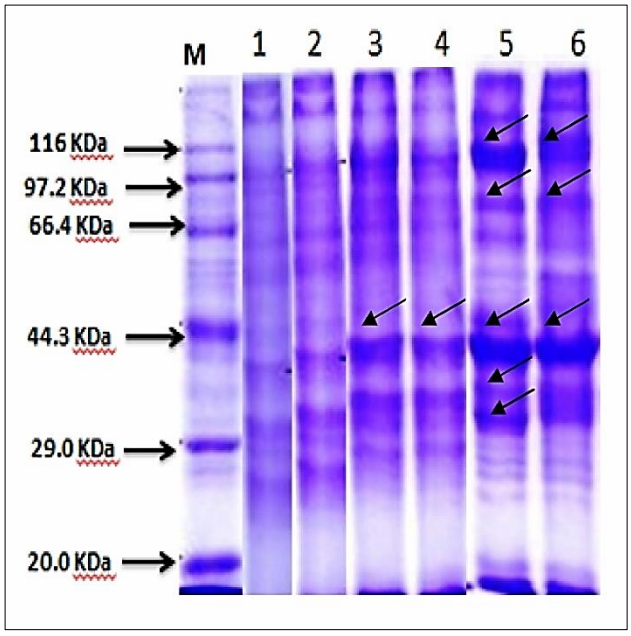
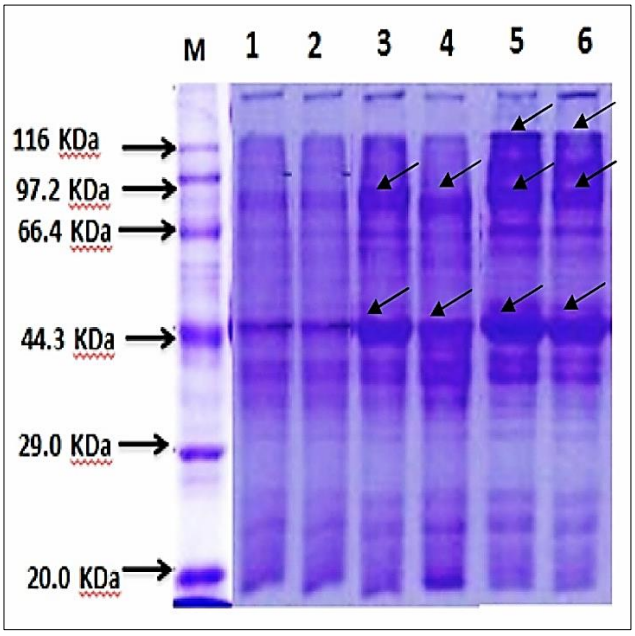
Profiling of proteins present in endosperm of germination (at 10 °C) seeds hardened with 20 mM $Ca(NO_3)_2$, 15 mM $CaCl_2$ and hydro hardened, has been presented in (Plate 2). Results revealed that protein of around size ~45 kDa and ~100 kDa were found to be present as broader bands, in the $CaCl_2$ HD2329 and $CaCl_2$ HD2643 and towards lesser size in $Ca(NO_3)_2$ HD2329 and 20 mM $Ca(NO_3)_2$ HD2643 hardened seeds, the bands were (~100 kDa and~45 kDa) were found to be less prominent in comparison to both $CaCl_2$ hardened seeds. Another major band of ~116 kDa is found to be present in $CaCl_2$ hardened seed of both the genotypes but not present in $Ca(NO_3)_2$ and hydro hardened of both the genotypes HD2329 and HD2643.

Germination of seedlings basically depend on seed reserve, moisture content in seed, enzyme activity and hormonal signaling etc. these are internal factors but some other external factors also affect early growth of seedlings like, biotic and abiotic stresses. Low temperature is one of the factors which reduce germination and early growth of seedlings. Due to late harvesting of rice, wheat sowing also delayed. Delayed sowing of wheat is one of the factors due to which wheat seed germination and crop establishment experiences low temperature. Wheat seed hardening increased their germination percentage in a field experiment conducted in Varanasi (U.P.) [10]. Seed hardening with $CaCl_2$ can induce seed germination and growth of seedlings because it helps in improving the activity and stability of $Ca^{2+}$-ATPase under 2 °C low temperature, which is a key factor in the development of cold resistance of winter wheat [11]. $CaCl_2$ has the potential to maintain α- amylase activity due to the characteristic feature of α-amylases in their requirement of calcium ions for activity and structural stability. All known α-amylases contain a structurally conserved calcium-binding site [12-13] and one or more additional calcium –binding sites have been identified depending on the origin of α-amylase. It was reported that when exposed to cold conditions, the $Ca^{2+}$ concentration in cold-insensitive plants has been transiently increased, suggesting that $Ca^{2+}$ acts as a second messenger during cold acclimation [14]. In protein profile of endosperm major band of size ~45 kDa is a vacuole membrane-associated calcium-binding protein, present in 15mM$CaCl_2$ hardened seeds (Plate 1). The presence of a ~45 kDa calcium-binding protein in vacuole-enriched membrane fractions was noted previously [15]. Another major band of size ~116 kDa proteins, which is a blue light, induced the phosphorylation of plasma-membrane-associated protein in dark-grown seedling. Minor bands which are presents in $CaCl_2$ hardened seeds, ~100kDa protein is heat-stable microtubule-associated protein, ~32 and ~37kDa are associated with cold regulated proteins, which are present in 15mM $CaCl_2$.




M = Protein marker (GeNei™ broad gauge)
1 = Control + V1,
2 = Control + V2,
3 = 20 mM $Ca(NO_3)_2$ + V1,
4 = 20 mM $Ca(NO_3)_2$ + V2,
5 = 15 mM $CaCl_2$ + V1,
6 = 15 mM $CaCl_2$ + V2

Plate 1 Effect of $CaCl_2$ (15 mM) and $Ca(NO_3)_2$ (20 mM) on endospermic protein profile (using SDS-PAGE) of wheat genotypes at 15°C




M = Protein marker (GeNei™ broad gauge)
1 = Control + V1,
2 = Control + V2,
3 = 20 mM $Ca(NO_3)_2$ + V1,
4 = 20 mM $Ca(NO_3)_2$ + V2,
5 = 15 mM $CaCl_2$ + V1,
6 = 15 mM $CaCl_2$ + V2

Plate 2 Effect of $CaCl_2$ (15 mM) and $Ca(NO_3)_2$ (20 mM) on endospermic protein profile (using SDS-PAGE) of wheat genotypes at 10°C

The major bands of size ~45kDa observed to be present in response to 15mM $CaCl_2$ seed hardening treatment of genotype HD2329, (minor bands of sizes corresponding to ~116 kDa, ~45 kDa proteins) but not in HD2643. These proteins may correspond to calcium-binding protein in vacuole-enriched membrane fractions was noted previously [15]. These new proteins may be associated with hydrolysis of aleurone layer intact proteins which are giving bands in endospermic aliquot. Minor bands which present in $CaCl_2$ hardened seeds of genotype ~100 kDa protein is heat stable microtubule-associated protein. ~32 kDa and ~37 kDa are associated with cold regulated proteins. Further studies proteins induced in response to seed hardening with calcium salts would help in identifying them using specific antibodies against the proteins.

## SUMMARY

Wheat (*Triticum aestivum* L.) is an important cereal crop for majority of world's populations. It is the major staple food of about two billion people (36% of the world population). Because of late sowing of wheat crop faces low temperature stress during germination stage. Seed hardening with calcium salt is one the process by which thermotolerance can be induced in seed for better germination, growth and yield. $Ca^{2+}$ has been found to be involved in the regulation of responses of plants to environmental stresses. Calcium salts namely calcium chloride ($CaCl_2$) and calcium nitrate ($Ca(NO_3)_2$) were used for present investigation. Results showed that 15mM $CaCl_2$ was more efficient in up regulating of HSPs and other proteins during low temperature. Seeds hardened with 15mM $CaCl_2$ can influence tolerance against cold temperature during the germination.

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