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BRIEF COMMUNICATION

Chilling induced oxidative stress in germinating wheat grains as affected by water stress and calcium

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Abstract

Wheat (*Triticum aestivum* L.) plants were subjected to mild water stress during grain filling at milk (early, medium, and late) and dough (early, soft, hard) stages. The grains harvested from stressed plants were subjected to low temperature stress of 10 °C for 24 h in presence or absence of 1 mM $CaCl_2$, and embryos were examined for oxidative injury. The embryos of grains water stressed at milk and soft dough stages showed lowest contents of H_2O_2 and malondialdehyde and highest membrane stability index, ascorbic acid content, and activities of catalase, ascorbate peroxidase, and superoxide dismutase as compared to control embryos or water-stressed at other stages. Presence of Ca^{2+} in the medium reduced H_2O_2 and malondialdehyde content and increased ascorbic acid content, and catalase, ascorbate peroxidase and superoxide dismutase activities.

Additional key words: ascorbate peroxidase, catalase, hydrogen peroxide, malondialdehyde, superoxide dismutase, *Triticum aestivum*.

The grain development in wheat is one of the most sensitive stages in relation to abiotic stresses (Ferris *et al.* 1998, Viswanathan and Khanna-Chopra 2001). Water stress has been known to affect the quality and quantity of wheat grains (Blum 1998, El Hafid *et al.* 1998). The grain filling in wheat occurs in distinctive phases as early-, medium-, late-milk, early-, soft-, and hard-dough. We hypothesized that each stage will respond differently to water stress to affect the performance of grains exposed to subsequent stress during germination. The late sown wheat in India experiences chilling stress during germination and seedling growth. Cytoplasmic calcium has been reported to increase in response to chilling stress (Sebastiani *et al.* 1999) and is suggested to have a key role as a stress sensor and transducer (Sanders *et al.* 1999). Water stress in wheat has been reported to elevate oxidative metabolism and induce damage to membranes (Sairam and Srivastava 2000, Sairam *et al.* 2001). Keeping these in view, the response of embryos of wheat grains, water stressed at different stages during filling, was examined under chilling stress in the presence of calcium.

Wheat (*Triticum aestivum* L.) genotype VL 616 was raised in first week of November in earthen pots having sandy loam soil and N, P and K was applied at recommended dose of 120, 60 and 60 kg ha^{-1}, respectively. The plants were irrigated regularly till the beginning of stress treatment. Water stress was applied by withholding water for 5 d at each of these stages: early-, medium- and late-milk, early-, soft- and hard-dough. The soil moisture was tested at the end of the treatment in each case and was found to be 25.1 % for control and 17.4 % to 18.1 % for stress treatments. The grains of each treatment were carefully harvested from basal part of the spike of main tiller at maturity and mass of grains was recorded for each treatment. The calcium content in the grains was estimated using flame photometer (Jackson 1962). The grains were germinated under 10 °C in dark in 10 cm glass Petri dishes having 3 cm^3 distilled water or 1 mM calcium chloride. The opted concentration of calcium chloride was based upon preliminary experiment involving 0.5, 1, 2, and 5 mM concentrations, evaluated for their efficacy on membrane stability in wheat seedlings growing under chilling stress. After 24 h,

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Abbreviations: APO - ascorbate peroxidase; ASC - ascorbic acid; CAT - catalase; MDA - malondialdehyde; MSI - membrane stability index; SOD - superoxide dismutase.
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embryos were excised carefully from the grains and used for investigation of ascorbic acid and hydrogen peroxide contents (Mukherjee and Choudhuri 1983), lipid peroxidation as malondialdehyde content (Heath and Packer 1968), ascorbate peroxidase (Nakano and Asada 1981), catalase (Teranishi *et al.* 1974), superoxide dismutase activitiy (Dhindsa *et al.* 1981) using *Shimadzu* (Kyoto, Japan) UV-visible spectrophotometer. The membrane stability index was measured with conductivity meter (*Elico*, Hyderabad, India) according to Premchandra *et al.* (1990).

A significant reduction in grain mass was observed due to water stress at all the stages (Table 1). The decrease from early milk stage (EM) to soft dough stage (SD) was progressive and significant. Hard dough stage (HD) was less affected. Stress at reproductive phase reduces the translocation of assimilates and nutrients to developing grains (Corbellini *et al.* 1998, Ahmadi and Baker 1999) resulting in decreased grain mass. The calcium content in the grain was also reduced considerably due to stress. The medium-milk (MM), late-milk (LM) and early-dough (ED) stages showed minimal content of Ca^{2+}. A reduction in Ca^{2+} partitioning into seeds under water stress was noticed in soybean (Sorooshzadeh *et al.* 1999). Membrane stability index (MSI) was higher in embryos of milk stages and maximum at ED stage followed by decline at later stages. Malondialdehyde (MDA), hydrogen peroxide (H_2O_2) and ascorbic acid (ASC) contents were low in embryos of MM, LM and ED stages and increased thereafter in embryos of dough stages. As ASC is involved in removal of H_2O_2 catalyzed by ascorbate peroxidase (APO), its decrease seems to be concomitant with higher activity of APO at milk stages. The activities of catalase and superoxide dismutase (SOD) increased in embryos of milk stages and were highest at LM stage. Catalase activity was relatively higher than APO and SOD activities. The presence of calcium during germination reduced the oxidative damage especially in milk stages as indicated by high MSI, low MDA and H_2O_2 contents and higher activities of antioxidants.

Table 1. Effect of short-term mild water stress during grain filling on grain mass [g], contents of calcium [mg g^{-1}(d.m.)], ascorbic acid, H_2O_2, and malondialdehyde [μmol g^{-1}(d.m.)], activities of ascorbate peroxidase [μmol(ascorbate oxidised) s^{-1} g^{-1}(f.m.)], catalase [μmol(H_2O_2 reduced) s^{-1} g^{-1}(f.m.)], and superoxide dismutase [U s^{-1} g^{-1}(f.m.)], and MSI [%] of germinating embryos under chilling stress. Wheat plants were water stressed at various stages of grain development. Controls were regularly irrigated. The harvested grains were given low temperature stress at 10 °C for 24 h during germination in the presence (+Ca) or absence (–Ca) of 1 mM $CaCl_2$ and embryos were examined for oxidative injury. LSD (P = 0.05) for ascorbic acid: 0.21, H_2O_2: 0.25, malondialdehyde: 13.4, catalase: 0.06, ascorbate peroxidase: 0.04, superoxide dismutase: 0.05. Means ± SE, n = 3.

Parameter		EM	MM	LM	ED	SD	HD	Control
Grain mass		62.2 ± 1.12	60.1 ± 1.17	57.2 ± 1.09	54.4 ± 1.11	52.2 ± 1.21	59.4 ± 1.13	64.7 ± 1.26
Calcium		0.20 ± 0.02	0.10 ± 0.017	0.09 ± 0.0014	0.10 ± 0.011	0.19 ± 0.014	0.25 ± 0.016	0.28 ± 0.013
ASC	+Ca	13.5 ± 0.56	4.60 ± 0.85	4.20 ± 0.92	10.2 ± 0.86	14.6 ± 0.82	14.1 ± 0.75	14.6 ± 0.91
	–Ca	11.8 ± 0.85	9.40 ± 0.56	7.30 ± 0.95	8.10 ± 0.56	13.2 ± 0.85	15.2 ± 0.93	16.7 ± 0.58
H_2O_2	+Ca	2.10 ± 0.11	0.91 ± 0.15	0.48 ± 0.14	1.54 ± 0.12	2.10 ± 0.13	2.40 ± 0.12	4.10 ± 0.12
	–Ca	2.80 ± 0.12	2.10 ± 0.21	1.80 ± 0.2	3.90 ± 0.15	2.60 ± 0.12	2.80 ± 0.14	3.10 ± 0.16
MDA	+Ca	260 ± 5.6	180 ± 6.2	125 ± 7.1	150 ± 7.4	286 ± 8.1	263 ± 7.2	270 ± 5.9
	–Ca	280 ± 5.4	210 ± 5.6	188 ± 7.4	216 ± 5.9	250 ± 6.3	235 ± 5.6	240 ± 5.9
APO	+Ca	0.73 ± 0.03	0.95 ± 0.04	1.06 ± 0.05	1.13 ± 0.04	0.20 ± 0.05	0.22 ± 0.03	0.31 ± 0.04
	–Ca	0.60 ± 0.05	0.75 ± 0.06	0.90 ± 0.06	0.90 ± 0.05	0.34 ± 0.06	0.31 ± 0.04	0.40 ± 0.03
CAT	+Ca	1.10 ± 0.14	1.60 ± 0.12	2.40 ± 0.13	1.80 ± 0.14	1.10 ± 0.13	0.80 ± 0.14	0.20 ± 0.12
	–Ca	0.80 ± 0.15	1.30 ± 0.12	1.80 ± 0.13	1.10 ± 0.13	0.70 ± 0.15	0.50 ± 0.12	0.40 ± 0.12
SOD	+Ca	0.41 ± 0.021	0.47 ± 0.024	0.61 ± 0.023	0.56 ± 0.024	0.32 ± 0.021	0.24 ± 0.024	0.28 ± 0.021
	–Ca	0.32 ± 0.020	0.42 ± 0.03	0.52 ± 0.024	0.47 ± 0.023	0.39 ± 0.021	0.29 ± 0.022	0.32 ± 0.023
MSI	+Ca	42 ± 1.12	51 ± 1.13	60 ± 1.15	62 ± 1.11	45 ± 1.23	41 ± 1.18	28 ± 1.15
	–Ca	38 ± 1.24	44 ± 1.13	51 ± 1.12	52 ± 1.41	41 ± 1.21	37 ± 1.23	32 ± 1.12

The oxidative injury due to chilling stress was found to be less in embryos of water stressed grains especially at milk stages. It appears that developing embryos exposed to mild water stress may get 'hardened' by accumulating antioxidants which may be conserved and carried over and activated at the time of future stress while the developed embryos (dough stages) may lack this ability hence show more oxidative damage. A prior exposure to water stress confer some degree of tolerance in tissues against chilling stress also by elevation of intracellular calcium (Kitagawa and Yoshizaki 1998) which offers protection against stress by stabilizing membranes (Abdel-Basset 1998) and through activation of specific protein kinases, whose mechanism of action is

yet to be defined (Cowan *et al.* 1997). According to Knight *et al.* (1998), altered Ca^{2+} responses due to previous stress encode a memory of Ca^{2+} 'signatures' that help in acclimation to subsequent stresses. We observed low Ca^{2+} content in the grains of milk stages which on the contrary were more tolerant to oxidative injury and were more sensitive to exogenous application of calcium to stimulate the activity of antioxidants. The grains of dough stages despite having high endogenous calcium showed more oxidative damage to embryos and were less responsive to exogenous Ca^{2+}. A higher tissue Ca^{2+} content alone may not be sufficient to induce protection but its intracellular concentration and functioning with stress related metabolism is more important (Sanders *et al.* 1999). In the present study, Ca^{2+} could partially ameliorate the oxidative damage in embryos by stabilizing the membranes and detoxification of oxidative molecules by increasing the activity of antioxidants especially catalase and ascorbate peroxidase and less so by superoxide dismutase. The activity of SOD was found to be relatively lower than APO and catalase implying that H_2O_2 may be dominating the oxidative injury and embryos may largely depend upon latter enzymes for its removal. In a recent study, SOD was reported to be insensitive to Ca^{2+} (Jiang and Huang 2001) but we observed some stimulation in SOD activity in the presence of Ca^{2+} in embryos of milk stages. In embryos of grains not exposed to water stress, Ca^{2+} added to increase the oxidative injury due to chilling indicating that exogenously applied Ca^{2+} is being perceived as osmotic stress in 'unhardened' tissues where it may act as a cytotoxin (Kauss 1987). Thus our studies conclude that wheat grains show stage-specific sensitivity to water stress and a mild stress of short duration to growing grains during embryo development may impart partial protection towards subsequent damage due to chilling during germination. Ca^{2+} could mitigate some damage of oxidative stress by activation of antioxidants but its effect was also stage-specific.

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