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Enhanced wheat germ agglutinin accumulation in the germinating embryos of wheat (*Triticum aestivum* L.) appears to be a general stress response

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The effect of osmotic-, heat- and salinity-stress on accumulation of wheat germ agglutinin (WGA) in the germinating embryos of wheat (*Triticum aestivum* L.) was studied. Imposition of different stresses correspondingly resulted in differential enhancement in WGA levels; the effect being maximum during the initial 4 h period. Exposure of germinating embryos to salt-stress resulted in highest accumulation of WGA. Our studies suggest that WGA enhancement in the germinating embryos appears to be a general stress response, and is differentially regulated by different stress conditions.

WHEAT germ agglutinin (WGA) is a *N*-acetylglucosamine specific lectin and is synthesized in developing and germinating embryos[1–3], roots and meristematic part of leaves of wheat[4]. WGA is the best characterized of all the gramineous lectins[5] and its accumulation in different tissues of wheat is enhanced by different stress conditions[3,6–8]. Although based on *in vitro* studies, WGA has been implicated in dormancy[3] and plant defence[9], but these roles fail to account for its characteristic biochemical properties, its predominance in growing and meristematic tissues, and its osmotic- and drought-stress inducibility. Therefore, based on earlier and our own studies, a novel *in vivo* role of this lectin in scavenging of free radicals, especially under stressful conditions, was proposed[8]. Since other stress conditions, viz. heat and salinity have also been reported to elevate the production of free radicals[10], and if WGA is involved in their scavenging, enhanced accumulation of WGA is also expected under these conditions as well. To test this possibility and to gain insight into the proposed role of WGA as a free-radical scavenger, WGA accumulation in the embryos germinated under osmotic-, heat-, and salinity-stress conditions was studied at regular intervals.

Wheat (*Triticum aestivum* L. cv PBW-154) seeds obtained from Department of Plant Breeding, Punjab Agricultural University, Ludhiana (Punjab), were washed and surface-sterilized with 1% (w/v) mercuric chloride followed by 70% ethanol for two min each. Seeds were thoroughly rinsed and imbibed in sterile deionized water for 6 h. After imbibition, 100 seeds in ten replicates each, were placed on sterile Whatman no. 1 filter papers in petri plates and incubated in the seed germinator at 25°C after irrigating with different solutions. Control seeds were irrigated with deionized water whereas osmotic- and salt-stresses were imposed by irrigating the seeds with 0.75 M mannitol and 0.41 M NaCl solutions, respectively. Osmotic potential of both the solutions, as calculated by von't Hoff equation, was –1.86 MPa. Effect of temperature stress was studied by incubating the plates at 35°C. Embryos were collected at regular intervals of 4 h for up to 24 h of germination. Samples were immediately frozen and stored in liquid nitrogen till further analysis.

Total acid soluble proteins (TASP) were extracted from 50–60 embryos by grinding in 50 mM HCl (3 ml g^{-1} fresh wt) according to Singh *et al.*[3] and estimated by Lowry's method[11]. WGA was quantified by enzyme-lined immunosorbent assay (ELISA) with polyclonal anti-WGA antibodies (Sigma Chemical Co., USA) in eight replicates in the presence of *N*-acetylglucosamine as described earlier[3]. Westren blotting was performed according to Bhaglal *et al.*[8] Ten µg of TASP were resolved on 15.5% discontinuous SDS-polyacrylamide gel electrophoresis (SDS-PAGE) (ref. 12) followed by transfer onto nitrocellulose membrane (Hybond) using semi-dry western blot system (Pharmacia-LKB Multiphor II). WGA protein bands were detected by using WGA-specific rabbit polyclonal antibodies (Sigma Chemical Co., USA) and sheep anti-rabbit IgG-alkaline phosphate conjugate, with nitroblue tetrazolium and X-phosphate as the substrates.

Relative to control embryos, where the TASP (µg per embryo) increased up to 8 h, imposition of different stress treatments resulted in highest TASP accumulation during the initial 4 h of germination (Figure 1).

*For correspondence.

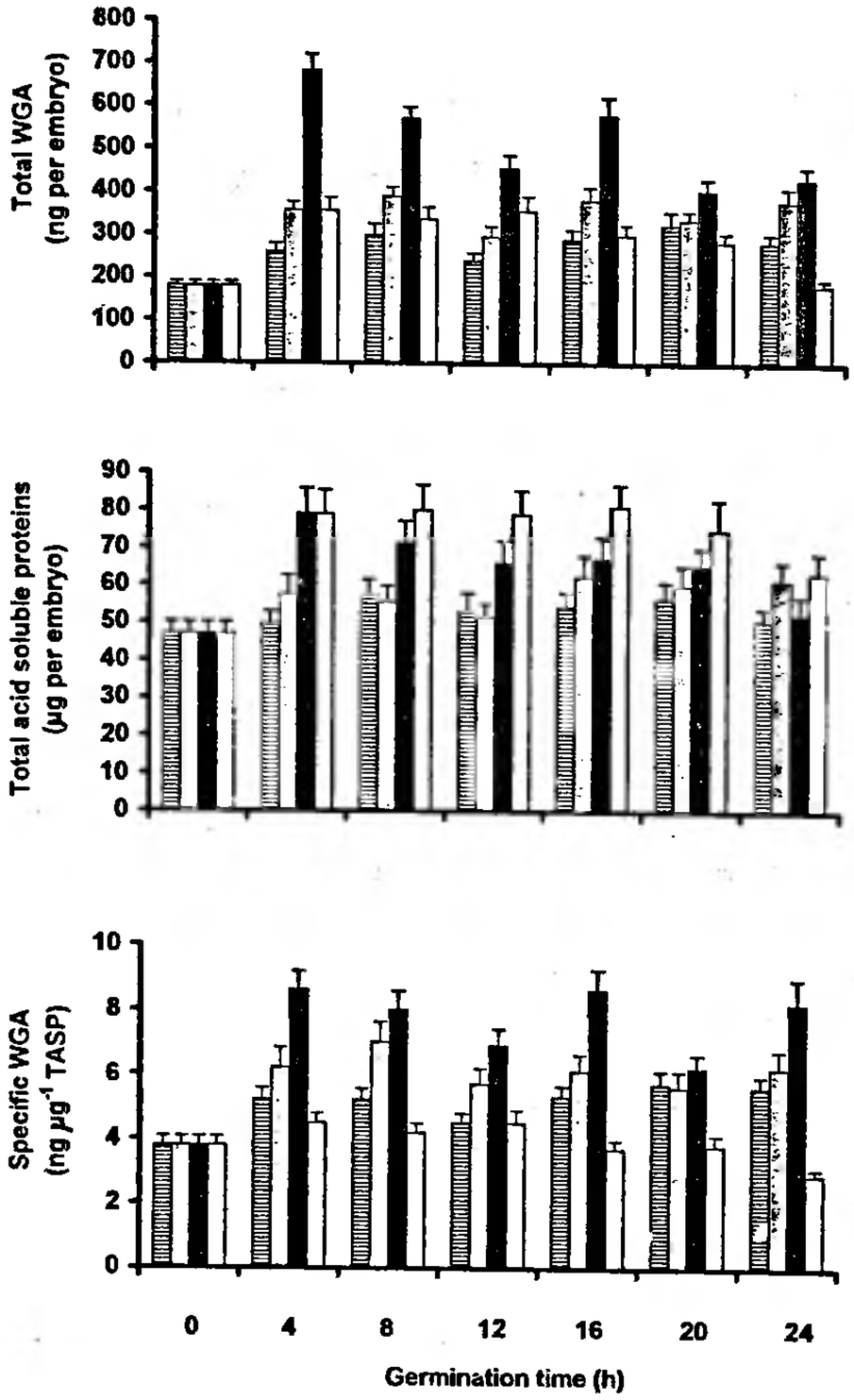

Figure 1. Effect of different stress treatments on total WGA content, total acid-soluble proteins (TASP), and specific WGA content of germinating wheat embryos. The data represent the mean ± SE of three experiments; control (▤), osmotic stress (□), salt stress (■), temperature stress (□).

Although in the initial 4 h, both high salt- and heat-stress caused almost identical increase in the TASP, the TASP content of the salt-stressed embryos showed a gradual decline with germination. During the initial 4 h of germination, total WGA content of the embryos under all stress conditions was higher than the control, with highest WGA content being observed in response to salt stress (Figure 1). Whereas enhanced total WGA content in response to osmotic- and salt-stress was also due to specific synthesis of WGA during the initial 4 h of germination, WGA synthesis in response to temperature stress was induced as part of overall protein synthesis since total WGA content increased without any concomitant increase in specific WGA under these conditions. After an initial increase, the total WGA content of the embryos exposed to salt- and heat-stress registered a substantial decline after 24 h of gemination. Total WGA content of the embryos germinated under osmotic stress, after an initial increase at 4 h, maintained almost constant level throughout the sampling period.

Immunoblotting studies were carried out to determine the extent of cross reactivity of the anti-WGA antibodies

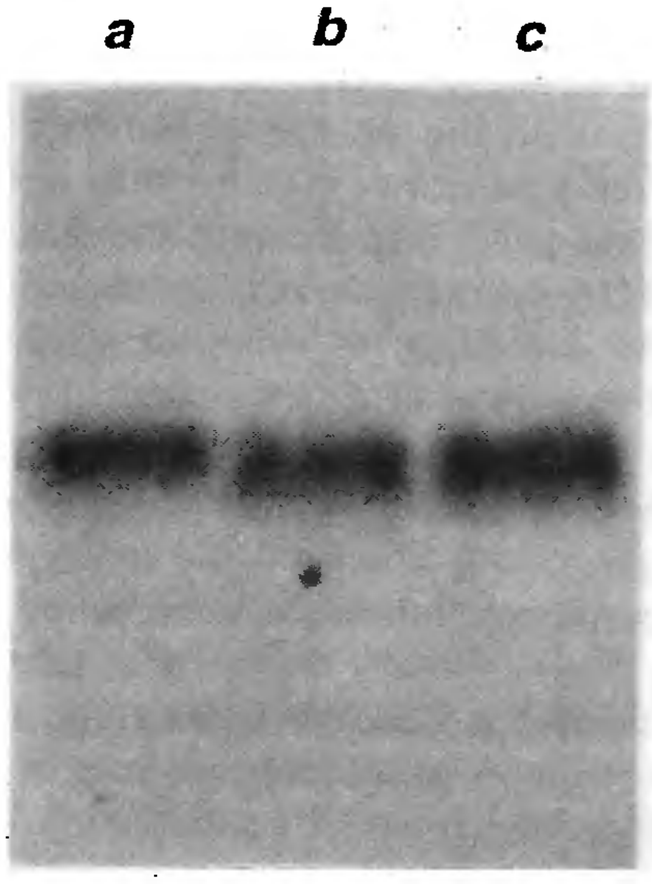

Figure 2. Western blot detection of pure WGA (lane **a**) as compared with control (lane **b**) and salt-stressed (lane **c**) embryos at 4 h after germination. Each lane was loaded with 10 µg of TASP and resolved on 15.5 % SDS-PAGE, transferred to nitrocellulose and probed with antibodies against WGA.

with other proteins. Presence of a single band of 18 kDa which co-migrated with the standard WGA implied lack of detectable cross-reactivity of anti-WGA antibodies (Figure 2).

In view of the recently proposed role of WGA in free-radical scavenging, especially under drought-stress conditions[8], we wanted to study the effect of other stress treatments, which enhance free-radical generation, on WGA accumulation. In the present study, imposition of different stress conditions resulted in differential enhancement in WGA accumulation in the germinating embryos. Despite equal osmotic potential (–1.86 MPa), irrigation with 0.41 M NaCl resulted in higher WGA content, on both protein and embryo basis, compared to 0.75 M mannitol solution thus signifying differential regulation of WGA gene expression by salinity- and osmotic-stress, respectively. The consistent pattern that emerged from this study was that the wheat embryos accumulated maximum WGA during the initial 4 h of germination (Figure 1) which is also the most active phase of WGA synthesis[2] and that the imposition of different stress conditions enhanced the steady-state levels of WGA during this period thus suggesting stage × stress interaction. Per embryo increase in WGA content implied *de novo* synthesis of WGA under stress conditions. These observations suggest that enhanced WGA accumulation, at least in the germinating embryos, may be a general stress response. WGA synthesis in the germinating wheat embryos has been attributed to the pre-existing mRNAs rather than *de novo* transcription and its rate of synthesis after being higher in the initial stages decreases with germination[2]. Stress-induced in-

crease in WGA levels in the initial 4 h of germination could be due to increase in ABA levels[13,14] which enhances the half-life of the already existing lectin mRNA, as reported in rice[15], rather than enhanced transcription of the gene. It is also possible that, as reported earlier[16], WGA degradation at latter stages of germination may be exceeding its synthesis, thus causing an overall decrease in total WGA content. Further studies on changes in WGA levels in response to stress imposition in embryos at different stages of germination, which are in progress, will elucidate the molecular basis of stage × stress interaction in the germinating embryos of wheat.

Although direct evidence is lacking, our observations lend further support to its proposed role in free-radical scavenging[8] as higher WGA accumulation in germinating embryos in response to salt-, osmotic- and heat-stress may be a part of the adaptation process for protecting the cells against free radicals, since a major cause of cellular damage due to stress is the production of free radicals[10].

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Artificial seed technology: Development of a protocol in *Geodorum densiflorum* (Lam) Schltr. – An endangered orchid

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The research communication reports the production of artificial seeds through encapsulation of protocorm-like bodies (PLBs) of *Geodorum densiflorum* (Lam) Schltr. – an endangered orchid taxon of Terai Hills, North-eastern Himalaya. 30-day-old PLBs were encapsulated in sodium alginate. Germination and regeneration capacity of the encapsulated seeds were tested by germinating such seeds in modified Knudson C (KnC) medium supplemented with coconut milk 15% (v/v), peptone (2 g l^{-1}), 6-benzylaminopurine (2 mg l^{-1}), and α-napthaleneacetic acid (1 mg l^{-1}). 88% Germination was recorded. Artificial seeds stored at 4°C for 120 days showed no reduction in viability. Non-encapsulated PLBs showed no viability after 30 days at 4°C. Artificial seeds showed 28% viability when directly transferred to nonsterile soil condition after incorporating food preservative and fungicide in its encapsulating gel.

PRODUCTION of artificial seeds has unravelled new vistas in plant biotechnology. The artificial seed technology is an exciting and rapidly growing area of research in plant cell and tissue culture. The idea of artificial seeds was first conceived by Murashige[1] which was subsequently developed by several investigators. Initially, the development of artificial seeds had been restricted to encapsulation of somatic embryos in a protective jelly. It had been considered that the induction of somatic embryogenesis (SE) and/or pollen embryogenesis which genetically differs from zygotic embryogenesis, is the prerequisite for the preparation of artificial seeds. Their induction has been reported in a number of cereals, millets, tuberous plants, vegetables, and other commercially important plants like soybean, mustards, coffee, tobacco, and cotton. However, because of certain inherent problems, the rate of production of uniform and high quality embryos is much lower as a result of which the preparation of efficient and quality seeds has been successful in only a few crop plants like carrot[3] and alfalfa[4].

Recent advances in the area have revealed that besides somatic embryos, encapsulation of cells and somatic tissues obtained following tissue culture techniques has

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