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The Roles of Germin Gene Products in Plants Under Salt Stress

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1. Introduction

The members of plants response various internal and external signals differently. The responses of plants to biotic and abiotic stress factors involve biochemical, physiological, morphological and developmental changes. Among the various circumstances salt stress is particularly under extensive studies due to low salt tolerance of crop plants. Germin and germin-like gene products were previously announced to be involved in various aspects of plant development such as plant defence, embryonic development and they are responsive to biotic and abiotic stress including salt. The responses of germin and germin like genes to salt stress are found to be various in different plants.

The salinity of soil is an important problem in agriculture, particularly since the majority of crop plants have low salt tolerance. The response of plants to salt stress is a complex phenomenon that involves biochemical and physiological processes as well as morphological and developmental changes (Flowers et al., 1977; Greenway & Munns, 1980). The identification of genes whose expression enables plants to adapt to or tolerate to salt stress is essential for breeding programs, but little is known about the genetic mechanisms for salt tolerance. One approach in clarifying the molecular mechanisms involved in salt stress is to identify the genes whose levels change as a result of salt stress. In this aspect, Hurkman et al. (1989) reported that in barley, gene regulation is altered by salt stress and the levels of translatable mRNAs change with salt treatment. Among the salt stress responsive gene products, germin and germin-like proteins (GLP) were identified (Caliskan, 1997; Hurkman et al., 1989). Cereal germin protein is a homopentameric apoplastic glycoprotein whose synthesis is associated with the onset of growth in germinating wheat embryos (Lane, 1991). Germin genes and their proteins were first detected in germinating cereals (Grzelczak et al., 1985), but subsequently, germin-like proteins were also identified in dicotyledonous angiosperms (Michalowski and Bohnerd, 1992), gymnosperms (Domon et al., 1995) and mosses (Yamahara et al., 1999). Germins are suggested to be a member of "superfamily" which comprises various growth-related genes (Dunwell et al., 2000).

Cereal germin proteins display strong oxalate oxidase activity (Lane et al., 1993), an activity that generates one mole of H_2O_2 and two moles of CO_2 from the degradation of oxalic acid. It is reported that H_2O_2 might act as a signaling molecule at low concentration (Luthell, 1993) or a component of cell wall modifications at high concentrations (Showalter, 1993). Another germin-like protein isolated from the cells of a moss, *Barbula unguiculata*, was

shown to have manganese superoxide dismutase activity (Yamahara et al., 1999). Germin genes and proteins have been shown to be associated with various aspects of plant development (Caliskan, 2000; Lane, 2002) such as defense system (Berna and Bernier, 1999; Donaldson et al. 2001), embryonic development (Caliskan and Cuming, 2001), photoperiodic oscillations (Ono et al. 1996), and hormonal stimuli (Berna & Bernier, 1997). The accumulation of germin gene products in wheat and barley seedlings in the presence of NaCl was analyzed previously, but little is known about the possible role of germin gene products during salt stress (Hurkman et al., 1991, 1994; Hurkman & Tanaka, 1996a; Berna & Bernier, 1999).

In this paper, we have brought together the results of our studies and the results of other researchers who are working in the field of germin, germin-like proteins and their enzymatic activities.

2. The stress factors and the early plant development

Plants are a distinct kingdom of organisms that possess unique properties of reproduction, development, physiology and metabolism. The early development of plants is also quite different from that of animals in various ways. The main features that distinguish embryogenesis in angiosperms from the related pathways in animal systems are (1) the process of double fertilization and the subsequent interaction between embryo and endosperm, (2) the totipotency of certain plant cells by contrast with the uniqueness of the zygote in animals, (3) the activation of large numbers of zygotic genes during very early stages of embryogenesis, (4) the formation of apical meristems which produce the basic body plan of the "adult" plant, (5) the absence of a germ line established early in development, (6) the absence of cellular migration during embryo development. Due to the above discrepancies, studies of animal systems (*e.g. Drosophila*) may not be directly applicable to plants.

Embryogenesis is a developmental stage which covers the time period beginning with the formation of the zygote and ending with the formation of a mature embryo within a seed. Embryogenesis has a central role in the life cycle of flowering plants since it results in the production of a structurally and functionally organized miniature adult plant which is called the embryo. The conversion of an ovule into a seed is triggered by fertilization. The cells in male (anther) and female (ovary) organs of the flower undergo meiotic and mitotic divisions which give rise to a male gametophyte (pollen grain) and a female gametophyte (embryo sac). The pollen grain contains two sperm cells, whereas the embryo sac contains only a single egg cell in association with a number of accessory haploid cells. Upon penetration of the embryo sac by the pollen tube, one of the sperm nuclei fertilizes the egg cell, while the other unites with the two additional haploid nuclei of the embryo sac to form the triploid endosperm. Therefore, unlike animal fertilization, plants undergo a double fertilization process. As the ovule develops into a seed after fertilization, the zygote enters a pathway of cell division and differentiation to produce an embryo. At the end of this pathway, the embryo is composed of an embryonic axis that bears the root meristem at one end, the cotyledon(s) and the shoot meristem at the other end. Based on the cotyledon number, angiosperms are defined as monocotyledonous or dicotyledonous plants. The endosperm tissue may comprise the bulk of the mature seed, as a storage tissue, as in the case of cereal grains, or it may be reduced in size and importance in seeds where the principal storage reserves are kept within the cotyledons, in dicotyledonous seeds.

In seeds of dicotyledonous plants, the embryo is typically comprised of an axial region (root and shoot) and two large cotyledons. The other organs are rudimentary, at best. By contrast, in monocotyledonous seeds the embryonic organs are usually highly differentiated at maturity. Primary root, numerous leaf primordia, shoot apex and coleoptile are well developed in cereal embryonic axes. Moreover, in monocots the cotyledon is often modified to form a relatively diminutive scutellum; whilst the endosperm is a large structure at maturity and is the main storage tissue. In wheat embryogenesis, five characteristic morphological stages have been identified by Rogers & Quatrano (1983). Fertilization and the subsequent stages of cell proliferation and differentiation end in the production of a functionally mature embryo. This embryo can germinate immediately, but normally it remains in a state of suspended growth (dormancy). The early stages of plant embryo development are characterized by cell division and morphogenesis. For instance, during wheat embryogenesis (~7 weeks), the zygote (1 cell) gives rise to a mature embryo (~10^5 cells) and the DNA content of the organism increases correspondingly (10^5-fold) (Lane, 1988). This is followed by a period of cell specialization and embryonic maturation in preparation for coming dormancy and germination periods.

Dormancy is not a period of metabolic inactivity because this period is characterized by the massive accumulation of storage materials in the appropriate tissues or organs, causing an increase in size and weight, the maturation stage. There are some proteins which characterize this stage of embryo development such as Em "**E**mbryo **m**aturation" and LEA "**L**ate **E**mbryogenesis **A**bundant" proteins. LEA polypeptides are non-storage proteins and synthesized during stage 3 of embryogenesis. It has been suggested that non-storage LEA proteins might be involved in the acquisition of desiccation tolerance, during stage 4, which is a characteristic feature of the development of most seeds (Galau et al., 1986). The most abundant cytosolic wheat embryo protein is the Em protein in mature embryos (Grzelczak et al., 1982). Levels of the Em polypeptide increase rapidly in the cytosol and continue throughout desiccation of the embryo. Upon subsequent imbibition, both the mRNA and its polypeptide product are rapidly degraded, and completely disappear by 24 hours post-imbibition (Cuming, 1984). It was suggested that Em could have a limited storage function but was more likely to be associated with the acquisition of desiccation tolerance of embryo during embryogenesis and it is considered to be an example of the "group-1" LEA polypeptides (Butler & Cuming, 1993; Galau et al. 1986). The dormant condition is relieved only by the final stage in seed development, dehydration. Dehydration is an essential part of embryo development, being both necessary to maintain seeds in a viable form for long time periods and to effect a switch in their pattern of development. Seed desiccation also effects a permanent change in metabolism, in that, upon rehydration, synthesis of proteins associated with development ceases, and that associated with germination and seedling establishment commences. During the maturation period, the embryo accumulates reserve compounds in an effectively anabolic lifestyle. As a result of dehydration, the embryo is potentiated to germinate immediately upon the rehydration, its metabolic activity switched towards the massive catabolism of storage compounds to support growth.

During the early cell division stage of development, very little storage material is synthesized. Most of the synthesis of storage proteins, carbohydrates and lipids takes place during subsequent cell expansion. The final stage of seed development is characterized by the loss of water during maturation drying, when reserve synthesis stops and the seed becomes metabolically inactive. Drying of the seed enables the embryo to be a dispersal structure that is resistant to environmental disturbances and that remains quiescent until

conditions are suitable for germination and growth. Germination of the seed starts with the imbibition of water, resulting in the rapid resumption of protein synthesis, using those components of the synthetic complex conserved within the dry seed. Upon emergence of the radicle from the seed, germination is complete and the seedling becomes established, with the rapid elaboration of the clearly defined root and shoot regions. During the early development (2 days) of a mature wheat embryo (<1 mg) into a seedling (> 10mg), the rapid and striking (> 10 fold) increase in mass is accompanied by limited cell division (Lane, 1988), and must therefore be a consequence of cell expansion driven by water uptake. This change in the size of the cells must necessarily involve significant adjustments in the structure of the extra cellular matrix (ECM) (apoplast, cell walls) in the growing seedling.

The role of plant growth regulators in the germination process is uncertain although it is known that exogenous application of abscisic acid inhibits germination in many species, while gibberellic acid promotes germination. However, the relationship between these experimental phenomena and the embryo's endogenous hormonal metabolism remains obscure (Bryant & Cuming, 1993). Gibberellic acid has a well-established role in the mobilization of the seed's nutrient reserves in the cereals during germination, and the mobilization of reserves is clearly essential for successful seedling development in the longer term, but for the first 48 hours of germination, very little of the endosperm reserves is mobilized, and the nutrients utilized must therefore derive from the stores held within the embryo (principally lipids and storage proteins). When a seed is provided with water and oxygen at an appropriate temperature, water is taken up by imbibition in order to initiate the germination. Protein synthesis must play an important role in germination and early plant development. It is important for the growth of the embryonic axis and in the synthesis of hydrolytic enzymes as well as for the other cellular processes involved in the mobilization of food reserves. Germination can therefore be recognized as a catabolic stage because of this breakdown of reserves. Dry seeds contain residual mRNAs which were synthesized during embryogenesis. The mRNAs translated during early germination are soon replaced as germination progresses by the synthesis of new mRNA and of additional proteins. Not surprisingly, investigations of these new proteins have characterized many of them to be hydrolytic enzymes including amylases, proteases and lipases (for the mobilization of starch, protein and oil respectively) (Fincher, 1989; Bewley & Black, 1985).

A close examination of the *in vitro* translation products from embryo mRNA sets isolated at different times of development has led to the identification of classes of genes expressed with characteristic patterns. The majority of genes are described to be produced constitutively, such as those encoding actins and tubulins (Dure, 1985), the remaining sets are expressed in a stage specific manner (Goldberg et al., 1989). Jendrisak (1980) reported that *de novo* mRNA synthesis was needed for resumption of growth in germinating wheat embryos. He suggested that the importance and significance of stored mRNA in the dry seed and its role in germination was minimal, since stored mRNA was by itself insufficient to allow for the resumption of germinative growth. As an imbibing seed undergoes the rapid transition from quiescence to vigorous metabolism many biochemical and physiological processes are activated. An obvious expectation is an increase in the expression of many different genes whose products provide for the higher levels of steady-state metabolic rate reached during this period (Caliskan et al., 2003). Whilst some of the biochemical events might be peculiar to this period, the majority would be expected to be related to normal growth-maintenance. Undoubtedly there are genes coding for proteins that control the activation of genes whose products provide for the higher steady-state

metabolic rates seen. Moreover, there are probably a number of genes that code for proteins involved in the more complex developmental aspects of germination and seedling establishment. Seedling establishment needs the mobilization of stored reserves, involving such supporting tissues as the cotyledons and endosperm in dicotyledons seeds and the aleurone layer and endosperm in seeds of monocots. These tissues will undergo increases in expression of growth-maintenance genes, but there will be major activation of growth regulatory genes in accord with the role of these tissues in supporting germination and growth, *e.g*. the postgerminative production of hydrolytic enzymes by storage tissues.

An early event upon rehydration of seed tissues is an increase in protein synthesis and the proliferation of cellular organelles. The first consequence of imbibition may be an increase in ATP to an appropriate threshold level allowing this basic metabolic process to commence (Moreland, 1974). Axis germination would then proceed through an initial phase during which substrates and enzymes already present in the dry seed provide the required biochemical activity, to a final phase in which the primary interactants are mobilized substrates and proteins synthesized from newly transcribed mRNAs. After germination of both wheat and barley, the cells of the aleurone layer (the secretory cells that surround the nonliving starchy endosperm) synthesize several hydrolytic enzymes, including, characteristically, α-amylase which increases its activity 100-fold after germination. It was shown that activation of α-amylase is under control of GA (gibberellic acid). In addition, a number of other hydrolytic activities are induced in the barley aleurone by GA, including nucleases, β-glucanases, and proteases (Bewley & Marcus, 1990).

Although the induction of hydrolytic enzyme activities is essential during germination, plants need to synthesize some other proteins and enzymes which are required for a successful seedling development and autotrophic life. Typically, these are those associated with the establishment of photosynthetically competent tissues, and include all those structural proteins and enzymes found within the chloroplasts, in aeriel parts of the plant. However, although these gene products are needed for sustained growth, they can not be considered as germination-specific. A number of uncharacterized polypeptides have been shown to be uniquely associated with the onset of germination. One such protein has been isolated in 4 day old pea seedlings and it is called "C3 protein". This displays a shoot-specific pattern of expression (de Vries et al., 1983). The absence of its mRNA in any other organs and also the insensitivity of its accumulation to illumination have indicated that the protein was not related to photosynthesis but rather is associated with shoot elongation. The gene for phytochrome is also stimulated in expression in germinated pea axes (Konomi et al., 1987). The amount of phytochrome mRNA and that of the mRNA for glutamine synthetase (glutamate-ammonia lyase EC 6.3.1.2) (clone 2A2; Datta et al., 1987), peaks shortly after the onset of increased embryo fresh weight, suggesting that these mRNAs can be regarded as functioning primarily in the maintenance of steady-state growth. Glutamine synthetase gene expression is also abundant in soybean seedling root tips (where the predominant activity is cell division and early elongation) and in rapidly growing soybean cell cultures (very little or no differentiation occurs), a conclusion again consistent with the notion that this gene is particularly involved in growth-maintenance, presumably through its role in nitrogen assimilation. By contrast, two other soybean seedling mRNAs have been identified (clones 4D7 and 2E2; Datta et al., 1987) which are not expressed in seedling root tips, nor in growing cells in culture, and thus were presumed to be involved in more subtle aspects of seedling development. Another defined mRNA, whose expression commences with the onset of germination, occurs in the wheat seedling. The product of this mRNA was

called germin to indicate that it was associated in germinative growth (Grzelczak & Lane, 1984). Germin (G) was not found in immature embryos or in mature, dry embryos before their hydration, nor was it found in mature wheat organs (Lane et al., 1992).

2.1 The possible roles of germin gene products

Germination is a critical period in plant development in which the rapid growth of the embryo is driven by water uptake. The water content of a mature wheat embryo in an ungerminated grain is less than 5%; upon germination it rises to about 60% in less than 1 hour. Between 1 and 5 hours of imbibition there is no further in fresh mass or water content. A "secondary water uptake" phase then raises the water content from 60% to 85% by 24 hours postimbibition (Marcus, 1969). Biochemical analysis of wheat embryo germination indicates that there is only a limited accumulation of new gene products during germination (Thompson & Lane, 1980) and until recently, just one had been observed to signal the onset of early plant development (Lane & Tumaitis-Kennedy, 1981). The synthesis and translation of the mRNA for a soluble protein initially called "g" (Thompson & Lane, 1980) and later called "germin" (Grzelczak & Lane, 1984) was concomitant with the initiation of growth in germinating wheat embryos (Rahman et al., 1988). The appearance of germin mRNA was coincident with the secondary water uptake phase and the accumulation of the germin protein reached its highest level between 24-48 hours postimbibition (about ~ 40ng/embryo in 40 hour germinated embryos (Grzelczak et al., 1985).

Germin was first detected in germinating cereals, but subsequently, germin-like proteins were also identified in a protist (Lane et al., 1991), dicotyledonous angiosperms (Michalowski & Bohnert, 1992; Hofte et al., 1993; Delseny et al., 1994; Heintzen et al., 1994), and gymnosperms (Domon et al., 1995). Wheat germin is a relatively rare water-soluble glycoprotein (less than 0.1% of the mass of soluble proteins in germinating wheat embryos) which in homogenates exists as an oligomeric complex even which does not dissociate when analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) unless boiled in the presence of the detergent (Grzelczak & Lane, 1984). Three isoforms of germin have been defined. These are G, G' and ΨG (pseudogermin). The G and G' oligomers are water soluble, resistant to digestion by pepsin and to dissociation in aqueous SDS solutions at room temperature (Grzelczak & Lane, 1983, 1984). Amino acid composition and sequencing indicate that G and G' have the same apoprotein and differ only in that G has two further N-acetylglucosamine units attached to the basic core of its N-glycans. (Jaikaran et al., 1990). Both forms of germin were defined as glycoproteins based on their positive Schiff reaction, which is a characteristic of glycoproteins as well as by the incorporation of radioactive glucosamine, mannose and fucose into the germin oligomer following labelling, *in vivo*. The carbohydrate content of germin is about 10% by weight (Lane et al., 1987; Jaikaran et al., 1990).

One of the most striking characteristics of germin is its resistance to proteolysis: it was found to be resistant to digestion by a broad range of proteases, including pepsin. By exploiting this property, milligram quantities of highly purified protein may be prepared from pepsinized supernatants of an homogenate, or simply from an aqueous wash fraction of germinated embryos, in a very short time (Grzelczak & Lane, 1984). It seems possible that the oligosaccharide substituents in germin play a role in the remarkable stability of protein towards protease (Lane et al., 1987). In addition to the germination related germin isoforms (G and G'), an antigenically related homotetrameric form of germin (pseudogermin - ΨG)

has also been detected, particularly in the cell walls of immature wheat embryos at a time when the maximum cellular enlargement associated with embryogenesis and maturation is occurring (20-25 dpa) (Caliskan et al., 2004). Unlike germin, pseudogermin is thermostable; the oligomer remains undissociated even when boiled in the presence of SDS, so long as non-reducing conditions (*e.g.* in the absence of mercaptoethanol) are maintained (Lane et al., 1992). Physicochemical studies indicated that germination-related germin (G) was a homopentamer and its oligomeric mass was calculated to be ~ 130 KDal when measured by three different methods. Therefore a molecular mass of 130/5 = 26 KDal was calculated for the monomer (McCubbin et al., 1987).

A virtually full length germin cDNA was isolated (Rahman et al., 1988) and its polynucleotide sequence was determined (Dratewka-Kos et al., 1989). This germin cDNA has been used as a probe to indicate that germin is encoded by a multigene family which has ~5 copies on chromosome 4A, ~3 copies on chromosome 4B and ~9 copies on chromosome 4D in hexaploid wheat. This cDNA was also used to screen a genomic wheat DNA library and the nucleotide sequences of a 2.8 kbp fragment (gf-2.8) from one genomic clone, and of 3.8 kbp fragment (gf-3.8) from another clone were determined. The protein coding-regions of these two genes are intronless and 87% identical (Lane et al., 1991). The association of germin isoforms with apoplast cause speculation that germin may have a role in embryo-specific desiccation/hydration processes (Lane et al. 1991). It was observed that even if germin was purified free of other proteins, there was a selective association between germin and the highly substituted glucuronogalactoarabinoxylans (HS-GGAX) (Jaikaran et al., 1990) whose synthesis was reported to be closely associated with cell wall extension in cereals and grasses (Gibeaut & Carpita, 1991). Upon this observation, it was suggested that germin-like oxalate oxidase might play a crucial role in early plant development by controlling integration of cell wall extension, for example by transporting extending wall material (*e.g.* HS-GGAX) into the cell wall to support extension and at the same time promoting cross-linking between wall polymers to restrict extension (Lane, 1994).

In gymnosperms, three germin-like proteins were discovered among the extracellular proteins produced by cells grown in liquid tissue culture. These proteins were found to be present in cultures which retained embryogenic potential, but to be absent in non-embryogenic cell lines. These proteins had high N-terminal amino acid sequence homology with other germins and were immunologically cross-reactive with an antiserum raised against the *apo*-protein component of cereal germin. Their molecular weight was about ~ 26 KDal. Pine germin-like proteins have not yet been shown to have oxalate oxidase activity (Domon et al., 1995). It was also demonstrated that wheat embryo callus induction by auxin is associated with a rapid accumulation of germin-like oxalate oxidase (Caliskan et al., 2004). Photoperiodic treatments of the short-day plant (SDP) *Pharbitis nil* seedling resulted in synthesis of a germin-like protein during darkness-induced flowering. This germin-like protein had a molecular mass of 22 KDal in SDS-PAGE analyses, and it reached the highest level of accumulation after the critical length of the dark period (~ 10 hours after the light was turned off). The cotyledons and leaves, known to be the two major organs that perceive the photoperiod and produce the floral stimulus, were the only expression sites for this protein. Sequence analysis showed that the *Pharbitis nil* germin-like protein shared the highest homology a germin-like protein in another dicotyledonous plant, *Sinapus alba* (white mustard). No oxalate oxidase activity was found for *Pharbitis nil* germin-like protein (Ono et al., 1996).

The similarities between wheat germin and the barley germin-like polypeptides may imply that both of these proteins have some protective function during early plant development. In wheat embryos, germin increases significantly during seed germination. Based on the increase in carbohydrate synthesis during germination and the presence of adventitious arabinoxylans in wheat germin preparations this has prompted speculation that germin could have a function in cell wall expansion (Jaikaran et al., 1990). The identification of cereal germins as oxalate oxidases (Dumas et al., 1993; Lane et al., 1993) has caused us to modify this view (Caliskan & Cuming, 1998). The principal product of the degradation of oxalic acid is hydrogen peroxide which is a highly reactive compound known to be involved in several metabolic processes in higher plants. The discovery of its oxalate oxidase activity instantly suggested several specific ways in which germin might function. Specifically, linkage of the developmental appearance of cell wall bound germin (Lane et al., 1992) to oxalate degradation suggests that germin might have a role in cell wall reinforcement by producing Ca^{++} and H_2O_2 for pectic cross-linking and peroxidase mediated cross-linking of cell wall polymers respectively. Proteins could be cross-linked through tyrosine side-chains, lignin via -OH groups and carbohydrates via -COOH groups (Cassab & Varner, 1988; Showalter, 1993). The anchorage of at least a proportion of the germin-like oxalate oxidase in the cell wall with GGAX oligomers also implicates it in the cross-linking of these components within the cell wall matrix; notably, arabinoxylan comprises a substantial fraction of monocotyledonous cell walls, in which the oxalate oxidase activity of germin-like proteins is characteristic. Although germin-like proteins have been identified in dicotyedonous plants, these, for the most part, have not been shown to possess oxalate oxidase activity. An exception to this is the oxalate oxidase of *Beta vulgaris*, which has been found to be germin-like in its properties (a protease-resistant SDS-stable oligomer): notably, the cell walls of this species, and other members of the *Chenopodiaceae*, are more "cereal-like" than "typically dicotyledonous", being enriched in arabinoxylan content (Bacic et al., 1988). Germin synthesis also appears to be auxin responsive (Berna & Bernier, 1997). Typically, auxins stimulate cell wall loosening and bring about cell wall expansion. On the other hand, germin (oxalate oxidase activity) produces hydrogen peroxide which is believed to be required for the peroxidase mediated cross-linking reactions in the cell wall. Thus, it was suggested that germin synthesis might be associated with both initiation and termination of cell wall expansion in early plant development (Lane, 1994).

Germin-like oxalate oxidase is also a pathogen inducible enzyme (Zhang et al., 1995; Dumas et al. 1995; Hurkman & Tanaka, 1996b). The production of H_2O_2 (the "oxidative burst") is the primary response of some higher plants to pathogen (*Erisyphe graminis)* infection. H_2O_2 can be implicated in the development, differentiation, vascularization, defence and signalling processes of higher plants. The action of oxalate oxidase, in generating H_2O_2, could be an especially potent defence mechanism. The pathogen-responsive oxalate oxidase was different from both the commercially available barley root oxalate oxidase, and barley salt stress-induced root oxalate oxidase in its molecular weight. Commercial oxalate oxidase was reported, variably, to have a molecular mass 80 KDal in 8% SDS-PAGE (or, Dumas et al. (1995) report 100 KDal) whereas the pathogen-related oxalate oxidase is 100 KDal - 95 KDal. This may therefore represent a specific, individual member of the germin gene family. In a parallel study of transcripts induced in response to *Erisyphe* infection, two novel germin-like sequences were identified as pathogen-responsive (Zhang et al., 1995).

Dumas et al., (1995) demonstrated an increase in the activity of germin-like oxalate oxidase in association with the response of barley to *Erisyphe graminis*. In normally growing seedlings, oxalate oxidase activity was detected in a tissue specific manner in 3 day germinated seed (in roots) and 10 day germinated seedlings (in the residual coleorhiza). These activities were demonstrated to be associated with a germin-like protein by immunoblotting using anti-germin antibody. In 10 day old seedling coleoptiles a 26 KDal polypeptide reacted with anti-germin serum but appeared to lack oxalate oxidase activity. This suggested that an inactive form of oxalate oxidase could accumulate. On infection by the fungus, oxalate oxidase was induced in barley leaves (5 day), especially along the vascular bundles. This suggested that oxalate oxidase belonged to a class of proteins that responds to pathogen attack. The production of H_2O_2 by oxalate oxidase might be significant in two ways: (1) By further enhancement of the defence response (through induction of further plant defence responses as a signalling molecule), (2) Through H_2O_2 being used as a substrate by peroxidase to mediate cross-linking of cell wall polymers (e.g. lignin) in the hypersensitive response, causing the sealing of infected lesions (Dumas et al., 1995).

Similarly, in wheat, germin mRNA, germin and oxalate oxidase activity were induced in leaves of wheat upon infection with *Erisyphe* (6 day old seedling +2, 4, 6, 8 days after inoculation). The control leaves at the same age gave negative results. An increase in expression of peroxidase was also detected. These results reinforce the suggestions that germin activity has a role in plant defence through the local production of H_2O_2 for the hypersensitive defence response (Hurkman & Tanaka, 1996b). This suggests that the genes encoding germin-like oxalate oxidase might have potential in transgenic approaches to plant defence. Another peroxide generating enzyme, a fungal glucose oxidase, has been shown to enhance the resistance of transformed plants to fungal infection, when introduced as a transgene (Wu et al., 1995). In experiments designed to protect *Brassica napus* plants from the oxalate secreting fungus, *Sclerotinia* transgenic oilseed rape plants, transformed with barley oxalate oxidase, were found to express a 25 kDal protein reactive with anti-germin antiserum and to express oxalate oxidase activity which protected plants against potentially toxic applications of oxalic acid (Thompson et al., 1995). In a current study, 36 expressed sequence tags (ESTs) encoding GLPs from peanut (*Arachis hypogaea* L.) were identified. The purified AhGLP2 has displayed superoxide dismutase (SOD) activity in enzymatic assay, but not oxalate oxidase activity. It was reported that the SOD activity of AhGLP2 was stable up to 70A ^{o}C and resistant to hydrogen peroxide, suggesting that AhGLP2 might be a manganese-containing SOD and likely protects peanut plants from reactive oxygen metabolites (Chen et al., 2011). The rice germin-like protein (OsGLP1) being a cell wall-associated protein involved in disease resistance also revealed to possess superoxide dismutase (SOD) activity as recognized by heterologous expression in tobacco (Banerjee et al., 2010).

It was discovered that both germins and spherulins had statistically significant sequence similarity with plant seed storage globulin domains. The germins were clearly related both to seed globulins (Baumlein et al., 1995) and spherulins (Lane et al., 1991). On the basis of these similarities, it has been proposed there is a superfamily (groups of gene families encoding structurally related but functionally distinct proteins) of related genes encoding vicilins, legumins, SBPs (sucrose-binding proteins), germins and spherulins. SBPs are proteins associated with the plasma membrane and which have a role in sucrose transport, and it is known that spherulins have a function in the cellular desiccation process, including

osmotic regulation. It has been suggested that sucrose may serve as one of the principal agents in the acquisition of desiccation tolerance in seeds and other plant tissues, where the role of disaccharides in the assumption of a "glassy state" by the cytosol has been inferred (Leopold et al., 1992). The legumin-like 11S and the vicilin-like 7S seed proteins are synthesized and accumulated during seed maturation, and are stored in protein bodies in mature seeds. In the timing of their accumulation, and their regulation by agents such as abscisic acid, they are at least associated with the acquisition of desiccation tolerance by seeds, which occur during the maturation phase of seed development (Braun et al., 1996).

2.2 The enzymatic activity of germins: oxalate oxidase

Oxalic acid is one of the strongest organic acids with pKa values of 1.3 and 4.3 (Lane, 1994). Oxalic acid has a wide variety of industrial and household applications for instance it is used as an analytical reagent. Oxalic acid is also a constituent of cleaning solutions for removing paint, varnish, rust and ink stains as well as being used extensively in laundries as a scouring agent to remove excess alkalinity remaining in washed fabrics. It is also used for cleaning or bleaching wood and straw, as a chrome stripper and as a bleach in leather manufacture.

In plants, the highest oxalate concentrations commonly occur in the leaves and the lowest in roots. Meanwhile, the oxalate content of plants can vary according to their age, the season, the climate and the type of soil. Wide variations can occur in the oxalate content of plants. In some plants, such as rhubarb, oxalate content tends to increase as the plants mature, whereas, in other plants, e.g. spinach, sugar beet leaves, oranges, bananas, there is a large increase in oxalate content during the early stages of development, followed by a decrease as the plants mature.

Although the origin of oxalic acid in plants is controversy, the early studies on plant organic acids, including oxalic acid, yielded evidence that the production of these acids was related to photosynthesis and carbohydrate metabolism. Myers (1947) noted that oxalate concentration in rhubarb leaves increased in parallel with the growing seasons, being correlated with the seasons of most active photosynthesis. Later, experiments on rhubarb and Begonia indicated that oxalic acid was not a direct product of photosynthesis but it was synthesized from precursors synthesized in the photosynthetic pathway (Stuta & Burris, 1951; Tavant, 1967).

Now, it is well known that oxalic acid is synthesized via several major pathways. Although glyoxylate and L-ascorbic acid appear to be the major precursors of oxalic acid in plants (Davies & Asker, 1983; Yang & Loewus, 1975), some other possible pathways have been reported. Glucose, acetate and some acids of the tricarboxylic acid cycle were determined to be involved in oxalate biosynthesis in red beet roots and young spinach leaves (Chang & Beevers, 1968). Moreover, glycolic and isocitric acids (Millerd et al., 1963), oxaloacetic acid (Chang & Beevers, 1968) are known to donate carbon to oxalic acid in plants. The relative significance of these metabolites as precursors of oxalic acid has not been established (Wagner, 1981).

The enzymes involved in the synthesis and degradation of oxalic acid are of interest. In lettuce, two enzymes identified to have a role in the oxidation of glycolate and glyoxylate to oxalic acid were lactate dehydrogenase and glycolate oxidase (Davies & Asker, 1983). Since oxalic acid could be formed from several precursors, there is no clear evidence of its synthesis and deposition site. However, it was observed that the primary site of deposition of oxalic acid formed from L-ascorbic acid was the vacuole in barley which is a low level oxalic acid accumulator (Wagner, 1981).

Most of the plants and animals produce oxalic acid, and it is of interest that they share some common pathways of oxalic acid synthesis. Oxalate may be present in the tissues as the free oxalic acid, as soluble sodium and potassium salts or as insoluble calcium oxalate crystals. Calcium oxalate crystal formation in animals is generally considered to be pathological. The pathological role of oxalic acid in the formation of urinary stones in animals and humans has been known since the early 18th century. For example, excess consumption of oxalate-rich foods leads to hyperoxaluria which is recognized as a key risk factor for calcium oxalate stone formation (Sharma et al., 1991). Furthermore, there is evidence that high ascorbic acid intake increases urinary oxalate levels which could lead to the formation of calcium oxalate stones in the kidneys and other regions of the urinary system (Roth & Breitenfield, 1977). Oxalic acid is regarded as an undesirable component of our food not only because it raises the risk of urinary stones but also because it sequesters calcium, which is one of the essential ions, as insoluble calcium oxalate. The toxicology of oxalic acid in humans was reviewed by several researchers (Polson & Tattersall, 1959; Hodgkinson, 1977). In medicine, the knowledge of the oxalate concentration in blood and other body fluids can be very important in certain clinical situations such as primary hyperoxaluria (Pertrarulo et al., 1990). Various methods have been formulated for the assay of oxalate from different sources, for instance, gas-liquid chromatography, ion chromatography, high performance liquid chromatography, mass spectrometry and enzymatic determination methods have all been developed. Of these, the oxalate oxidase activity-based determination of oxalate has become very popular and is used widely because of its simplicity, specificity and sensitivity (Pundir et al., 1985).

Oxalic acid has usually been seen as an inert end product of metabolism and only plants have been reported to be able to metabolize oxalic acid and oxalates. However, recent studies indicate that the levels of oxalate are too high for the substance to only be an end-product of metabolism in animals (Emsley, 1994). Therefore, it has been suggested that there could be an oxalate oxidase pathway in animals which uses oxalate to produce H_2O_2, which could than be used to promote a "burst" of phagocytes: cells that engulf and break down foreign particles, cell debris and disease-producing microorganisms. Unlike animals, plants are highly tolerant of oxalic acid and oxalates. Oxalic acid and oxalates have been detected in varying quantities in all parts of most plants' leaves, leaf stalks, flowers, tubers and roots (Srivastava & Krishnan, 1959). It is well established that plants are capable of metabolizing oxalate by observing fluctuations in oxalate concentrations under certain conditions (Vincent & Harry, 1980), and enzymes degrading oxalic acid have been detected in numerous plants. Several functions have been proposed for the presence of oxalic acid in plants. It has been implied that oxalic acid might be related to ionic balance, since it can combine with various plant ions to form soluble or insoluble compounds. It was suggested that oxalate synthesis occurred to balance the excess of inorganic cations (represented by K^+, Na^+, NH_4^+, Ca^{++} and Mg^{++}) over anions (represented by NO_3^-, Cl^-, $H_2PO_4^-$, SO_4^{-2}) normally present in the plant - the ability of nitrate ions and chloride ions to inhibit oxalic acid oxidase activity in *Beta vulgaris* results in an accumulation of oxalate.

Calcium oxalate crystals were amongst the first objects observed in plants in the early days of light microscopy in the late 17th century (Lane, 1994). Insoluble calcium oxalate formation enables plants to control the concentration both of ionically active oxalic acid and calcium. Both of these molecules might have a toxic effect when accumulating in excess quantities. Thus plants could induce calcium oxalate crystal formation to remove excess oxalic acid or calcium. Although calcium is essential to biological growth and development, free calcium

at high concentrations is toxic to cells. So it was suggested that calcium oxalate precipitation serves to sequester excess calcium and remove it from active metabolism (Webb et al., 1995). The rapid induction of calcium oxalate crystal formation by calcium in Lemna plants suggests that the crystals may serve as a storage form for calcium for future needs (Hepher & Wayne, 1985). Further support for this came from the observation that in some plants the crystals appear to be dissolved during calcium deficient conditions, presumably to supply calcium for growth and cell maintenance (Franceschi, 1989). Calcium is required for the activation and/or stabilization of certain enzymes; for example plant cells need calcium to release peroxidases which are related to the control of cell elongation since they can rigidify walls by their cross-linking activity and their ability to participate in the formation of lignin. Thus, for this role they are under the control of cellular calcium levels (Sticher et al., 1981). One of the major roles of calcium in plant cells is its action in the formation of the middle lamella where Ca^{2+} ions form stabilising ionic bridges between pectin chains. Although it has been suggested that calcium oxalate crystals are a means of detoxifying excess oxalic acid, the fact that many plants are able to retain high concentrations of soluble and free oxalic acid within their vacuoles indicates that oxalic acid may not be particularly toxic to plant tissues. However, accumulation of oxalic acid may have some toxic consequences (for example in causing osmotic problems and destabilization of cells), unless it is readily metabolized (Raven & Smith, 1976).

It is well known that certain plant pathogenic fungi secrete oxalic acid as part of the process for invasion of plant tissues. For example, *Sclerotium rolfsii* Sacc., a fungus, causes diseases of plants in nearly 100 plant families. Considerable quantities of oxalate were detected in infected but not in healthy tissues. Oxalic acid produced by this pathogenic fungus played an essential role in its pathogenic capabilities (Maxwell & Bateman, 1968). Another pathogenic fungus infecting a wide range of plant species is *Sclerotinia sclerotiorum*. During infection, the fungus produces high levels of a necrosis phytotoxin identified as oxalic acid (Noyes & Hancock, 1981). The role of oxalic acid in the pathogenicity process is still unclear. However, oxalic acid may have a number of functions in the infection process including chelating calcium from the cell wall thus making the pectic fraction more available to fungal hydrolases, and providing an acid pH needed for maximum activity of the wall degrading enzymes released by the pathogenic fungus (Keates et al., 1996). Furthermore, it was suggested that oxalic acid produced by fungi played a key role in lignin biodegradation through its stimulation of lignin-degrading enzymatic activities (e.g. Mn-peroxidase activity) (Kuan & Tien, 1993). One part of the plant defence response to this may be the production of oxalate oxidase which is an oxalic acid degrading enzyme. A second response would be the induction of deposition of oxalic acid in the form of soluble or insoluble salt.

Oxalic acid and its salts, oxalates, are widely distributed within the cells and cell walls of plants and probably they play an important role in tissue metabolism. There are two possible enzymatic reactions for the degradation of oxalate in plants: (1) decarboxylation by oxalate decarboxylase (oxalate carboxy-lyase, EC 4.1.1.2) which catalyzes conversion of one mole of oxalate to one mole of CO_2 and formate, (2) oxidation by oxalate oxidase. Oxalate oxidation has been found to occur in fungi, mosses and higher plants, but the biological significance of oxalate oxidation is not yet clear. The discovery that germin, a protein marker of early plant development, is an oxalate oxidase suggested that oxalate oxidase, the enzymatic formation of H_2O_2, and Ca^{++} release from poorly soluble calcium oxalate might play an important role in metabolic regulation, particularly in cell wall modification during germination and seedling development.

Oxalate oxidase is an oxidoreductase (oxalate: oxygen oxidoreductase EC 1.2.3.4) which catalyzes the formation of one mole of H_2O_2 and two moles of CO_2 from one mole of oxalate and aerobic oxygen. Oxalate oxidase has been found in Pseudomonas sp. OX-53 (Koyama, 1988), *Tilletia controversa* (Vaisey et al., 1961), banana peel (Raghwan & Devasagayam, 1985), mosses (Laker et al., 1980), *Bougainvillea* leaves (Srivastava & Krishnan, 1962), barley seedlings and roots (Chiriboga, 1966; Pietta et al., 1982), spinach, beet stem and leaves (Obzansky & Richardson, 1983; Leek et al., 1972), and leaves and roots of CSH-1 and CSH-5 varieties of grain sorghum (Pundir & Nath, 1984; Pundir & Kuchhal, 1989).

An oxalate oxidase was purified from sorghum leaves with a molecular weight of 62 kDal and pH optimum of 4.3. This enzyme had maximum activity at 40 °C and it was insensitive to Na^+ . This property made it well suited for medical diagnostic use in the detection of oxalate in urine - the principal cause of kidney stone formation. The enzyme was suggested to be a flavoprotein on the basis of stimulation of its activity by FAD. However, neither the barley oxalate oxidase nor that of Pseudomonas sp. OX-53 was classified as flavoproteins (Pundir, 1991).

Of the other oxalate oxidases purified from plant sources, that from grain sorghum leaves (*Sorghum vulgare* L hybrid CSH-5) (Kuchhal et al., 1993) was active in a range of pH from 4-6, with an optimum of pH: 5 and optimal temperature of 40° C. The optimum pH for barley oxalate oxidase was pH: 3.2, and for mosses pH: 4, whilst the bacterial (Pseudomonas) enzyme was maximally active at pH: 4.8. The enzyme purified from grain sorghum leaves was heat sensitive, as it lost 80% of its activity when heated at 80 °C for 3 min whilst the inhibition of the enzyme by EDTA, and its stimulation by metal ions (Cu^{++}, Mg^{++}), led it to be subsequently classified as a metalloprotein and not a flavoprotein. Both the enzymatic activity and oxalate content showed progressive decrease with the advance of germination in sorghum. These data suggested that the high enzyme activity during initial growth was required for degradation of endogenous oxalate to form H_2O_2 which might play an important role in cellular regulation, such as glucose transport, glucose incorporation into glycogen, lipid synthesis, and release of Ca^{++} (Kuchhal et al., 1993).

The oxalate oxidase purified from leaves of grain sorghum hybrid CSH-5 was reported to have a molecular mass of 120 KDal and to be composed of 62 KDal monomeric subunits, unlike the enzymes purified from moss, barley, and banana peel, it was unaffected by Cl^-, in the physiological concentration range, which made it particularly suitable for urinary diagnostic tests, since the removal of Cl^- from urine, prior to oxalate assays was not required (Satyapal & Pundir, 1993). More recently, an oxalate oxidase has been purified from beet stems (*Beta vulgaris* L.) (Azarashvili et al., 1995). When the activity of this enzyme was determined as a function of pH and temperature, the optimal conditions were found to be at pH: 4 and 30 °C. This enzyme has been incorporated into an automated method for determination of urinary oxalate - a rapid (3 hours) and reliable procedure based on the measurement of H_2O_2 produced by oxidation of oxalate. The chromogen MBTH-DMA was used to measure the hydrogen peroxide produced (Obzansky & Richardson, 1983). Nitrate ions (at concentrations as low as 5x10-5 M) was identified as the sole factor in crude beet extracts responsible for the inhibition of oxalate oxidase activity in vitro (Meeuse & Campbell, 1959). This would lead to accumulation of oxalate in cells.

Barley seedling oxalate oxidase was purified by Sugiura et al. (1979). It was originally reported to have a monomeric molecular mass of 75 KDal and to assemble as a homodimer of 150 KDal. The optimal pH and temperature were found to be 3.2 and 37 °C, respectively. The enzyme was found to be extremely stable at temperatures up to 70 °C and to be strongly

inhibited by 2-mercaptoethanol and halogen ions (Cl^-, F^-). This enzyme appeared to differ in its properties from the sorghum leaf enzyme, which in addition to its different pH and temperature optima, and molecular mass, was not inhibited by halogen ions (Satyapal & Pundir, 1993).

Oxalate oxidase activity in plants may vary between species, and differ between organs and tissues of a plant even though these may have similar oxalate contents. For example, a close examination of germinated cereal embryos indicated that there are only modest differences between the oxalate contents of different varieties. However, there may be gross differences between their oxalate oxidase activities. For example, cold-tolerant maize (C0255) contains ~ 20 fold higher levels of oxalate oxidase activity than cold-sensitive maize (C0286), whilst in 6 day germinated wheat seedlings, the coleoptile and leaf have the similar oxalate content but the coleoptile has ~ 16 times higher levels of oxalate oxidase activity than the leaves (Lane, 2000).

Analysis of beet shows that the oxalate content of this dicotyledonous plant is ~ 10-fold greater than that determined in wheat, but that the oxalate oxidase/oxalate ratio is 100-fold smaller than for hexaploid wheat. Beet oxalate oxidase shares several properties with the wheat enzyme: typically the "germin-like" characteristic of resistance to SDS-denaturation, allowing its activity to be determined directly in SDS-PAGE gels. However it was sufficiently immunologically similar to be recognized by wheat anti-germin in Western-blots. By contrast, the sorghum oxalate oxidase, although derived from a monocotyledonous source did not have "germin-like" stability, but it did react weakly with the anti-serum raised against wheat germin (Lane 2000).

2.3 The expression of germin genes under salt stress

Although there are plenty of organisms need salt to carry out their life (Ozcan et al., 2006, 2007) excessive salt is a stress factor for most of the living beings. Salt stress is also an important agricultural problem, particularly since the majority of crop plants have low salt tolerance. The response of plants to salt stress is a complex phenomenon that involves biochemical and physiological processes as well as morphological and developmental changes (Flowers et al., 1977; Greenway & Munns, 1980). The identification of genes whose expression enables plants to adapt to or tolerate to salt stress is essential for breeding programs, but little is known about the genetic mechanisms for salt tolerance. One approach in clarifying the molecular mechanisms involved in salt stress is to identify the genes whose levels change as a result of salt stress.

Isolation and examination of the two germin genomic clones (Rahman et al., 1988) and the determination of the predicted amino acid sequences have revealed high homology with spherulin 1a/1b proteins of the slime mould *Physarum polycephalum* (Lane et al., 1991). The synthesis of these proteins occurs during spherulation: a transition leading to developmental arrest imposed by environmental conditions such as osmotic stress and starvation (Bernier *et al*., 1987), this similarity led to the suggestion that another possible function for germin might be related to the changing osmotic properties of cells. In support of this notion, synthesis of germin-like proteins was discovered to be altered upon salt stress in barley (Hurkman et al., 1991) and in the halophytic "ice plant", *Mesembryanthemum crystallinum*, (Michalowski & Bohnert, 1992). In salt-stressed barley, these proteins, like wheat germin, were resistant to protease and were glycosylated and heat stable. They were detected in barley roots (but not in tips) and coleoptiles but not leaf of 6 day seedlings. Their synthesis increased in roots upon salt stress, but decreased in coleoptiles. On the other hand,

it was observed that the synthesis of a germin-like protein in the ice plant declined after salt stress (Michalowski & Bohnert, 1992). Thus, these different studies implied that germin might represent a family of proteins of which individual members may have different biochemical functions related to changes of the osmotic properties of the cell.

Experiments based on the extraction of mRNA from different organs suggest that wheat and barley have different spatial distributions of germin mRNA expression (Hurkman & Tanaka, 1996a). The vascular transition region was reported to contain the highest levels of germin mRNA in wheat, whereas roots displayed the highest germin expression levels in barley seedling. It was additionally observed that salt stress caused an increase in germin mRNA in roots at an early developmental stage (3 days on 0.2M NaCl), whereas, in the whole seedling, salt stress cause the "normal" expression of germin to be prolonged, relative to that in control seedlings. Overall, it was concluded that germin gene expression in barley seedlings was developmentally regulated in a tissue-specific manner, and also, potentially, by various plant hormones (IAA, ABA). Interestingly, it was discovered that a decline in germin expression occurred after 3 days in control seedlings and after 4 days in stressed seedlings - in both case seedlings were at a similar developmental stage, as assessed by seedling weight (approximately 25 mg in each treatment). Thus one interpretation of the experimental data is that NaCl treatment prolongs germin gene expression for an additional 1 day indirectly, through a slowing of seedling growth (Hurkman & Tanaka 1996a).

Among the salt stress responsive gene products, germin and germin-like proteins (GLP) suggested to be a member of "superfamily" which comprises various growth-related genes were identified (Caliskan, 1997; Hurkman et al., 1989). Cereal germin proteins have strong oxalate oxidase activity (Lane et al., 1993), an activity that produces one mole of H_2O_2 and two moles of CO_2 from degradation of oxalic acid. It is reported that H_2O_2 might act as a signaling molecule at low concentration (Luthell, 1993) or a component of cell wall modifications at high concentrations (Showalter, 1993). Another germin-like protein isolated from the cells of a moss, *Barbula unguiculata*, was shown to have manganese superoxide dismutase activity (Yamahara et al., 1999). Germin genes and proteins have been shown to be associated with various aspects of plant development (Caliskan, 2000; Lane, 2002) such as defense system (Berna and Bernier, 1999; Donaldson et al. 2001), embryonic development (Caliskan, 2001), photoperiodic oscillations (Ono et al. 1996), and hormonal stimuli (Berna and Bernier, 1997). The accumulation of germin gene products in wheat and barley seedlings in the presence of NaCl was analyzed previously, but little is known about the possible role of germin gene products during salt stress (Hurkman et al., 1991; 1994; Hurkman & Tanaka, 1996a; Berna & Bernier, 1999).

Germin and germin-like proteins are suggested to be salt-responsive gene products and their response to salt stress seems to be various. For example, accumulation of germin mRNA is up-regulated during the growth of germinating barley seedlings in the presence of NaCl (Hurkman & Tanaka, 1996a). In contrast, it is reported that in ice plant (*Mesembryanthemum crystallinum*) the synthesis of GLPs declined after salt stress (Michalowski and Bohnert, 1992). On the other hand, it was reported that in wheat seedlings germin synthesis was remained unchanged in the presence of NaCl (Berna & Bernier, 1999; Caliskan, 2009). The addition of NaCl to the cells of moss, *Barbula unguiculata*, during the logarithmic phase increased both the *BuGLP* mRNA levels and total SOD activity of BuGLP, but decreased the SOD activity bound to the cell wall due to release of most of the SOD activity into the medium. On the other hand, the addition of NaCl to the cells during the

stationary phase hardly affected *BuGLP* mRNA levels or SOD activity levels bound to the cell wall. These results suggest that the induction of *BuGLP* gene by salt stress is caused by dissociation of BuGLP protein from the cell wall into the medium in the cells during the logarithmic phase (Nakata et al., 2002).

In situ RNA hybridization is one of the most powerful techniques developed for localizing the expression site of particular gene products at the cell, tissue and organ levels. This method is particularly useful in understanding the function of specific gene products in particular tissues and the relation between tissue function and its localization in the whole structure of an organ (Ranjhan et al., 1992). This technique was employed for analyzing the possible germin functions and it was shown that germin mRNAs synthesized in the cells of coleorrhiza in wheat seedlings and it was considered that the enzymatic activity of germin, oxalate oxidase, might play an important role in metabolic regulation, particularly in cell wall modification during germination and seedling development (Caliskan & Cuming, 1998). It is well known that stress factors alter the synthesis of gene expression. Indeed, upon salt stress the localization pattern of germin gene expression was changed (Caliskan, 2009). It is shown that although the water grown embryos and salt stress grown embryos accumulate the similar amount of germin mRNAs, the synthesis site of germin mRNAs are completely different from each other. In germinating wheat embryos the salt stress somehow caused germin mRNAs to be synthesized in coleoptile instead of coleorrhiza cells (Caliskan, 2009). It is possible to envisage that an explanation for the reasons of this shift will help us to have a better understanding of stress physiology of plants.

3. Conclusion

Although germin and germin-like proteins have been studied extensively since the 1980s, their biological importance and functions remain confusing. Furthermore, germin, germin-like proteins and oxalate oxidase have been found in a broad range of various plant species under different circumstances, and related with different aspects of plant development. For example, germin-like protein and oxalate oxidase enzyme activity have been identified in plants in relation to salt stress, pathogen infection, photoperiodic oscillations, germination and embryogenesis. It has also reported that germin and germin-like proteins and oxalate oxidase are responsive to various plant growth regulators such as auxin and abscisic acid. Germin-like oxalate oxidase therefore seems to fulfil some crucial functions in plants. In addition to these properties, recently it was suggested that germins and various other proteins which are involved in early plant development might belong to an evolutionarily ancient superfamily: cupin superfamily (Dunwell et al., 2000; 2008).

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5. References

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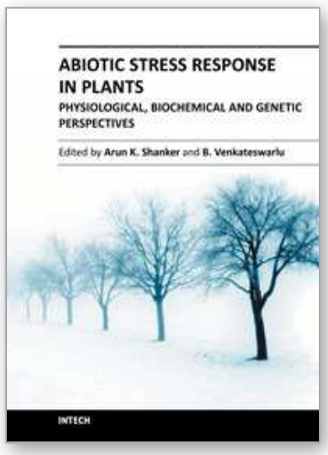

Abiotic Stress Response in Plants - Physiological, Biochemical and Genetic Perspectives

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Plants, unlike animals, are sessile. This demands that adverse changes in their environment are quickly recognized, distinguished and responded to with suitable reactions. Drought, heat, cold and salinity are among the major abiotic stresses that adversely affect plant growth and productivity. In general, abiotic stress often causes a series of morphological, physiological, biochemical and molecular changes that unfavorably affect plant growth, development and productivity. Drought, salinity, extreme temperatures (cold and heat) and oxidative stress are often interrelated; these conditions singularly or in combination induce cellular damage. To cope with abiotic stresses, of paramount significance is to understand plant responses to abiotic stresses that disturb the homeostatic equilibrium at cellular and molecular level in order to identify a common mechanism for multiple stress tolerance. This multi authored edited compilation attempts to put forth an all-inclusive biochemical and molecular picture in a systems approach wherein mechanism and adaptation aspects of abiotic stress are dealt with. The chief objective of the book hence is to deliver state of the art information for comprehending the effects of abiotic stress in plants at the cellular level.

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