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INDUCTION OF MATURATION PROTEINS IN GERMINATING SEEDS OF GROUNDNUT BY EXOGENOUS APPLICATION OF ABA AND SODIUM CHLORIDE

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SUMMARY

The induction of maturation proteins in the germinating seeds by exogenous application of abscisic acid (ABA) and sodium chloride (NaCl) was examined in groundnut. SDS-PAGE protein analyses revealed that proteins of 65, 31 and 22 kDa accumulated in abundance in embryos during late seed maturation. The expression of these proteins declined to undetectable level by fourth day during seed germination. The relative amounts of 65, 31 and 22 kDa proteins varied in the groundnut seeds germinated in the presence of ABA and NaCl at different concentrations. Proteins of 65 and 31 kDa accumulated to a higher level in the presence of 75 µM ABA, while 22 kDa protein was present in relatively lower amounts at this concentration. There was a gradual decrease in the amount of 65 kDa protein with further increase in the concentration of ABA (100 and 125 µM). Application of higher concentrations of NaCl (100, 150 and 200 mM) inhibited seed germination and promoted the accumulation of 65, 31 and 22 kDa proteins.

Key Words: **ABA stress, groundnut, maturation proteins, salt stress**

INTRODUCTION

Environmental stresses such as drought, high soil salinity, extreme temperature, cold etc. are serious threats for the crops influencing their growth and productivity. Plants have developed many strategies to withstand such stresses that include expression of some novel proteins (Bray 1997, Wang *et al.* 2003). Heat-shock proteins (Hsps) and late embryogenesis abundant (LEA) type proteins are two major types of stress-induced proteins that accumulate upon water, salinity, and extreme temperature stresses. They have been shown to play a role in cellular protection during the stress (Close 1996, Ingram and Bartels 1996, Thomashow 1998). LEA proteins are a broad family of plant proteins that are stored in dry seeds and have been shown to exhibit developmental and organ-specific expression (Hong *et al.* 1992). They were induced by drought, salt, and cold stresses in the vegetative tissues of various plants (Dure *et al.* 1989, Ingram and Bartels 1996). One common characteristic of LEA-type proteins is that, in most cases, their related gene expression is transcriptionally regulated and responsive to ABA (Mundy and Chua 1988, Skriver and Mundy 1990, Leung and Giraudat 1998). Abscisic acid increases during stress conditions and has important roles in the tolerance of plants to drought, high salinity, and cold (Chandler and Robertson 1994, Swamy and Smith 2000). In rice, the ABA-responsive *Rab21* gene was also induced by treatment with NaCl (Mundy and Chua 1988). Expression of these proteins in young seedlings of rice

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during salinity stress has been reported (Jayaprakash *et al.* 1998, Chourey *et al.* 2003). Xu *et al.* (1996) have shown the relevance of LEA3 proteins in imparting tolerance by over-expressing *HVA1* gene, coding for LEA3 protein, which conferred a small increase in tolerance to water deficit and salt tolerance in transgenic rice. In groundnut, little is known about the biochemical and molecular basis of stress response. Sharma *et al.* (1990) examined the biochemical changes in groundnut seedlings grown under polyethylene glycol induced stress. They reported that water stress decreased protein contents and increased amino acid and proline contents in leaves of 15-day-old seedlings. In this study, we were interested to determine if maturation proteins accumulate in the germinating seeds grown in the presence of ABA and NaCl.

MATERIALS AND METHODS

Groundnut (*Arachis hypogaea* L.) seeds of cultivar DRG-12 were obtained from Directorate of Oil Seed Research, Hyderabad, India. It is a high yielding cultivar belonging to Spanish bunch type and matures in 110-115 days in post-rainy season. Pods from field grown plants were collected at different days after pollination corresponding to different stages of seed development. They were surface sterilized with 70% ethanol (v/v) for 1 min followed by treatment with 0.1% mercuric chloride (w/v) for 20 min and rinsed with sterilized water 3-4 times. Embryo axes were dissected out from the seed of different developmental stages and the protein was extracted for analyzing the changes in protein profiles during seed maturation.

To check the induction of the proteins under stress conditions, dry seeds were surface sterilized and germinated in culture bottles with filter paper moistened with different concentrations of ABA (25, 50, 75, 100, 125 µM) and NaCl (50, 100, 150, 200 mM), separately. Seeds were placed on ABA or NaCl for 4 days and the germination responses were recorded in comparison to control seeds grown for the same duration in sterile distilled water. After these treatments, the sprouted seeds from which the cotyledons were removed were used for protein extraction. In case of seeds grown on 75-125 µM ABA or 100-200 mM NaCl germination was not observed, and hence the embryo axes were excised from the seeds imbibed for 4 days and used for protein extraction.

Protein Extraction

The various protein samples (100 mg each) were ground in a prechilled mortar and pestle with 1000 µl extraction buffer containing 50 mM Tris-Cl pH- 7.5, 5 mM $MgCl_2$, 2 mM KH_2PO_4, 10 mM NaF, 2 mM PMSF, 5 mM DTT, 2% PVP, 10 mM 2-mercaptoethanol, 20% glycerol and 500 µl n-hexane was added. N-hexane helps in dissolving the lipids that interfere during protein isolation (Stegemann and Pietsch 1983). Homogenized protein samples were centrifuged at 15,000 rpm for 20 min at 4°C. Supernatant was collected and protein concentration was estimated using Lowry's method (1951).

SDS-PAGE was performed according to Laemmli (1970) using 10% resolving gel and 5% stacking gel. Protein samples were treated with 4 X sample loading buffer containing 0.248 M Tris-Cl (pH 6.7), 8% SDS, 40% glycerol, 10% 2-mercaptoethanol, 0.004% bromophenol blue and boiled for 3 min and then stored at -80 °C. Equal amount of protein (100 µg) was loaded per well. Electrophoresis was performed at 75 V in stacking gel and 110 V in resolving gel. Gels were stained in coomassie brilliant blue R-250 solution (0.25 g coomassie brilliant blue R-250 in 50 ml methanol, 12 ml acetic acid and 38 ml water) overnight and destained with destaining solution (50 ml methanol, 12 ml acetic acid, 38 ml water) until the background was clear. Medium range (97.4 to 20 kDa) molecular weight markers (Bangalore Genei Pvt. Ltd.) were used as standard.

RESULTS AND DISCUSSION

SDS-PAGE analysis of proteins from immature embryo axes at different stages of development revealed striking differences in the expression of three proteins (Fig.1). Proteins of 65, 31 and 22 kDa were expressed at very low levels during early developmental stages and increased in abundance during seed maturation. It is hypothesized that these three proteins play a role in protecting the mature seed against desiccation damage in groundnut. The levels of maturation proteins rapidly declined during the seed germination and were almost undetectable in 4-day old germinating seeds (Fig. 2). Kermode (1990) contended that dehydration in developing seeds is a critical switch from a developmental to germination program. The developmental switch is acquired at seed ages closer to full maturity, during which desiccation

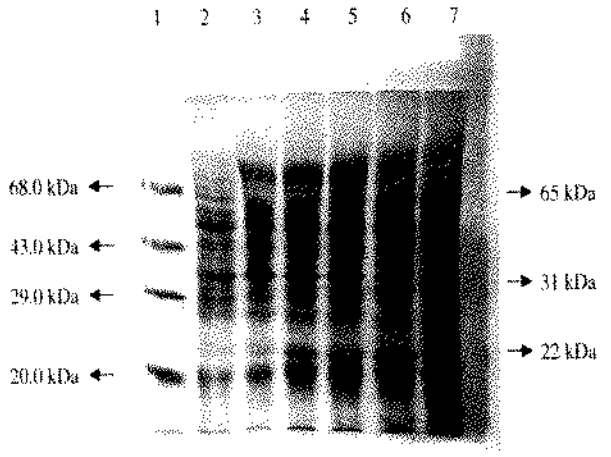

Fig.1. SDS-PAGE profiles of protein extracts of embryo axes of DRG-12 cultivar at different developmental stages
Lane 1: protein molecular weight markers (in kDa), lane 2-6: protein extracts of immature embryo axes collected from seeds after 20-25, 25-30, 30-40, 40-50 and 50-60 days after pollination, respectively, lane 7: protein extract of mature embryo axes from dry seed

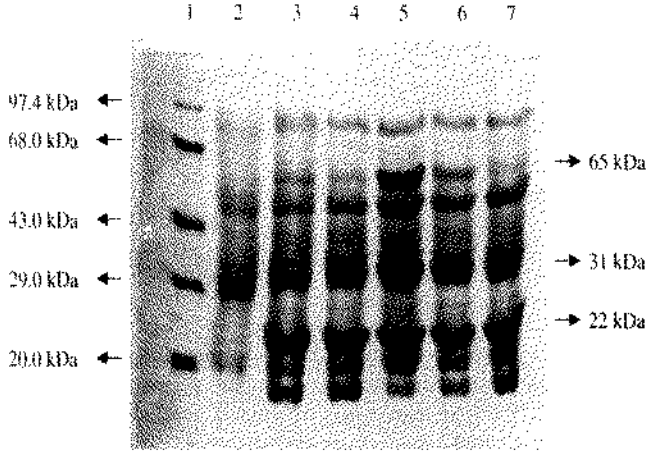

Fig. 2. SDS-PAGE profiles of protein extracts of germinating/ imbibed seeds grown on ABA
Lane 1: protein molecular weight markers (in kDa), lane 2: protein extracts of 4-day old germinating seeds grown on sterile distilled water (control), lane 3-7: protein extract of 4-day old germinating/imbibed seeds grown on ABA (25, 50, 75, 100 and 125 µM, respectively)

occurs and specific proteins accumulate. ABA responsive late embryogenesis abundant (LEA) proteins that accumulate during embryo maturity and degrade on rehydration of the seeds have been reported in several species (Skriver and Mundy 1990, Reid and Walker-Simmons 1990, Robertson *et al.* 1989).

Some of the characterized *lea* genes were expressed in vegetative tissues in response to osmotic stress and are ABA responsive (Bray 1993, Bracale *et al.* 1997). These proteins are expected to play important role in imparting stress tolerance (Skriver and Mundy 1990, Chandler and Robertson 1994). In the present study, the induction of 65, 31 and 22 kDa proteins was examined in 4-day old germinating seeds grown in the presence of ABA and NaCl. The germination percentage was influenced by the concentration of ABA and NaCl used. Application of ABA at 25 and 50 µM reduced the germination percentage to about 50% and delayed the emergence of radicle whereas higher concentrations of ABA (75-125 µM) inhibited seed germination. In the case of seeds grown in the presence of NaCl, germination percentage and seedling growth was not affected up to 50 mM, but was completely inhibited in the presence of 100-200 mM NaCl. Walker-Simmons (1987) demonstrated that 0.05 to 0.5 µM ABA is sufficient to block germination of wheat embryos from dormant grain, while 5 to 50 µM ABA is required for embryos from non-dormant grain. Growth inhibition under saline stress is one of the first responses described in barley (Ramagopal 1990). In the present study, detectable differences were noticed in the expression of maturation proteins in the seeds that were germinated in the presence of ABA (Fig. 2). Proteins of 65 and 31 kDa were expressed maximally at 75 µM ABA and further increase in the concentration of ABA reduced the expression of these proteins. There was a detectable increase in the expression of 22 kDa protein in the seeds grown in the presence of 25 and 50 µM ABA while it decreased at 75 µM ABA. Interestingly, further increase in the level of ABA resulted in enhanced expression of 22 kDa protein. From these results it is concluded that higher concentrations of ABA (100 and 125 µM) down regulated the expression of 65 and 31 kDa proteins whereas 22 kDa protein was up regulated. Exogenous application of ABA has been shown to induce the synthesis of many maturation proteins in *Arabidopsis* (Delseny *et al.* 2001), *Craterostigma plantagineum* (Ditzer *et al.* 2001) and alfalfa (Xu and Bewley 1991). ABA effects on protein levels may include both the stimulation of synthesis of specific proteins as well as prevention of their degradation (Reid and Walker-Simmons 1990). Blackman *et al.* (1991) showed that the development of desiccation tolerance in soybean seeds is associated with an increase in the level of a set of

maturation proteins although they may not be sufficient to induce tolerance by themselves. Jayaprakash *et al.* (1998) found a positive correlation between LEA2 and LEA3 protein levels and the growth of seedlings during stress and recovery in both rice and fingermillet, indicating a possible relevance of these proteins in stress tolerance.

SDS-PAGE on protein extracts from seeds germinated in the presence of NaCl showed that the accumulation of 65, 31 and 22 kDa proteins was influenced by its concentration (Fig. 3). The levels of maturation proteins were maintained in the seeds imbibed on 50 mM NaCl whereas higher concentrations of 100, 150 and 200 mM resulted in a remarkable increase in the level of proteins. It was noticed that the level of these maturation proteins were higher than those detected in mature embryos excised from dry seed. The physiological function of these proteins and their precise role in stress induced response in groundnut remains to be determined.

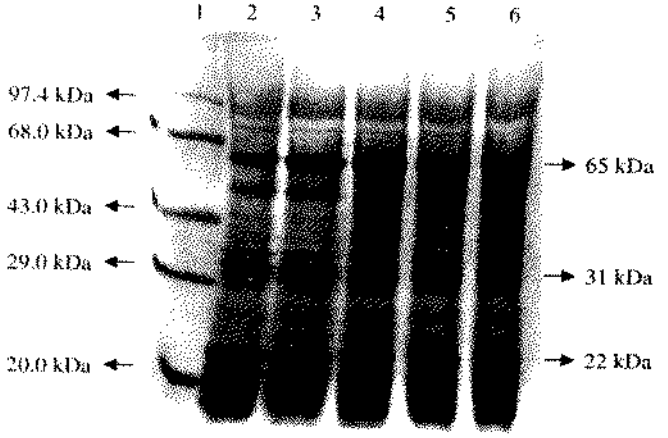

Fig.3. SDS-PAGE profiles of protein extracts of germinating/ imbibed seeds grown on NaCl
Lane 1: protein molecular weight markers (in kDa), lane 2: protein extracts of mature embryo axes excised from dry seed, lane 3-6: protein extract of 4-day old germinating/imbibed seeds grown on NaCl (50, 100, 150 and 200 mM, respectively)

Chourey *et al.* (2003) reported that salinity stress-induced LEA proteins accumulate during the salt stress-triggered growth arrest of young rice seedlings and the recovery from salt stress is accompanied by degradation of these proteins. Uma *et al.* (1993) showed that pretreatment of finger millet seedlings with ABA and non-lethal concentration of NaCl resulted in the accumulation of ABA-responsive proteins and that these seedlings exhibited tolerance to lethal salinity. Radha Rani and Reddy (1994) reported that two polypeptides (15 and 26 kDa) were specifically induced in shoots of NaCl treated rice seedlings. They further demonstrated that 15 and 26 kDa salt responsive polypeptides had a unique property of boiling stability.

In the present study, we have shown that 65, 31 and 22 kDa proteins accumulated in abundance during final stages of seed maturation in groundnut and declined during germination. Exogenous application of ABA or NaCl during germination resulted in the induction of maturation proteins although the levels varied depending on the concentration. Additional studies are needed to determine how maturation proteins function during seed development and stress.

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