BIOLOGIA PLANTARUM 42 (2): 241-248, 1999

Alterations in protein and esterase patterns of peanut in response to salinity stress

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Abstract

The ability of peanut (*Arachis hypogaea* L.) to grow at high concentrations of NaCl may be due to the alteration in gene expression. SDS-PAGE analysis has revealed that plants grown under NaCl showed induction (127 and 52 kDa) or repression (260 and 38 kDa) in the synthesis of few polypeptides. In addition, nine different esterase isoenzymes were detected in embryos of seeds germinated in 105 mM NaCl, whereas only five of them were detected in the embryos of untreated seeds. On the other hand, in the cotyledons, the esterase pattern was not affected by NaCl concentration. The esterase patterns of both stems and leaves were less influenced by NaCl in comparison to those of roots. The lipid contents, and fresh and dry masses were increased up to 45 mM NaCl and decreased at higher concentrations.

Additional key words: *Arachis hypogaea*, gene expression, lipids.

Introduction

The ability of a plant to grow under salinity stress is largely the result of alteration in gene expression and the relationships between different gene products (mRNA or proteins) and the degree of adaptation to NaCl were studied. Roots usually contain more induced proteins than other plant organs (King *et al.* 1986, Lin *et al.* 1997). The oil producing plants showed a certain degree of salinity tolerance. The accumulation of lipids in these plants under stress condition could be regarded as a means of osmoregulation (Ahmed *et al.* 1977, 1979, 1987, Younis *et al.* 1987, Abdel-Rahman and Hassanein 1988). It is well known that during germination of seeds rich in lipids, the fatty acids derived from of triglycerides undergo β-oxidation in the glyoxysome (Devlin and Witham 1986). The increase of lipid hydrolysis due to the increase in lipase activity under salinity was stated by Younis *et al.* (1987). Therefore, the question now is: could the increase in lipid hydrolysis be due to expression of new

Received 27 October 1998, *accepted* 21 January 1999.
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isoenzyme forms and/or increase in the activity of some existed isoenzyme forms. Consequently, the aim of this work was to study how the protein, lipid and esterase patterns in peanut are influenced by salt stress.

Materials and methods

Seeds of peanut (*Arachis hypogaea* L.) were pretreated with 5 % *Clorox* solution for 5 min followed by 5-min dip in 75 % ethanol and washed three times in autoclaved distilled water. Then the seeds were germinated on filter paper in Petri dishes containing 10 % Hogland's solution and 15, 30, 45, 60, 75, 90 and 105 mM NaCl. Three replicates were prepared for each salinization treatment. After one week the percentage of seed germination was determined. Seeds were considered to be germinated when the radicle emerged from the seed. Seeds were germinated and plants were grown at 16-h photoperiod (irradiance of 100 $\mu mol\ m^{-2}\ s^{-1}$) and temperature of 25 ± 1 °C, without humidity control. The embryos were separated from the cotyledons, and the both were subjected to esterase analysis.

Seeds were also sown into plastic pots containing 600 g of air dried soil and moisture was adjusted to 26 % of full field capacity. The cotyledons of plants in some pots were removed 1, 2, 4, 6, 8, and 15 d after sowing to study esterase pattern and lipid content in early seedlings. Plants were treated with 0, 15, 30, 45, 60, 75, 90, and 105 mM NaCl when they were 2-week-old. The saline solutions were added once. The salinized and non-salinized plants (four replicates) were daily irrigated with distilled water to reach soil moisture content of full field capacity. After two weeks, the plants were harvested and subjected to different analyses. During the experimental period 20 % Hoagland solution was used four times (once per week) to irrigate the plants.

One gramme of the plant material (cotyledons, embryos, roots, stems or leaves) was ground on ice in a mortar using 1 cm^3 0.04 M Tris-HCl, pH 7.0, containing 0.002 M cysteine and was used for esterase detection. In case of SDS-PAGE the buffer contained 0.25 M Tris-HCl, pH 8.5, and 0.3 % (v/v) 2-mercaptoethanol. In both cases, the homogenate was centrifuged at 15 000 *g* at 4 °C for 15 min. Native PAGE was performed in 7.5 % acrylamide slab gels. Protein samples were loaded onto the gel wells for electrophoresis. Gels were run at 10 mA per gel for 6 h at 4 °C with 0.025 M Tris-HCl + 0.192 M glycine buffer, pH 8.9. The esterase bands were stained by α- and β-naphthyl acetate with blue RR salt as described by Brewer (1970) and Chibbar *et al.* (1988).

SDS PAGE was performed using 15 % acrylamide gels. Protein samples containing 40 μg of protein were mixed with an equal volume of buffer containing 0.125 M Tris-HCl, pH 6.8, 4 % SDS, 20 % glycerol, 10 % 2-mercaptoethanol and bromophenol blue as a tracking dye. The mixture was heated in a water bath (96 °C) for 90 s and loaded onto gel wells for electrophoresis (*Bio Rad, Protean II XI Cell*). Gels were run at 18 mA per gel for 6 h at 4 °C in run buffer containing 0.025 M Tris,

0.192 M glycine and 0.1 % SDS. Protein bands were visualized by Coomassie Brilliant blue.

At the end of the experimental period, the lipid content in various plant parts was determined gravimetrically after extraction of lipids into petroleum ether according to Meara (1955). The fresh and dry matters of plants were also determined (for detail see Hassanein 1985).

Results

Salt stress caused an induction or inhibition in the synthesis of some polypeptides in the roots of peanut plants. SDS-PAGE (Fig. 1) showed the appearance of one band which was undetected in untreated control plants (127 kDa). The synthesis of other polypeptide (52 kDa) was increased in the NaCl treated plants. On the other hand, the syntheses of two polypeptides (260 and 38 kDa) was suppressed.

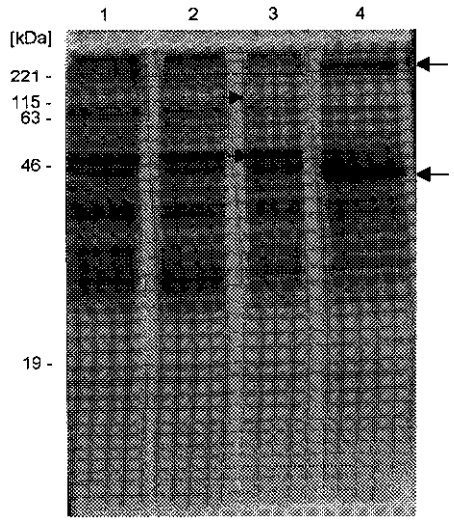

Fig. 1. Protein pattern in roots of peanut grown for two weeks under different NaCl concentration [45 mM NaCl (*lane 1*), 75 mM NaCl (*lane 2*), 105 mM NaCl (*lane 3*) and untreated control plants (*lane 4*)]. Proteins were separated on 15 % polyacrylamide gel and stained by Coomassie blue. Molecular mass distributions is indicated on the vertical axis. The syntheses of some polypeptides was enhanced (→) or repressed (←) by salinity stress.

In cotyledons of control peanut progressive decrease in the number and staining intensity of esterase (EST) bands with time after seed sowing were found (Fig. 2). While eight dark stained isoenzyme bands were detected after 1 d (*lane* 1), only five were detected after 15 d. The esterase pattern in embryos of germinated seeds was not changed during the first 7 d (data not shown).

The highest number of EST bands was found in embryos of seeds germinated in the highest NaCl concentration (105 mM, Fig. 3). Where nine different molecular forms could be detected in embryos of treated seeds, only five of them could be detected in the embryos of untreated seeds. EST-G1 and EST-G2 bands were only detected in the embryos of germinated seeds. In addition, a progressive increase in the staining intensity of EST bands with the increase in NaCl concentration was detected in the embryos. On the other hand, in cotyledons, the esterase pattern was not influenced by salt treatments.

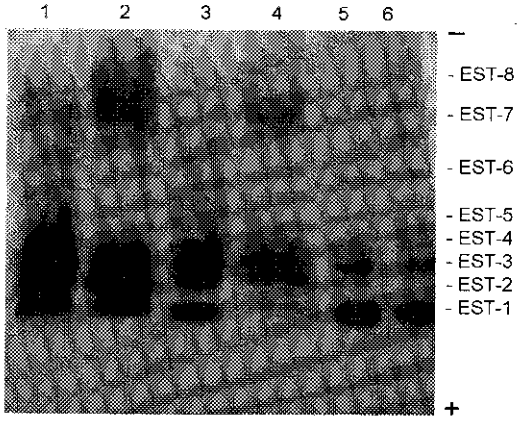

Fig. 2. Esterase isoenzyme pattern (on 7.5 % polacrylamide gel) in cotyledons of peanut seeds germinated for 1 d (*lane 1*), 2 d (*lane 2*), 4 d (*lane 3*), 6 d (*lane 4*), 8 d (*lane 5*), and 15 d (*lane 6*).

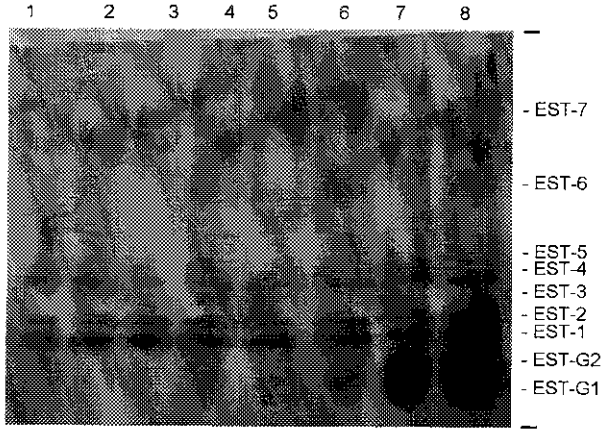

Fig. 3. Esterase isoenzyme pattern in embryos of germinated seeds treated with 15 mM NaCl (*lane 2*), 30 mM NaCl (*lane 3*), 45 mM NaCl (*lane 4*), 75 mM NaCl (*lanes 5, 6*), 90 mM NaCl (*lane 7*) and 105 mM NaCl (*lane* 8), compared with untreated plant (*lane 1*).

Differences in the changes of polypeptide patterns in roots, stems and leaves induced by NaCl were found (data not shown). In addition, under salinity stress the

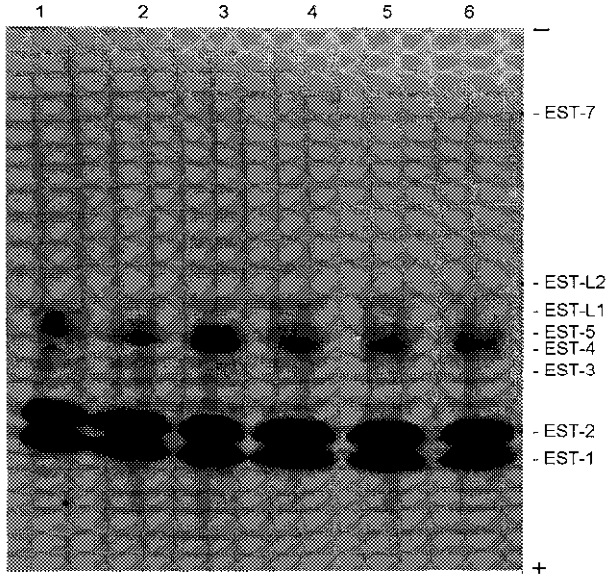

Fig. 4. Esterase isoenzyme pattern in leaves of peanut plants treated with 30 mM NaCl (*lane 2*), 45 mM NaCl (*lane 3*), 75 mM NaCl (*lane 4*), 90 mM NaCl (*lane 5*) and 105 mM NaCl (*lane 6*), compared with untreated plant (*lane 1*).

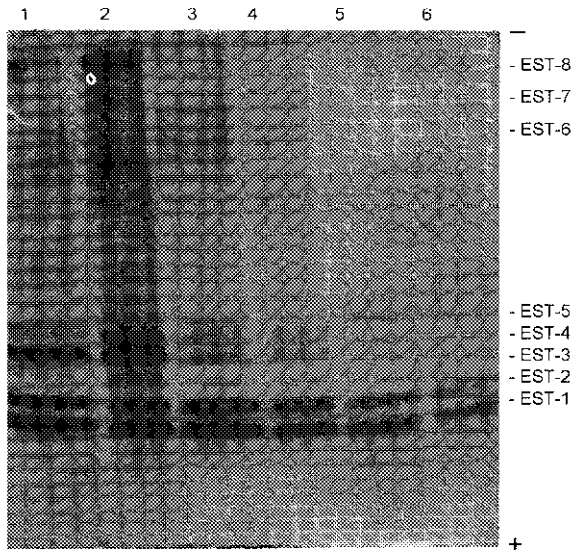

Fig. 5. Esterase isoenzyme pattern in roots of peanut plants treated with 105 mM NaCl (*lane 1*), 90 mM NaCl (*lane 2*), 75 mM NaCl (*lane 3*), 45 mM NaCl (*lane 4*), 30 mM NaCl (*lane 5*), compared with untreated plant (*lane 6*).

esterase patterns in both stems and leaves were less influenced by NaCl in comparison to those in roots. In the leaves of untreated plants, five esterase isoenzyme forms were detected (Fig. 4, *lane 1*) but eight isoenzyme forms were detected when peanut plants were subjected to salinity stress, where EST-L1 and EST-L2 (appeared only in leaves) as well as EST-7 could be detected.

The roots of untreated control plants (Fig. 5) were characterized by the expression of three esterase forms (EST-1, EST-2 and EST-8). With the increase of NaCl concentrations the number of esterase isoenzymes in plant roots increased. Seven or eight isoenzymes could be detected when the peanut plants were grown under the relatively high concentrations of NaCl.

The final germination percentage of peanut seeds was considerably reduced by NaCl: under the highest NaCl concentration (105 mM) to 50 %, whereas the germination percentage of untreated seeds was 96 %. The values of lipid content as well as fresh and dry masses increased with the rise of salinization up to 45 mM NaCl. Under higher NaCl concentrations, the growth parameters tended to decrease but the lipid content decreased only under high NaCl concentrations (90, 105 mM) (Fig. 6).

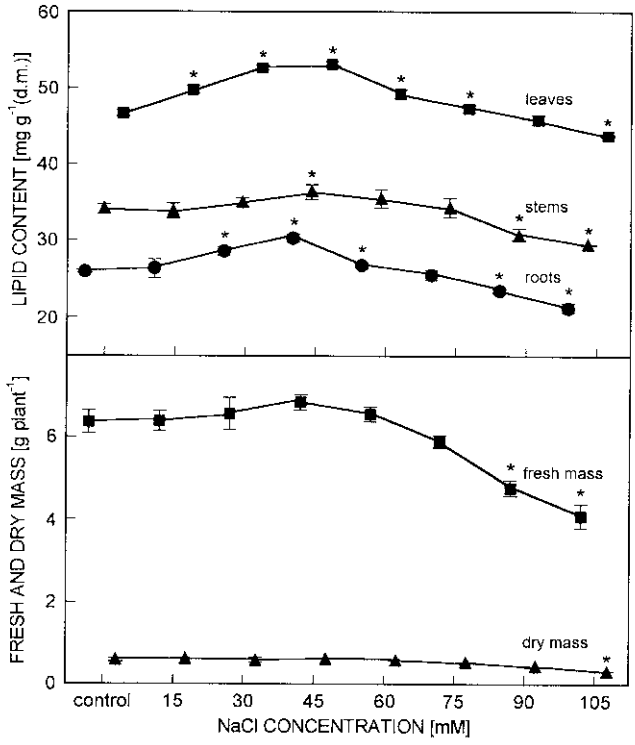

Fig. 6. Lipid content, and fresh and dry masses of peanut plants grown for two weeks under different NaCl concentrations (* - means significantly different from control at $P < 0.05$, $n = 4$).

Discussion

Peanut plant respond to salt stress by induction and repression in the synthesis of few polypeptides. In the root, the syntheses of two 127 and 52 kDa polypeptides was increased, which is generally in accordance with the results obtained previously by Ramagopal (1988). Salinity induced polypeptides may play direct or indirect role in the cellular adaptation to salt stress (Ramagopal 1988). Peanut tolerates relatively high salinity (75 mM NaCl added suddenly) without significant reduction in lipid content and grows.

During seed germination lipids were hydrolyzed by esterase to glycerol and fatty acids. These components were used by cells to synthesise different metabolites or provide the germinated embryos by energy. In this work, the maximum activity of esterases in peanut cotyledons was detected 1 d after seed sowing, but after 15 d most of the lipid content of cotyledons may be hydrolized by esterases, therefore the high number of isoenzyme forms and/or activity of esterases were not needed for the plants. This was in agreement with Ismail (1997) who was found in *Glycine max* and *Ricinus communis* that esterase activity was reached a maximum after 1 d and reached a minimum after 7 d from seed soaking. On the other hand, esterase pattern in germinated peanut embryos did not change during seed germination.

Salinity decreased the ability of peanut seeds to germinate. The number of esterase isoenzymes increased with the increase of NaCl concentration in the embryos. On the other hand, cotyledons of NaCl treated seeds were not affected. Thus esterase pattern in peanut displayed tissue specificity. The number of the isoenzymes in leaves or stems was higher than in roots of untreated plants. Generally, the number of isoenzymes increased under the influence of NaCl

The fresh and dry masses as well as lipid content of peanut plants increased to maximum at 45 mM NaCl and decreased thereafter. These results are in accordance with the results obtained previously (Ahmed *et al.* 1977, 1979, Erdei *et al.* 1980, Younis *et al.* 1987, Evans *et al.* 1998, Quartacci *et al.* 1998). Some relations appeared between the lipid metabolism and stress in higher plants (Ahmed 1977, 1979, 1987, Hassanein 1985, Abdel-Rahman and Hassanein 1988, Ben-Raïs *et al.* 1993, Barrachina *et al.* 1996). Ahmed *et al.* (1979) reported that the oil producing plants may have their own mechanism to counteract the effect of moderate salinity. The peanut plant subjected to moderate salinity increased the lipid content. On the other hand, treatment the peanut plants with highest NaCl concentration lowered the lipid content. This decrease in lipid content was in agreement with increased number and/or activity of esterase isoenzymes: the highest number of esterase isoenzymes, and the lowest lipid content were detected under the highest NaCl concentration used. Coordination between the increase of esterase activity and the decrease in lipid content and was also detected in cotyledons of untreated seedlings, where about 87 % of lipid content in the cotyledons was hydrolysed in two weeks.

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