

Jasmonic acid induced changes in protein pattern, antioxidative enzyme activities and peroxidase isozymes in peanut seedlings

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Abstract

Protein pattern, ammonia content, glutamine synthetase activity, lipid peroxidation, superoxide dismutase, catalase, peroxidase and peroxidase isoforms were studied in the leaves and roots of 7-d-old peanut (*Arachis hypogaea* L. cv. JL-24) seedlings treated by 25, 100 and 250 μ M jasmonic acid (JA). SDS-PAGE protein profile of leaves and roots after JA application showed a significant increase in 18, 21, 30, 45, 47 and 97.4 kDa proteins and significant decrease in 22 and 36 kDa proteins. Pathogenesis related PR-18 was specific in leaves at 250 μ M JA and PR-21 have cross reacted differently with 21 and 30 kDa proteins in leaves and roots treated by all JA concentrations. Further, the immunoblot analysis with glutamine synthetase, GS-45 antibodies revealed a specific cross reaction with 45 and 47 kDa proteins of both control and JA treated leaves, however, higher at 100 and 250 μ M JA treated leaves than control ones. Further, the malondialdehyde (MDA) content significantly increased in leaves and roots treated with JA, indicated membrane damage with JA treatments that led to the generation of peroxidation products. The peroxidase isozymic pattern showed two specific isoforms. Besides, the activities of SOD and catalase were significantly elevated in JA treated leaves.

Additional key words: *Arachis hypogaea*, catalase, lipid peroxidation, PR-proteins, SDS-PAGE.

Introduction

JA and MeJA are linoleic acid derived cyclopentanone based compounds and key molecules of the octadecanoid-signalling pathway (Meyer *et al.* 1984). Jasmonates are one of the simplest nontraditional plant hormones with diverse roles and functions, including a potential role in plant defense as part of the complex signalling pathways (Creelman and Mullet 1997a,b). The wound and elicitors has been known to cause JA and MeJA accumulation in plants, these results implicate a role for jasmonate in plant defense during biotic stress (Creelman and Mullet 1995). It has been showed that exogenous application of JA or MeJA antagonistically regulated expression of salt

stress-induced proteins associated with water stress and defense responses (Moons *et al.* 1997). Jasmonates at concentrations above 50 μ M in rice induced the degradation of chloroplast proteins, ribulose biphosphate carboxylase/oxygenase subunits and synthesis of novel proteins, including a novel basic 28 kDa Bowman-Birk proteinase inhibitor (BBPI) protein, acidic 17 kDa pathogenesis-related class 1 protein (PR-1) and a 19 kDa pathogenesis-related class 5 protein (PR-5) (Rakwal *et al.* 1999, Rakwal and Komatsu 2000, 2001, Agrawal *et al.* 2002). JA application also resulted in necrotic lesions and the programmed cell death (Repka 2002).

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Abbreviations: CAT - catalase; GDH - glutamate dehydrogenase; GOGAT - glutamate synthase; GS - glutamine synthetase; JA - jasmonic acid; MDA - malondialdehyde; MeJA - methyl jasmonate; POD - peroxidase; PR - pathogenesis related; PVDF - polyvinylidene difluoride; ROS - reactive oxygen species; SDS-PAGE - sodium dodecyl sulfate polyacrylamide gel electrophoresis; SOD - superoxide dismutase.

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In plants ammonium was released from organic compounds by several metabolic processes, including the deamination of amino acids during seed germination, the synthesis of specific amino acids or lignin and photorespiration. It was found that a significant accumulation of ammonia has been detected in several plants subjected to various abiotic and biotic stresses (Turner and Debbage 1982, Ramanjulu and Sudhakar 1997, Ashraf and Harris 2004). Plant glutamine synthetase (GS) and glutamate synthase (GOGAT) assimilate ammonia into organic compounds (Lea and Miflin 1974). The GS/GOGAT cycle is the principal route of ammonium assimilation in plants (Temple *et al.* 1998).

Increased endogenous jasmonate contents have been found in wounded plants, in plants suffering from drought and osmotic stress (Creelman *et al.* 1992, Baldwin *et al.* 1997), after treatment with fungal elicitors (Gundlach *et al.* 1992, Farmer and Ryan 1992) or upon pathogen attack (Xu *et al.* 2003). In recent years jasmonates have been the focus of much attention because of their ability to provide protection to salinity stress (Tsonev *et al.* 1998), UV irradiation (Mackerness *et al.* 1999), or to increased freezing tolerance of bromegrass (Wilén *et al.* 1994) which led to the suggestion that jasmonate could mediate the defense response to various environmental stresses.

The common link among different stresses was that they all produce an oxidative burst (Popova *et al.* 2003). These toxic oxygen species cause extensive lipid peroxidation (Babbs *et al.* 1989), chlorophyll breakdown (Rakwal and Komatsu 2000), loss of photosynthetic activity (Rakwal and Komatsu 2001), leakage of electrolytes (Fedina and Benderliev 2000), and loss of cell membrane integrity (Jyothsnakumari and Sudhakar 2003/4). According to Moons *et al.* (1997) JA markedly

induced a cationic peroxidase in rice seedlings by salt stress.

Antioxidative enzymes include catalase and peroxidase that remove H_2O_2 and superoxide dismutase that catalyze the disproportion of superoxide radicals to hydrogen peroxide and dioxygen. Activity of SOD, CAT and POD increased during biotic and abiotic stresses to protect cells from the potential hazardous effects of reactive oxygen species (Menezes-Benavente *et al.* 2004). Similarly Popova *et al.* (2003) has reported the accumulation of SOD, CAT and POX isoforms in barley under MeJA treatment. Catalase (CAT) efficiently scavenges H_2O_2 and does not require a reducing substrate to perform the task. In leaf tissue, catalase was localized in peroxisomes, to scavenge the H_2O_2 produced by glycolate oxidase in the C_2 photorespiratory cycle. Catalase isozymes differ in biochemical properties, as well as in developmental specificity, some related to germination, but their principal role involving probably fatty acid conversion, where as others were related to lignification, photorespiration or aging process.

MDA was produced when polyunsaturated fatty acids in the membrane undergo peroxidation (Meloni *et al.* 2003). MeJA stimulates MDA accumulation and inhibition of the Fe-induced release of chelators, intracellular chelators counteract the salt stress and it suggests that MeJA trigger some other protective mechanisms (Jyothsnakumari and Sudhakar 2003/4). Rakwal *et al.* (1999) observed that high concentrations of JA in wounded leaves caused significant changes in protein pattern of rice plants.

Since JAs are mediator of cellular responses in defense or stress, we studied changes in the protein pattern, MDA content and induction activity of antioxidative enzymes in peanut.

Materials and methods

Plants: Peanut (*Arachis hypogaea* L. cv. JL-24) seeds obtained from Agricultural Research Station, Anantapur were surface sterilized with 0.1 % mercuric chloride solution for 30 s and rinsed with deionized water. Seeds germinated in Petri plates lined with two layers of filter papers, moistened with distilled water (control) and 25, 100 and 250 μ M JA solution. The plates were kept under irradiance of 150 Wm^{-2} and temperature of 22 ± 2 °C in plant growth chamber for 7 d. Roots and leaves were separated, frozen in liquid nitrogen and stored at -80 °C until further use.

Protein analysis: Plant material (1 g) was frozen and powdered by using liquid nitrogen and extracted in 50 mM Tris-HCl (pH 7.4) buffer contained 10 mM Na EDTA, 5 % β -mercaptoethanol and 10 % glycerol. The homogenate was centrifuged at 5 000 g for 10 min in a refrigerated high speed centrifuge and removed the

cellular debris. The resultant supernatant was centrifuged at 10 000 g for 20 min and obtained clear supernatant. The supernatant was used as crude extract for the isolation of proteins by resolving on electrophoresis. The concentration of proteins was estimated by the method of Lowry *et al.* (1951) using bovine serum albumin (BSA) as the standard. For one-dimensional separation, proteins (100 μ g per lane) were electrophoresed on 12 % linear sodium dodecyl sulphate (SDS)-polyacrylamide slab gels (1 mm thickness) overlaid with stacking gel (Laemmli 1970). The electrophoresis was carried out at 50 V for 1 h followed by 100 V for 2 h. Proteins and molecular mass markers were visualized by staining with Coomassie Brilliant Blue R-250 followed by destaining appropriately.

Immunoblotting: After SDS-PAGE, the proteins from the gel were transferred to polyvinylidene difluoride

(PVDF) membrane using (25 mM Tris, 40 mM amino caproic acid and 20 % methanol) at 60 mA for 1 h. After rinsing the membrane in Tris-buffered saline (TBST; 100 mM Tris, 1500 mM NaCl, 0.05 % Tween-20, pH 7.5), the unoccupied sites on membrane were blocked with 1 % BSA in TBST. The membrane was washed in TBST and then incubated with 1:1000 dilution of anti-PR and anti-GS antisera. Antiserum of PR-18 was raised against *Phaseolus lunatus* primary leaves infected with tobacco ring spot nepovirus. PR-21 was raised against *Phaseolus vulgaris* cv. Pinto infected with the same viruses (generously supplied by Prof. O.P. Sehgal, Columbia, Missouri). Antisera of GS raised against the *Escherichia coli* recombinant proteins (generously supplied by Dr. Hans Peter Mock, IPK, Gatersleben, Germany). After incubation for 1 h on a rotary shaker, membrane was washed and incubated in 1:1 000 dilution of goat-anti-rabbit IgG-peroxidase conjugate (*Sigma*) for 45 min. After washing, colour was developed using diaminobenzidine as colour developing reagent and cobalt metal as catalyst. The reaction was stopped after the desired colour intensity was obtained by rinsing the membrane in distilled water.

Ammonia content was estimated according to Bergmeyer (1965). 5 % tissue homogenate was prepared in cold distilled water and centrifuged at 10 000 *g* for 15 min. To 1.0 cm³ of the supernatant 2.0 cm³ of 15 % perchloric acid was added and the content was centrifuged and then 2.0 cm³ of 10 % sodium hydroxide was added to it. To this 0.5 cm³ of Nessler's reagent was added and centrifuged. The colour developed was read against reagent blank at 495 nm in Shimadzu 1601 UV spectrophotometer (Kyoto, Japan).

Glutamine synthetase (EC. 6.3.1.2) activity was determined by the method of O'Neal and Joy (1973). 1 g frozen plant material was homogenized in 0.05 M Tris-HCl buffer (pH 7.9) containing 10 mM β -mercaptoethanol and centrifuged at 3000 *g* for 15 min, 4 °C. The supernatant was passed through Sephadex G-25 columns and active fractions were collected and used as enzyme source. The standard reaction mixture (1.0 cm³) contained 0.05 M Tris-HCl buffer (pH 7.9), 20 μ M magnesium sulphate, 80 μ M α -ketoglutarate, 6 μ M hydroxylamine, 8 μ M ATP and 0.5 cm³ of enzyme extract. The reaction was started after an initial 2 min pre-incubation at 35 °C by the addition of enzyme. After 30 min incubation, the reaction was stopped with 10 cm³ of a solution containing 0.37 M FeCl₃, 0.67 M HCl and 0.2 M TCA. The reaction mixture was centrifuged at 3 000 *g* for 10 min to remove precipitated proteins. The colour of ferric hydroxamate complex was measured at 540 nm.

MDA content was determined by the thiobarbituric acid (TBA) reaction as described by Heath and Packer (1968). 1 g of frozen tissue from leaves and roots of peanut

seedlings was homogenized in 5 cm³ of 0.1 % (m/v) TCA. The homogenate was centrifuged at 10 000 *g* for 5 min and 4 cm³ of 20 % TCA containing 0.5 % (m/v) TBA was added to 1 cm³ of the supernatant. The mixture was heated at 95 °C for 30 min and then quickly cooled on ice. The contents were centrifuged at 10 000 *g* for 15 min and the absorbance was measured at 532 nm. The concentration of MDA was calculated using a coefficient of absorbance of 155 mM⁻¹ cm⁻¹.

Superoxide dismutase: 1 g of frozen plant material was homogenized in 50 mM phosphate buffer (pH 7.0) containing 1 % polyvinyl pyrrolidone. The homogenate was filtered and then centrifuged in a refrigerated centrifuge at 15 000 *g* for 15 min, and the supernatant obtained was used as source of enzyme. All steps in the preparation of enzyme extract were carried out at 4 °C. The activity of superoxide dismutase was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium as described by Beauchamp and Fridovich (1971). The reaction mixture (3 cm³) consisted of 50 mM phosphate buffer (pH 7.8), 15 mM methionine, 75 μ M nitroblue tetrazolium, 0.1 M EDTA, 2 μ M riboflavin and 0.1 cm³ of enzyme extract. Riboflavin was added as the last, and test tubes were shaken and placed 30 cm below a light source (30 W fluorescent lamps). The reaction was started by switching on the lights. The reaction was stopped after 30 min by switching off the lights. The tubes were covered with black cloth, control was the reaction mixture, which was not exposed to light. The absorbance was measured at 560 nm. Log absorbance at 560 nm was plotted as a function of the volume of enzyme extract used in the reaction mixture. The volume of the enzyme extract corresponding to 50 % inhibition of the reaction was considered as one enzyme unit.

Peroxidase and catalase: The frozen plant material was homogenized in 50 mM Tris-HCl buffer, pH 7.0 and centrifuged at 1 000 *g* to remove cellular debris. The resultant supernatant was recentrifuged at 10 000 *g* for 20 min. The supernatant was passed through a Sephadex G-25 column and fractions containing enzyme were pooled and used as enzyme source for the assay of catalase, peroxidase and for peroxidase isoforms. All the steps were carried out in cold room. Peroxidase activity was assayed by the method of Hammerschmidt *et al.* (1982). The reaction mixture (3 cm³) contained 0.25 % (v/v) guaiacol in 10 mM sodium phosphate buffer (pH 6.0) and 10 mM hydrogen peroxide. The reaction was started by adding 0.025 cm³ of enzyme solution and measured spectrophotometrically at 470 nm. The enzyme activity was expressed as change in absorbance.

Catalase activity was assayed by the method of Barber (1980). The reaction mixture consisted of enzyme extract, 5 mM H₂O₂ and 50 mM Tris-buffer, pH 7.0. After 1 min incubation at 25 °C, the reaction was stopped by

adding 1.0 cm³ of 2.5 M H₂SO₄. The residual H₂O₂ was titrated with 0.01 M KMnO₄.

Peroxidase isozymes: A non-denaturing gel (native PAGE without SDS) was carried out to separate the peroxidase isozymes. It was carried out on 7 % separating and 4 % stacking gel. Stock buffers and electrode buffers were used without SDS. Other electrophoretic conditions were same as SDS-PAGE mentioned earlier. After electrophoresis the gels were incubated for two minutes in benzidine solution to visualize isozymes. Gels were

Results

Quantitative differences in the protein profiles were observed as the JA concentration was increased (Fig. 1). The amount of 18, 21, 30, 45, 47 and 97.4 kDa proteins increased, whereas amount of 22, 36 and 42 kDa proteins decreased in JA treated seedlings.

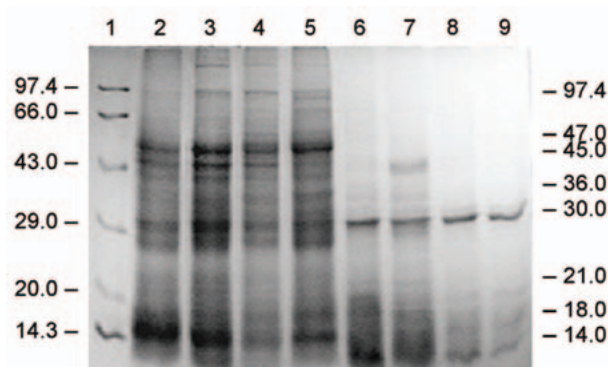


Fig. 1. SDS-PAGE protein profile of leaves and roots of 7-d-old peanut seedlings treated by different concentrations of jasmonic acid. Lane 1: molecular mass markers; phosphorylase B - 97.4 kDa; bovine serum albumin - 66 kDa; ovalbumin - 43 kDa; carbonic anhydrase - 29 kDa; soybean trypsin inhibitor - 20 kDa; lysozyme - 14.3 kDa; lane 2: leaf control; lane 3: leaf 25 μM JA; lane 4: leaf 100 μM JA; lane 5: leaf 250 μM JA; lane 6: root control; lane 7: root 25 μM JA; lane 8: root 100 μM JA; lane 9: root 250 μM JA. Arrows indicate proteins with higher expression.

The same protein-gels were transferred on the membranes and were probed with anti PR-18, 21 and GS-45 kDa antibodies. PR-18 antibodies specifically cross-reacted only with 14.3 and 18 kDa proteins of 250 μM JA treated leaves, but did not showed cross-reaction with proteins of root samples and leaves treated with 25 and 100 μM JA (Fig. 2A). PR-21 antibodies showed the cross reaction with 21, 30 kDa proteins of both leaves and roots of control and JA treatments (Fig. 2B). However, the higher intensity was in 250 μM JA treated leaves. The immunoblot of GS antibody detected 45 and 47 kDa protein bands (Fig. 2C) in the control, 25, 100 and 250 μM JA treated leaves. The cross-reaction was

immediately transferred into distilled water to arrest reaction after visualizing the bands to the required intensity on electrophoregrams. The gels were photographed immediately using *Image Analysis System* (Alpha Innotech DE-400, San Leandro, CA, USA).

Statistical analysis: The data obtained in all cases were subjected to analysis of variance (ANOVA), and the mean values ($n = 5$) were compared by Duncan's Multiple Range Test (DMRT) at 0.05 % level (Duncan 1955).

comparatively higher in JA treated leaves than in controls. The cross-reaction of the antibodies in leaves increased with increasing concentration of JA applied. In roots the cross-reaction was observed only in control and 25 μM JA treatments. This revealed that the GS activity increased specifically in leaves with JA treatments.

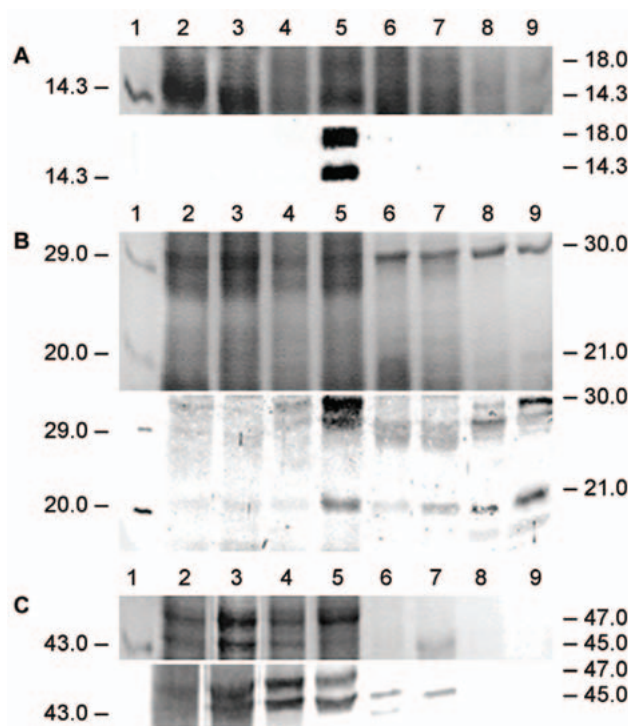


Fig. 2. Immunoblot analysis of peanut leaf and root proteins probed with anti-PR (18 kDa) antibodies (A), with anti-PR (21 kDa) antibodies (B) and with anti-GS (45 kDa) antibodies (C). The position of standard molecular mass markers is shown on the left and the positions of PR-14.3 and 18 kDa (A), PR-21 and PR-30 kDa (B) or 45 and 47 kDa (C) are shown by arrows on the right. Lane 1: Molecular mass protein marker 14.3 kDa; lane 2: leaf control; lane 3: leaf 25 μM JA; lane 4: leaf 100 μM JA; lane 5: leaf 250 μM JA; lane 6: root control; lane 7: root 25 μM JA; lane 8: root 100 μM JA; lane 9: root 250 μM JA. The top panel is SDS-PAGE protein profile.

Table 1. Ammonia content [$\mu\text{g g}^{-1}(\text{d.m.})$], activities of glutamine synthetase, GS [$\text{mmol}(\text{hydroxymate}) \text{g}^{-1}(\text{protein}) \text{min}^{-1}$], superoxide dismutase, SOD [$\text{U g}^{-1}(\text{f.m.}) \text{min}^{-1}$], catalase, CAT [$\text{mg}(\text{H}_2\text{O}_2 \text{ oxidized}) \text{g}^{-1}(\text{f.m.}) \text{min}^{-1}$] and peroxidase, POD [$\text{U g}^{-1}(\text{f.m.}) \text{min}^{-1}$] and malondialdehyde content [$\text{mmol g}^{-1}(\text{f.m.})$] as affected by 25, 100, and 250 μM concentrations of jasmonic acid in 7-d-old seedlings of *Arachis hypogaea* L. cv JL-24. Means from 5 experiments $\pm$ SE. The means in a row followed by a different letter for each species were significantly different ($P \leq 0.05$) according to Duncan's multiple range test.

Parameters	Organs	Control	25 μM JA	100 μM JA	250 μM JA
Ammonium	leaf	80.24 $\pm$ 7.68a	82.28 $\pm$ 10.23b	86.82 $\pm$ 10.23c	90.63 $\pm$ 10.51d
	root	124.67 $\pm$ 10.51a	142.96 $\pm$ 12.70b	155.53 $\pm$ 13.10c	160.36 $\pm$ 13.76d
GS	leaf	0.13 $\pm$ 0.90a	0.18 $\pm$ 0.13a	0.24 $\pm$ 0.16c	0.26 $\pm$ 0.11c
	root	0.22 $\pm$ 0.22a	0.21 $\pm$ 0.19b	0.14 $\pm$ 0.09c	0.13 $\pm$ 0.05d
MDA	leaf	7.57 $\pm$ 0.39a	7.62 $\pm$ 0.27b	9.22 $\pm$ 0.39c	10.43 $\pm$ 0.23d
	root	5.36 $\pm$ 0.26a	5.75 $\pm$ 0.23b	7.21 $\pm$ 0.29c	9.65 $\pm$ 0.29d
SOD	leaf	0.11 $\pm$ 0.02a	0.12 $\pm$ 0.01b	0.20 $\pm$ 0.01c	0.30 $\pm$ 0.17d
	root	0.09 $\pm$ 0.01a	0.10 $\pm$ 0.21b	0.16 $\pm$ 0.48c	0.25 $\pm$ 0.06d
CAT	leaf	1.45 $\pm$ 0.31a	1.52 $\pm$ 0.23b	3.59 $\pm$ 0.45c	4.96 $\pm$ 0.70d
	root	0.70 $\pm$ 0.20a	0.83 $\pm$ 0.37b	1.06 $\pm$ 0.60c	1.66 $\pm$ 0.67d
POX	leaf	10.46 $\pm$ 0.20a	10.65 $\pm$ 0.15b	34.10 $\pm$ 0.10c	34.50 $\pm$ 0.10d
	root	2.30 $\pm$ 0.20a	2.70 $\pm$ 0.20b	4.90 $\pm$ 0.15c	6.40 $\pm$ 0.15d

Ammonia content in the leaves and roots increased significantly (Table 1) with increase of JA concentrations. The increase was higher in roots than in leaves.

A significant increase in the glutamine synthetase activity was registered in leaves with increase of JA concentration (Table 1). It was further supported from the immunoblot analysis, however in roots GS activity was decreased.

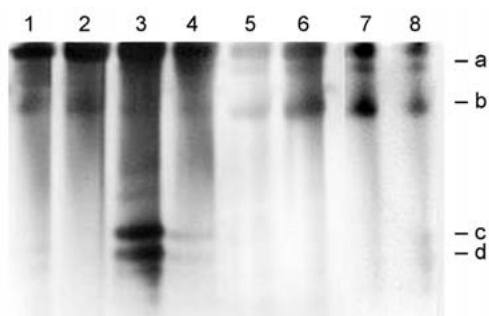


Fig. 3. Peroxidase isoforms on 7 % native PAGE in 7-d-old peanut seedlings under control and after jasmonic acid treatment. Lane 1: leaf control; lane 2: leaf 25 μM JA; lane 3: leaf 100 μM JA; lane 4: leaf 250 μM JA; lane 5: root control; lane 6: root 25 μM JA; lane 7: root 100 μM JA; lane 8: root 250 μM JA. Arrows indicate the increase of peroxidase isoforms in JA treated seedlings.

Discussion

Recently role of JA in plants have received considerable attention and various modes of JA action have been discussed. This study aimed at evaluating the role of exogenously applied JA to the peanut seedlings in counteracting the stress induced responses such as

JA treatments caused a significant increase in the MDA content both in leaves and roots of peanut seedlings. Accumulation was higher in roots than in leaves indicating a high rate of lipid peroxidation at high concentrations (100 and 250 μM) of jasmonic acid in roots (Table 1). Superoxide dismutase activity significantly elevated in JA treated seedlings in comparison to control plants both in leaves and roots at all JA concentrations. At 100 and 250 μM JA there was a significant increase in the enzyme activity. The increase in enzyme activity was higher in leaves than in roots (Table 1).

The catalase activity significantly elevated in the leaves and roots of peanut seedlings at all JA treatments. The increase was dependent on JA concentration. The enzyme activity was more increased in leaves than in roots (Table 1).

Also total peroxidase activity of the crude enzyme extracts was considerably higher in leaves than roots of 100 and 250 μM JA treated seedlings (Table 1). Four peroxidase isoforms (namely, a, b, c, d) were detected in the extracts of both leaves and roots of peanut seedlings. Among them two isoforms (c and d) were specifically induced in leaves by 100 μM JA treatments (Fig. 3). The activity of these isoperoxidases was more in leaves than in roots by 100 μM and 250 μM JA treatments.

changes in total protein content, PR proteins, ammonia content, GS activity, MDA content, antioxidative enzymes and peroxidase isoforms.

Protein pattern changes due to environmental stress. The assumption is that exogenously applied jasmonates

provoke alterations of the protein content (Rakwal and Komatsu 2001). The JA treatments resulted in several quantitative changes in polypeptide profiles. Changes were reproducible in individual experiments, and changes in the individual polypeptide bands can be considered as reliable. As compared to the control, JA-treated plants showed the accumulation of 18, 21, 30, 45, 47 and 97.4 kDa proteins. Among them 18 and 97.4 kDa proteins were specific in leaves while 30, 42 kDa proteins were present in only roots. In roots the quantity of 22, 36 kDa proteins were gradually decreased with JA treatments. Increased accumulation of proteins was observed in 250 μ M JA treated leaves. These results are in accordance with those reported by Rakwal and Komatsu (2001) who reported the rapid accumulation of a specific set of proteins the so-called jasmonate induced stem proteins (JISP) in rice seedlings.

Immunoblotting with anti PR-18 cross-reacted with 14.3, 18 kDa proteins (Fig. 2A). These proteins were found to be PR-1 like proteins as evidenced from the immunoblot, this could be a first report on the induction of PR-1 proteins by JA in peanut. Furthermore, this PR-1 proteins were observed only in 250 μ M JA treated leaves and not in roots (Fig. 2A). PR-1 proteins were the first discovered pathogenesis-related proteins of 15 - 17 kDa molecular mass, but some members have recently been shown to have antifungal activity, with a possible involvement in defense and infusibility by pathogens, *PR-1* gene (encoding an 17 kDa protein) from rice was described as pathogen-inducible and its expression was found to be a hypersensitive response (Rakwal and Komatsu 2001).

In the present investigation, PR-21 kDa anti-sera cross-reacted with 21 and 30 kDa proteins (Fig. 2B) and the cross reactivity was more at 250 μ M JA treated leaves and roots. PR-21 kDa was found to be PR-5 family protein and the N-terminal sequence of JISP 11 (19 kDa) was determined as ATFTITNRCS with 100 % identity to the rice thaumatin-like protein (PR-5) family from the earlier reports (Reimmann and Dudler 1993). The enzymatic function of PR-5 proteins remains unknown though a role in plant defense was attributed to some of its members. This finding of PR-5 family proteins in peanut after JA treatments was quite interesting, as a similar PR-5 protein was identified from mature rice leaves treated with 500 μ M JA earlier (Rakwal *et al.* 1997). These results were further supported by Moons *et al.* (1997) in rice seedlings after JA treatment under salt stress, where JA induced two acidic PR-1, and PR-10 pathogenesis-related proteins in rice roots.

Ammonia has been found to be accumulated in several plants under stress. A significant accumulation of ammonia has been detected in leaves of salt sensitive plants subjected to saline stress (Ashraf and Harris 2004), a substantial increase of ammonia levels was noticed under water stress in mulberry (Ramanjulu and Sudhakar 1997) and in horse gram (Reddy *et al.* 1990). Similarly,

JA treatments caused a significant increase in ammonia content both in leaves and roots of peanut seedlings, however, the increase was higher in roots than in leaves. Givan (1979) suggested that either rapid nitrate reduction or enhanced proteolysis might be the cause for the increased content of ammonia in plants under stress.

Immunoblot analysis of GS antisera (45 kDa) showed the cross-reaction with two proteins with 45 and 47 kDa (Fig. 2C) and reveals the increased activity of defensive enzyme under JA treatments. The enhanced activity of glutamine synthetase (Table 1) and the reaction with anti-GS antibody in peanut seedlings evidenced for the increase in amino acid pool. The GDH and GS/GOGAT and transamination activities largely decide the fate of accumulated ammonia during stress periods. Glutamine synthetase, key enzyme for the assimilation of ammonium into amino acids, catalyses the incorporation of NH_4 into glutamate to produce glutamine (Lea and Mifflin 1974). Increased GS activity was reported in mulberry under NaCl salinity (Ramanjulu *et al.* 1994, Giridarakumar 2002).

It was reported that the JA application results in disturbances in the photosynthetic efficiency (Reinbothe *et al.* 1993) which results in an increase in the ROS. JA also increases lipid peroxidation that was harmful to the cells (Orozco-Cardenas and Ryan 1999). We also noticed the enhancement of malondialdehyde, a product of lipid peroxidation, in peanut seedlings subjected to JA treatment and similar results were observed in *Scenedesmus incrassatulus* (Fedina and Benderliev 2000) after application of JA.

The increase in the activity of SOD in peanut seedlings under JA treatments revealed its involvement in plant tolerance to the oxidative stress caused under biotic and abiotic stresses. SODs were differentially regulated and respond to a variety of stress conditions.

In the present study, the greater increase in catalase activity of leaves than in roots may suggest its effective scavenging mechanism to remove H_2O_2 produced in leaves under JA treatment. A significant elevation in the activity of peroxidase was also recorded in roots and especially in leaves of peanut seedlings. In addition to chloroplasts, the peroxisomes produce H_2O_2 in association with oxidative metabolisms, such as photo-respiration and β -oxidation of lipids. Peroxisomal ROS production was increased during senescence and the derived ROS decompose cellular compartments (Babbs *et al.* 1989).

High peroxidase isozyme content was seen in peanut leaves after treatment by 100 and 250 μ M JA (Fig. 3). Similar results (increase in two isoperoxidases) were reported in 12-d-old barley seedlings treated with MeJA (Popova *et al.* 2003). The peroxidase system of higher plants exists in multiple isoforms that were developmentally regulated and highly reactive in response to exogenous stimuli (Gaspar *et al.* 1982). An increase in total peroxidase activity was a common

response to various oxidative stress factors (Gaspar *et al.* 1991). Increased total peroxidase activity and induction of peroxidase isoforms might be a useful adaptation in the defense mechanism of plants against ROS.

In conclusion, exposure of peanut seedlings to JA resulted in induction of pathogen related (PR) proteins,

increase of ammonia content, GS activity, lipid peroxidation, and activities of antioxidative enzymes such as SOD, CAT, POD and specific induction of two peroxidase isoforms. These results clearly indicate the role of JA in plant self-defense mechanism and contributes to investigation of JA role in the peanut plant.

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