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BRIEF COMMUNICATION

Effects of jasmonic acid on groundnut during early seedling growth

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Abstract

Exposure of groundnut seeds and seedlings to 25, 100, and 250 μM concentrations of jasmonic acid resulted in a reduction of germination percentage, growth, fresh mass, dry mass, chlorophyll content, chlorophyll stability index, proteins and an increase in free proline content and cell membrane injury. The results suggest the inhibitory effect of jasmonic acid on growth of the groundnut seedlings.

Additional key words: malondialdehyde, photosynthesis, proline, chlorophyll stability index, cell membrane stability.

Jasmonic acid (JA) and its methyl ester (JA-Me) have been isolated from a large number of plant species and for their inhibitory effects on growth and a variety of other activities in plants have been studied (Anderson 1989). Jasmonate can induce senescence, leaf abscission and inhibit germination (Sembdner and Parthier 1993, Creelman and Mullet 1997, Bogatek *et al.* 2002). It was shown that the concentrations between 0.1 and 1 μM JA-Me stimulated the cell division and microcalli formation and positively affected the development of potato plantlets *in vitro* (stem length) as differentiated root systems, whereas concentrations of 10 μM and higher caused compaction of the stem, roots and root hairs (Reinbothe *et al.* 1993). At concentrations > 50 μM, jasmonate induce senescence in plant cell cultures and excised leaves, senescence response which includes a loss of chlorophyll, degradation of chloroplast proteins such as ribulose bisphosphate carboxylase (Rakwal and Komatsu 2001, Agrawal *et al.* 2002). The presence of necrotic lesions was observed with applied JA resulting in necrosis and the programmed cell death (Rakwal and Komatsu 2000, 2001), exogenously applied methyl jasmonate (JA-Me) might induce the formation of necrotic lesions that closely resemble hypersensitive response lesions (Repka 2002).

This study aimed at evaluating the role of exogenously applied JA to the groundnut seeds and seedlings in counteracting the changing and stress-induced responses such as seedling growth, intracellular accumulation of proline, total proteins and cell membrane stability.

The seeds of groundnut (*Arachis hypogaea* L. cv. JL-24) were procured from Agricultural Research station, Anantapur. The healthy and uniform size seeds were surface sterilized with 0.1 % mercuric chloride solution for 30 s were thoroughly rinsed with deionized water.

To detect the percentage of germination the Petri plates lined with filter papers were moistened with distilled water for control, and 25, 100, and 250 μM JA solution for treatment, kept under irradiance of 150 W m^{-2} and temperature of 22 ± 2 °C in plant growth chamber for 2 d. The growth of plants in terms of root and shoot length was measured and recorded. The seeds germinated under JA treatment and control plants were germinated for 7 d. Then plants were thoroughly washed with deionized water, and the root and shoot parts were separated and length and fresh mass were measured. The material was dried at 80 °C in a hot air-oven for 48 h and dry mass were recorded. The total chlorophyll content was estimated in the leaves of control and JA treated seedlings, according to the method of Arnon (1949). The leaf material was homogenized in a prechilled mortar using 80 % cold acetone. The homogenate was centrifuged at 10 000 *g* for 30 min and the supernatant was collected. The sediment was resuspended with 80 %

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Abbreviations: JA - jasmonic acid; JA-Me - methyl jasmonate; CMS - cell membrane stability; CSI - chlorophyll stability index.
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cold acetone and centrifuged. All the supernatant collceted at each time were pooled and made to known volume with 80 % cold acetone. The absorbance of the acetone extract was measured at 645 and 663 nm on spectrophotometer (*Shimadzu 1601*, Tokyo, Japan). Chlorophyll stability index was determined by the method of Koloyereas (1958). Leaves (5 g) from control and JA treated seedlings were harvested and placed in a 100 cm^3 conical flask with 50 cm^3 of distilled water and heated in a water bath at 56 ± 1 °C for 30 min. The leaves without heating served as control. The CSI was expressed as the difference between the chlorophyll content without heating - chlorophyll content after heating.

The total protein content was estimated according to Lowry *et al.* (1951). The extraction and estimation of proline was done according to Bates *et al.* (1973).

Cell membrane stability was determined according to the method of Premachandra *et al.* (1992). Thirty leaf discs were sampled from control seedlings and placed in a 100 cm^3 flask and washed three times with deionized water. For the JA treatment, discs were submerged in 30 cm^3 of 25, 100, and 250 μM concentration solutions for 24 h at 10 °C. Discs submerged in distilled water served as controls. The electrical conductivity of the bathing solution was measured at 25 °C (C_1 and T_1). Then leaf discs were autoclaved for 15 min, cooled to 25 °C and electrical conductivity was measured (C_2 and T_2). Cell membrane stability of leaf tissues was calculated as per cent membrane injury from the formula: Percent membrane injury = $1 - [(1 - T_1/T_2) / (1-C_1/C_2)] \times 100$, where T_1 and T_2 are the first and second conductivity of JA treatment (25, 100, and 250 μM), and C_1 and C_2 are the first and second conductivity measurements of the control.

The exogenous addition of JA affected germination, growth, physiological and biochemical parameters (Table 1). With increasing concentrations of JA the percentage of seed germination was decreased by 33 % at 100 μM and 40 % at 250 μM concentration. JA and JA-Me inhibited the germination of non-dormant seeds and stimulated the germination of dormant seeds (Creelman and Mullet 1997, Bogatek *et al.* 2002). JA, JA-Me, ABA and ethylene inhibited germination of the recalcitrant seeds of *Quercus robur* L. (Finch-Savage *et al.* 1996). There are, some reports JA inhibits the germination of oily non-dormant seeds (Corbineau *et al.* 1988).

The lengths of shoot and root and also their fresh mass and dry masses decreased significantly at 100 and 250 μM concentrations. JA strongly inhibited the root growth by a mechanism not mediated by ethylene (Berger *et al.* 1996). Similarly in the present study, the root length was significantly decreased in JA treated seedlings but the repression was more at 100 μM and 250 μM concentrations. These results were supported by Staswick *et al.* (1992) in *Arabidopsis thaliana.* Inhibition of root growth may be a direct effect of JA-Me. The shoot length was also decreased with increasing concentrations. The fresh and dry masses of the JA-treated seedlings decreased significantly in 100 and 250 μM concentration. This decrease in dry mass accumulation of shoot in the present study could be attributed to decreased rates of photosynthesis and chlorophyll content as evidenced by Reinbothe *et al.* (1994).

Table 1. Germination percentage, shoot and root length, fresh and dry mass, total chlorophyll content, chlorophyll stability index, total protein content, proline content, and cell membrane injury as affected by 25, 100, and 250 μM concentrations of jasmonic acid in 7-d-old seedlings of groundnut. Means from 5 experiments ± SE. The mean values in a row followed by a different letter for each species are significantly different ($P \leq 0.05$) according to Duncan's multiple range test.

Parameter	Organ	Control	25 μM	100 μM	250 μM
Germination [%]	seed	-	87.00 ± 0.20a	67.00 ± 0.10b	60.00 ± 0.50c
Length [cm $plant^{-1}$]	shoot	6.50 ± 0.60a	6.20 ± 0.70b	4.20 ± 0.36b	3.80 ± 0.60c
	root	5.20 ± 0.92a	5.00 ± 0.90a	3.20 ± 0.91b	3.10 ± 0.50c
Fresh mass [g $plant^{-1}$]	shoot	1.09 ± 0.58a	0.89 ± 0.14b	0.76 ± 0.01b	0.62 ± 0.14c
	root	0.96 ± 0.02a	0.55 ± 0.18b	0.54 ± 0.16b	0.47 ± 0.12c
Dry mass [g $plant^{-1}$]	shoot	0.12 ± 0.18a	0.09 ± 0.14b	0.07 ± 0.10c	0.07 ± 0.05d
	root	0.08 ± 0.10a	0.06 ± 0.05b	0.05 ± 0.07c	0.05 ± 0.04d
Total chlorophyll content [mg g^{-1}]	leaves	1.42 ± 0.14a	1.38 ± 0.12a	1.00 ± 0.10b	0.76 ± 0.06c
CSI [%]	leaves	-	89.00 ± 0.50a	68.00 ± 0.30b	45.00 ± 0.50c
Total protein content [mg g^{-1}]	shoot	70.92 ± 1.74a	65.34 ± 0.52b	58.29 ± 0.36c	42.24 ± 0.98d
	root	45.28 ± 1.32a	40.09 ± 0.06b	37.56 ± 0.76c	30.02 ± 0.36d
Free proline content [μg g^{-1}]	shoot	24.43 ± 0.78a	128.10 ± 2.06b	137.30 ± 0.37c	177.10 ± 1.60d
	root	50.70 ± 0.30a	52.90 ± 0.55b	61.80 ± 0.60c	129.00 ± 0.60d
Cell membrane injury [%]	leaves	-	46.00 ± 0.12a	82.00 ± 0.10b	95.00 ± 0.13c

The total chlorophyll content declined correspondingly with the decline in CSI in the leaves of JA treated 7-d-old seedlings of groundnut, indicating a role of JA in chlorophyll degradation and senescence. The treatment with JA to leaves decreased the expression of nuclear and chloroplast genes involved in photosynthesis and also caused a loss of chlorophyll (Weidhase *et al.* 1987, Bunker *et al.* 1995).

JA treatments decreased total shoot and root protein contents in groundnut seedlings. The shoot protein content was more decreased than in roots in JA treated seedlings. Changes in proteins can result from a variety of environmental stresses. The assumption is that exogenously applied jasmonates, as presumed stressors, provoke alterations in the protein content (Maslenkova *et al.* 1992).

The application of JA increased the free proline content 5-fold at 100 and 7-fold at 250 μM concentrations. The increase was more expressive in shoot than in root at all JA concentrations. Free proline accumulation under JA treatments could be due to an increased synthesis or to an inhibition in the oxidation of proline. Creelmann *et al.* (1992) reported an accumulation of mRNAs encoding proline rich proteins under low water potential with an increase of endogenous jasmonates in soybean seedlings.

The membrane injury was increased in treated seedlings due to the electrolyte leakage. The cell membrane injury leads to the increase of MDA content (Fedina and Benderliev 2000). Increased rates of lipid peroxidation, lipoxygenase induction, synthesis of defensive protease inhibitors and peroxidases were also reported to be induced by methyl jasmonate in several plant species (Farmer and Ryan 1990). The maintenance of membrane integrity and there by reducing the amount of solute leakage as a result of stress was considered as a stress tolerance. It was assumed that the amount of electrolyte leakage was a function of membrane integrity. Cellular membrane stability has been a commonly used physiological screening method for environmental stresses (Blum and Ebercon 1981). The increase in jasmonate was correlated with lipid peroxidation, in terms of MDA content, which suggests that the production of jasmonate may not be regulating germination but rather is a consequence of membrane damage.

The results indicates that JA acts as an inhibitory compound, affecting various morphological, physiological and biochemical parameters like CMS, CSI, total chlorophyll content, total proteins, and proline contents of groundnut (cv. JL-24) during seedling growth.

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