BIOLOGIA PLANTARUM 41 (2): 307-311, 1998

BRIEF COMMUNICATION

Effect of salicylic acid on nodulation, nitrogenous compounds and related enzymes of *Vigna mungo*

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Abstract

Plants of *Vigna mungo* raised from seeds presoaked in salicylic acid (0.0, 0.01, 0.1 and 1.0 mM) and nodulated with the cowpea strain of *Rhizobium leguminosarum* were analysed 15 and 30 d after sowing. The foliar nitrate and nitrite contents were varying but soluble protein and total nitrogen contents were lower in treated than control plants. Nitrate reductase activity was increased at the two lower concentrations of 0.01 and 0.1 mM but was inhibited at the highest concentration used (1.0 mM). The number of nodules, their leghemoglobin and protein contents and nitrogenase activity of roots were reduced.

Additional key words: black gram, plant growth regulator, nitrate reductase, nitrogenase, phenolics.

Salicylic acid (SA) is a secondary metabolite of microbes and higher plants. It was found to induce flowering in plants, to block ethylene synthesis in pear cell suspension cultures, to inhibit phosphate uptake in barley roots and potassium absorption in oat roots, to induce rooting in combination with indole acetic acid, to reverse abscisic acid-induced leaf abscission, to reduce transpiration in kidney bean and to increase yield in mung bean. SA or acetyl SA injected into or sprayed on to the plants or added to soil could protect plants against pathogenic infections (for review see Raskin 1992). The regulatory roles played by SA, whether generated endogenously or applied exogenously, prompted him to recommend it as another plant growth regulator (PGR). This paper focused on the effects of pre-soaking seeds of black gram with SA on nodulation and symbiotic nitrogen fixation, an aspect hitherto neglected in SA studies.

Received 15 September 1997, *accepted* 25 May 1998.
Acknowledgements: The authors thank Dr. B. Ambalanathan and Dr. Saroja Sundararajan of the Centre for Post Graduate Studies, Pondicherry for facilities and encouragement.

Seeds of black gram [*Vigna mungo* (L.) Hepper cv. T-9] were obtained from Tamilnadu Agricultural University, Coimbatore and presoaked in 0.01, 0.10, 1.00 mM salicylic acid, or in distilled water (control) for 20 h. They were then washed thoroughly, mixed with a commercial preparation of *Rhizobium leguminosarum* (cowpea strain), air dried, and then sown in earthen pots filled with a mixture of red soil:sand:farm-yard manure (2:1:1, v/v). Plants were maintained in greenhouse [temperature 32 ± 2 °C, relative humidity 65 ± 5 %, maximum irradiance 1400 μmol(PAR) m^{-2} s^{-1}, photoperiod 12-14 h] and watered daily just to keep the soil moist. The first trifoliate leaves were sampled at two stages 15 and 30 days after sowing (DAS) for analyses.

Soluble protein content was estimated by the Folin-phenol method according to Lowry *et al.* (1951). Total free nitrogen was estimated after digesting the samples with H_2SO_4 by a modified micro-Kjeldahl method (Lang 1958). Nitrate content was determined spectrophotometrically by reducing it to nitrite with Zn after conversion to an azo compound (Woolley *et al.* 1960). Nitrite was estimated similarly skipping the reduction step. The number of nodules was counted at both stages, but leghemoglobin (LHb) content was estimated only at 30 DAS by converting it to pyridine hemochromogen (Appleby and Bergersen 1980).

Nitrate reductase activity was assayed following method of Jaworski (1971) with modifications suggested by Muthuchelian *et al.* (1990). The acetylene reduction technique of Stewart *et al.* (1967) was followed for assaying nitrogenase activity. Data were analysed statistically by Tukey's multiple range test (Zar 1984).

Nitrate, nitrite and total nitrogen content of leaves of 15- and 30-d-old plants were quantified to examine the effect of SA on nitrogen assimilation. The content of nitrate in the leaves was not altered drastically after treatment though the values were slightly less than control ones in all cases. No significant changes in nitrite content due to treatment were evident in both stages (Fig. 1). On the other hand, the total nitrogen content in leaves was reduced markedly (15 DAS by 48.6 to 65.7 % and 30 DAS by 8.0 to 36.0 %). Soluble leaf protein content was also suppressed by SA treatments, the reduction being 12.5, 11.4 and 45.3 % (15 DAS) and 4.2, 6.4 and 22.2 % (30 DAS) for 0.01, 0.1, 1.0 mM SA, respectively (Fig. 1).

Low concentrations of SA (0.01 and 0.1 mM) stimulated the activity of nitrate reductase by 100 and 42.9 %, respectively (at 15 DAS); the highest concentration (1.0 mM) depressed it by 57.1 %. At 30 DAS, the similar trend prevailed but with less degree of stimulation or inhibition (Table 1).

The nodules were small and developing at 15 DAS but had matured to full size by 30 DAS. At both stages, plants pretreated with 0.1 and 1.0 mM SA had fewer nodules. In contrast, 0.01 mM SA induced more nodules in both stages (Fig. 1). The LHb content was comparable in treated and control plants. Nitrogenase activity was depressed by 46, 55 and 64 % in nodules of 0.01, 0.1 and 1.0 mM SA treated plants. The nodular protein content declined in all treatments (Table 1).

The stimulation at low concentrations of SA and inhibition at high concentrations has already been demonstrated by Jain and Srivastava (1981) since 0.1 mM SA triggered NRA in maize seedlings while 5.0 and 10.0 mM SA inhibited it. A number of phenolics appeared to behave so (Schrader and Hageman 1967, Ray and Laloraya

1984). Similarly, the decline in leaf protein content consequent to SA pretreatment was in agreement with the results of Asthana and Srivastava (1978) in oats. The inhibition at 1.0 mM SA is consistent with the inhibition of the NRA (Schrader and Hageman 1967). There was a correlation between protein content in nodules and

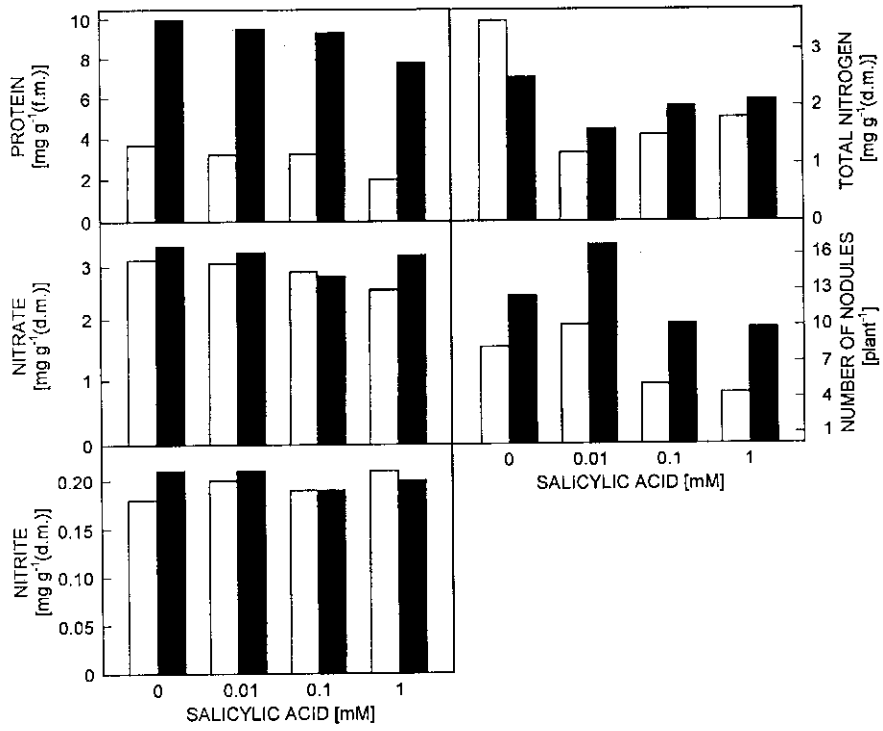

Fig. 1. Effect of presoaking of seeds with 0.0 (control), 0.01, 0.10, and 1.00 mM salicylic acid on protein, nitrate, nitrite, and total nitrogen contents of leaves and the number of nodules in black gram plants. Values are means of 10 determinations measured at 15 DAS (*empty columns*) or 30 DAS (*dark columns*).

Table 1. Effect of presoaking of seeds with 0.0, 0.01, 0.10, and 1.0 mM salicylic acid on foliar nitrate reductase activity (NRA [nmol(NO_2) kg^{-1}(f.m.) s^{-1}]), root nitrogenase activity [μmol(ethylene reduced) g^{-1}(f.m.) s^{-1}] and nodular protein and leghemoglobin (LHb) contents [mg g^{-1}(f.m.)]. Numbers followed by different letters differ by Tukey's multiple range test at 5 % level ($n = 4$).

Parameter	DAS	0.0	0.01	0.10	1.00
NRA	15	700a	1400b	1000c	300d
	30	2400b	3600c	3600c	1650a
Nitrogenase	30	0.31a	0.17b	0.14bc	0.11c
Protein content	30	15.0a	14.1a	12.4b	10.3c
LHb content	30	1.01a	1.25b	1.05a	0.95a

their nitrogenase activity. The content of LHb was not seriously depleted. Its content was not correlated with nitrogenase activity, though Johnson and Hume (1973) and Nash and Schulman (1976) have deduced that LHb is a prerequisite for induction of nitrogenase activity and thus initiates nitrogen fixation. However, nitrogenase is regulated by a number of factors independently. Since SA is implicated in inducing host resistance to bacterial, fungal and viral infections, its interference in black gram-*Rhizobium* symbiosis cannot be gainsaid. The supression of nodulation and nitrogenase activity could adversely affect the nitrogen economy. The foliar NRA might have been enhanced to compensate the insufficient supply of symbiotic nitrogen. That such a trend persisted after 30 DAS is a cause of concern since the presence of SA in the rhizosphere of several weed and crop plants has been sufficiently documented (Pareek and Gaur 1973, Shettel and Balke 1983). In this background, it would be interesting to study the role of SA on nitrogen assimilation as an allelopathic chemical.

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