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EFFECT OF HUMIC ACID ON ROOT NODULATION AND NITROGENASE ACTIVITY OF *SESBANIA SESBAN* (L.) MERRIL

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Abstract

Effect of different concentrations of sodium humate applied to the root system of hydroponically grown seedling of *Sesbania sesban* were studied on the seedling growth, root nodulation and nitrogenase activity. HA treatment at cotyledonary leaf stage at the time of inoculation increased the production of secondary laterals. Dry weight of root and shoot increased at 0.075 to 0.225% HA. Root nodules appeared 5 — 7d earlier and the number of nodules per plant and their fresh weight was significantly higher in HA treated seedlings. Activity of nitrogenase increased following HA treatments. HA appeared to affect both the infection process as well as the formation and function of nodules in *S. sesban*.

Introduction

The effect of humic acid (HA) in root morphogensis is well documented. Treatment with Na-humate promotes production of profused secondary laterals (Azam & Malik, 1983; Malik & Azam 1985; O'Donnel, 1973; Schnitzer, 1967; Schnitzer & Poapost, 1967) which is more efficient in exploiting nutrient resources from the soil particularly under stress conditions. A positive role of HA in water and ion uptake has been demonstrated (Azam & Malik 1983; Briggs & Robertson 1957; Cheng 1977; Guminski *et al.*, 1983). Rao (1977) observed positive effect of HA in the proliferation of *Rhizobium leguminosarum.* Stimulatory effects of HA on nodule mass and plant dry matter content has been reported in soybean, peanut and clover (Tan & Tantiwiramanond, 1984). However, role of HA on the correlative control of host plant and root-nodule development is not known. Results of the effect of HA on plant growth and root nodule differentiation in *Sesbania sesban* is reported.

Materials and Methods

Plant material and growth condition: Seeds of *Sesbania sesban* (L.) Merril surface sterilized with 10% sodium hypochlorite were germinated in 0.7% water-agar in Petridishes kept in a controlled environment growth room at a light intensity of 9,000 lux with 16h photoperiod and 30 ± 2°C day and 26 ± 2°C night temperatures.

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Humic acid preparation: Humic acid was prepared following the method of Malik & Azam (1985). The dialyzed HA solution was adjusted to pH 6.8 with 0.1N NaOH with C-content 1.25 mg ml^{-1} and N-content 0.0114 mg ml^{-1} as determined by colorimetric (Anon., 1979) and microkjeldhal methods (Bremner, 1965). The C-content of HA was used as a dose parameter for making HA solution of different concentrations.

Inoculation and HA treatments to the seedlings: Seedlins at the cotyledonary leaf stage were inoculated with a suspension of *Rhizobium* strain Ses D_2 isolated from the nodules of field grown *S. sesban* by the method of Vincent (1970). The seedlings were planted into laboratory made γ-sterilized (2.5 M rad) polyethylene pouches containing a double folded Whatman No. 1 filter paper. Twenty ml sterile culture medium (quarter strength N-free Hoagland solution pH 6.8) was added to the pouches in control treatment.

Filter-sterilized (Disposable filter 0.2 µm Schleicher and Schull, Germany) HA solution was added to the culture medium in the pouch at the time of inoculation. To protect the seedlings from exposure to direct light, the growth pouches were wrapped with black carbon paper and the pouches containing seedlings were placed on a thermopore tray in hanging position.

Determination of effect of humic acid on rhizobial growth: Rhizobium strain *Ses* D_2 was cultured on yeast mannitol (YM) broth at 30°C on a shaking water bath. After 8h, one ml aliquot of the culture was added to fresh yeast mannitol broth. Filter-sterilized HA was added to 3 of these flasks at the final concentration of 0.075% HA. Rhizobial growth was monitored at 1h interval for 24 h by measuring the optical density of the YM broth culture at 660 nm against blanks. The blanks consisted of (a) Yeast mannitol broth + 1 ml of distilled water (b) Yeast mannitol broth + 1 ml of HA at 0.075%.

Detection of infected root hairs: Infected root hairs were detected and counted following the methods of Phillips (1971).

Measurement of Nitrogenase activity: Nitrogenase activity was measured in a gas chromatograph (*Electrometer model 180 Carlo Erba*) following Bergersen (1980).

Results

Seedling growth: Humic acid promoted seedling growth from the very first day of HA treatment. Observations made at harvest indicated that the root portion was more affected than the shoot. The number of secondary laterals increased as compared to the control with an increase of HA concentration from 0.075% to 0.275%. The number of primary lateral roots remained unaffected except at 0.15% HA. HA treated plants showed an increase in the dry weight of root and shoot with a maximum increase noticed at 0.075% HA (Table 1).

Table 1. Effect of humic acid on seedling growth of *Sesbania sesban*.

Treatment	Shoot Length (cm)	Root Length (cm)	Number of lateral roots plant^{-1}		Dry weight of plant (g)	
			Primary	Secondary	Dry wt. of nodulated root plant^{-1} (mg)	Dry wt. of shoot plant^{-1} (mg)
Control	4.2	6.5	16 (± 3.24)	24 (± 2.425)	5	13
HA (0.075%)	5.1	7	11 (± 0.66)	33 (± 5.51)	8	17
HA (0.15%)	5	9	22 (± 5.81)	36 (± 4.70)	7	16
HA (0.225%)	5	10	10 (± 5.70)	45 (± 6.35)	7	15
HA (0.275%)	4.2	10	13 (± 2.84)	52 (± 2.99)	5.7	12

Treatments were made at the cotyledonary leaf stage (7d after germination) and the measurements were made at harvest (17-21d after treatment). Data are mean of 15 replicates per treatment, figures in parentheses; represent the standard error.

Table 2. Effect of humic acid (HA) on root nodulation and nitrogenase activity.

Treatment	Number of infected root hair	Number of nodules plant^{-1}	Fresh weight of nodules plant^{-1} (mg)	Nitrogenase[1] activity plant^{-1}	Specific[2] nitrogenase activity
Control	18	3.66	2.9	0.093 (± 0.024)	34.40 (± 2.87)
HA (0.075%)	20	6.33 (± 0.33)	7 (± 2)	0.309 (± .102)	53.77 (± 18.71)
HA (0.15%)	31	6.66 (± 0.33)	19 (± 3)	0.251 (± .144)	24.71 (±4.4)
HA (0.225%)	26	6.66 (± 0.33)	9.8 (± 2.6)	0.327 (± 0.039)	48.87 (± 12.28)
HA (0.275%)	24.5	5.33 (± 1.45)	5.4 (± 1)	0.226 (± 0.0039)	42.99 (± 14.40)

Number of infected root hairs were counted 72 h after inoculation and HA treatment, and other parameters were taken 17-21 d after inoculation and HA treatment. Data are mean of 15 replicates per treatment^{-1}. Figures in parentheses represent standard error.

1 = Nitrogenase activity expressed as μ mol C_2H_4 plant^{-1} h^{-1}.

2 = Nitrogenase activity expressed as μ mol C_2H_4 g^{-1} nodule h^{-1} plant^{-1}.

Nodule development and nitrogenase activity: Different concentrations of HA increased the number of infected root hairs with maximum increase of 72% obtained at 0.15% HA. HA stimulated proliferation of *Rhizobium* since in HA (0.075%) treatment OD was 0.55 as compared to 0.46 in the control. The nodules appeared 4-5d earlier with greater number of nodules in HA treated seedlings as compared to *Rhizobium* culture in distilled water control. No marked difference was observed among the treatments (0.075, 0.15 and 0.225%) for this parameter. The specific activity of nitrogenase increased in HA treatments except 0.15% HA which showed a decrease (Table 2).

Discussion

HA has been reported to increase root development and seedling growth (Azam & Malik, 1983; Lee & Bartlett, 1976; Malik & Azam, 1985). The effect of HA has been related to an increased water and ion uptake by the plant. Early nodulation and greater nodules per plant may be attributed to the stimulatory effect of HA on the infection process of nodules. The greater weight of nodules in HA treatment suggest the favourable effect of HA on the nodule formation. Our results corroborates the findings of Tan & Tantiwiramanond (1984) in soybean and peanut. HA significantly stimulated nitrogenase activity. The pivotal role of photosynthesis in nitrogen fixation is well established (Hardy & Havelka, 1975; Bethlenfalvay & Phillips 1977). Hence this increase in N_2-ase activity may be associated with the positive effect of HA on plant growth.

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