Humic Acid Seed and Substrate Treatments Promote Seedling Root Development

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Abstract. Seed of *Pelargonium ×hortorum* L.H. Bailey ‘Freckles’ (geranium) and *Tagetes patula* L. ‘Bonanza’ (marigold) were soaked for 12, 24, or 48 h in solutions containing 0 (deionized water), 5000, 10,000, or 15,000 mg·L⁻¹ humic acid (HA) or nutrient controls (NC) containing similar levels of nutrients prior to planting. Soaking in deionized water (DI) and NC treatments had no significant effect on root fresh weight. However, several of the HA treatments increased root fresh weight of marigold seedlings, and all increased geranium root fresh weight. Percentage of germination and shoot fresh weight were not significantly affected by treatment. Seed of *Cucumis sativus* L. ‘Salad Bush’ (cucumber), *Cucurbita pepo* L. ‘Golden Summer Crookneck’ (squash), ‘Freckles’ geranium and ‘Bonanza’ marigold were sown into 15-cell plug trays (5 mL volume), and the substrate was drenched with DI, 2500 or 5000 mg·L⁻¹ HA, or 2500 or 5000 mg·L⁻¹ NC. DI and NC treatments did not affect root fresh weight. However, cucumber, squash, and marigold seedlings germinated in substrate drenched with 2500 and 5000 mg·L⁻¹ HA and geranium seedlings germinated in substrate drenched with 2500 mg·L⁻¹ HA had significantly higher root fresh weight than did seedlings from all other treatments. Percentage of germination and shoot fresh weight were not significantly affected by treatment. ‘Salad Bush’ cucumber and ‘Golden Summer Crookneck’ squash seedlings germinated on germination towels soaked with 2500 or 5000 mg·L⁻¹ HA, had significantly higher root fresh weight than did seedlings germinated on towels soaked with DI or NC solutions. Treatment with HA did not affect shoot fresh weight or the number of lateral roots. However, HA treatment increased the total length of lateral roots. The increase in lateral root growth occurred primarily in lateral roots developing from the lower hypocotyl.

Humus acids including humic, fulvic, and hymatomelonic acids occur widely in mineral soils, peats, and natural waters. Humic acids (HA) are high molecular weight aromatic organic acids that have a polyphenolic backbone with numerous carbohydrate and peptide side chains that give rise to a complex array of structures (Hays, 1989).

One of the most common responses of plants to HA has been the promotion of root development. David et al. (1994) reported that tomato (*Lycopersicon esculentum* Mill.) plants grown in HA-containing solutions had higher root fresh and dry weights and contained higher levels of certain mineral elements in the shoots and roots than did plants grown in control solutions. Sanders et al. (1990) incorporated HA into a fluid drill gel used in carrot (*Daucus carota* L.) production and found that average root fresh weight of seedlings grown from

seed drilled with gel containing HA was more than twice that of seed drilled with a control gel. Mylonas and McCants (1980) reported that tobacco (*Nicotiana tabacum* L.) plants grown on filter paper saturated with HA solutions had higher root numbers and total root length than those grown on filter paper saturated with nutrient solutions or deionized water. Malik and Azam (1985) and Vaughan and Linehan (1976) reported increased root and shoot fresh and dry weights in wheat (*Triticum aestivum* L.) seedlings grown in nutrient solutions containing HA.

To date, HA has been applied through fluid drilling or by placing seedlings in nutrient solutions or on filter paper containing HA. The objective of this study was to determine if HA when applied to the seed or the substrate would increase root growth in seedlings.

Materials and Methods

Humic acid seed soak. Solutions containing 5000, 10,000, and 15,000 mg·L⁻¹ HA were prepared by adding the commercial HA preparation Enersol SC (American Colloid Co., Arlington Heights, Ill.) to deionized water (DI). The nutrient control solutions (NC) designed to contain similar mineral nutrients as the HA solutions were prepared by mixing (mg·L⁻¹) 5.0 NH₄NO₃; 3.2 NH₄H₂PO₄; 163.5 KCl; 62.7 Ca(NO₃)₂; 57.7 MgSO₄; 39.8

CaSO₄; 1.1 H₃BO₃; 114.9 FeNaDTPA; and 0.7 MnCl₂ in DI per 1000 mg·L⁻¹ HA. All solutions were adjusted to a pH of 9.5 using 1.0 N NaOH. Electrical conductivities (EC) were 1.8, 3.3, and 4.9 mS·cm⁻¹ and 4.7, 7.7, and 9.4 mS·cm⁻¹ for the 5000, 10,000, and 15,000 mg·L⁻¹ HA solutions and for the 5000, 10,000, and 15,000 mg·L⁻¹ NC solutions, respectively.

Seed of ‘Freckles’ geranium and ‘Bonanza’ marigold in 100 mL of each treatment solution were placed on an Innova 2100 shaker (New Brunswick Scientific, Edison, N.J.) and shaken at 150 rpm for 12, 24, or 48 h. After soaking, seeds were separated from the treatment solutions by vacuum filtration. A DI and nonsoaked treatment were included for each soaking duration to create a balanced experimental design. Seeds were sown into 15-cell plastic plug trays (#288 with a volume of 5 mL) filled with 70% sphagnum peat and 30% vermiculite (v/v) and adjusted to pH 5.5 using calcium carbonate. Trays were transferred to a mist house and placed in a completely randomized design. Overhead mist, controlled by a Gemini 6 timer (Phytotronics, St. Louis), was applied for 15 s every 10 min.

After 2 weeks, percentage of germination, root fresh weight (total weight of root tissue from one tray/number of seedlings germinated), and shoot fresh weight (total weight of shoot tissue from one tray/number of seedlings germinated) were recorded. A 15-cell tray served as a replication and each of the treatment solutions was replicated three times for each species. The experiment was repeated three times (blocked over time). An analysis of variance was conducted to determine if DI, HA, or NC treatments affected seedling development. Where significance differences occurred, a LSD mean separation test ($\alpha = 0.05$) was conducted to establish significant differences between means.

Humic acid substrate drench. Seed of ‘Salad Bush’ cucumber, ‘Golden Summer Crookneck’ squash, ‘Freckles’ geranium, and ‘Bonanza’ marigold were sown into 15-cell plastic plug trays filled with substrate as previously described. Solutions containing 2500 and 5000 mg·L⁻¹ HA and NC solutions were prepared as previously described. EC were 1.0, 1.9, 2.1, and 4.6 mS·cm⁻¹ for the 2500 and 5000 mg·L⁻¹ HA and 2500 and 5000 mg·L⁻¹ NC solutions, respectively. A DI treatment was also included for a total of five treatment solutions.

After seeds were sown, 3 mL of the appropriate treatment solution was pipetted into each plug cell to saturate the substrate. Trays were placed on 15 × 10 cm subirrigation mats in a growth chamber set at a constant 22 °C and arranged in a completely randomized design. A 12-h photoperiod was maintained with an average irradiance of 218 μ mol·m⁻²·s⁻¹ using a combination of incandescent and cool-white fluorescent lamps.

Data collection, replication, and statistical analysis were as previously described.

Humic acid-treated germination towels. Solutions were prepared as described for the substrate drench experiment, and each towel was soaked with 40 mL of treatment solution.

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Ten seeds each of 'Salad Bush' cucumber and 'Golden Summer Crookneck' squash were placed across each towel ≈ 4 cm from the top. Towels were rolled, sealed with a paper clip, and placed in an upright position in a plastic container. The container was covered with a clear plastic bag and placed in a growth chamber under the conditions previously described. After 2 weeks, root and shoot fresh weights, and number and total length of secondary roots >5 mm long were recorded. Replication and statistical analysis were as previously described.

Results

Humic acid seed soak. Marigold percentage of germination ranged from 77% to 87% and shoot fresh weight from 60 to 80 mg. Neither was significantly different among the treatments (data not shown). Root fresh weight was not significantly affected by soaking in DI or NC solutions (Table 1). However, it was significantly higher for geranium seed soaked in 5000 mg·L⁻¹ HA for 48 h, 10,000 mg·L⁻¹ HA for 12 and 24 h, and 15,000 mg·L⁻¹ HA for 12 and 48 h than for unsoaked seed and seed soaked in DI or their respective NC solutions (Table 1). Main effects of HA concentration, soaking duration, and block did not significantly affect marigold root fresh weight.

Geranium percentage of germination ranged from 81% to 91% and shoot fresh weight from 80 to 110 mg and neither was significantly affected by the treatments (data

not shown). Geranium root fresh weight for seedlings germinated from unsoaked seed or seed soaked in DI or NC solutions were not significantly different (Table 1). Geranium root fresh weight was significantly higher for seedlings developing from seed soaked in all HA treatments than for seedlings developing from unsoaked seed and seed soaked in DI or their respective NC solutions (Table 1). Main effects of HA concentration, soaking duration, and block did not significantly affect geranium root fresh weight.

Humic acid substrate drench. Percentage of germination did not vary significantly among the treatments and ranged from 80% to 95% depending upon species (data not shown). Marigold, squash, and cucumber seedlings germinated in substrate drenched with HA had significantly higher root fresh weights than those germinated in substrate drenched with DI or NC solutions (Table 2). Humic acid concentration did not significantly affect root fresh weight of marigold, squash or cucumber seedlings. Root fresh weight for geranium seedlings germinated in substrate drenched with 2500 mg·L⁻¹ HA was significantly higher than for seedlings germinated in substrate drenched with 5000 mg·L⁻¹ HA, DI, and NC solutions (Table 2). Shoot fresh weights ranged from 56 to 61 mg, 750 to 810 mg, 89 to 110 mg, and 430 to 500 mg for marigold, squash, geranium, and cucumber, respectively, and were not significantly different among treatments (data not shown).

Humic acid-treated germination towels. Root fresh weight for cucumber and squash

was significantly higher for seedlings germinated on towels soaked with HA than for those germinated on towels soaked with DI or NC solutions (Table 3). Humic acid concentration did not significantly affect root fresh weight. Number of secondary roots ranged from 58–62 and 64–72 for cucumber and squash, respectively, and were not affected by treatment (data not shown). There was no significant difference in secondary root length among the DI and NC treatments (Table 3). Seedlings germinated on towels soaked with HA had significantly higher secondary root lengths than those germinated in the respective NC solutions or seedlings germinated on DI-soaked towels. However, HA concentration did not significantly affect secondary root length. (Table 3). The increase in root length was primarily a result of the increased length of lateral roots developing from the lower hypocotyl (Fig. 1).

Discussion

Humic acid application as seed and substrate drenches consistently resulted in increased root fresh weight in seedlings. Our results are similar to those of David et al. (1994), Malik and Azam (1985), Sanders et al. (1990), Tan and Nopamornbodi (1979), and Vaughan and Linehan (1976), who reported increased root weight following HA application using other methods. Nutrient controls did not increase root fresh weight, which agrees with the conclusions of David et al. (1994) who proposed that HA affected

Table 1. Root fresh weight for marigold and geranium seedlings germinated from seed soaked in deionized water, humic acid, and nutrient solutions for varying durations.

Treatment ^z	Duration (h)	Root fresh wt (mg)	
		Marigold	Geranium
Unsoaked	12	50	80
	24	60	70
	48	50	70
Deionized water	12	70	80
	24	60	80
	48	60	80
5000 HA	12	70	110
	24	70	120
	48	80	110
10,000 HA	12	80	100
	24	80	110
	48	70	110
15,000 HA	12	80	110
	24	70	110
	48	80	110
5000 NC	12	60	80
	24	70	80
	48	60	80
10,000 NC	12	60	80
	24	60	70
	48	60	80
15,000 NC	12	50	70
	24	60	80
	48	60	70
<i>Significance of F</i>			
Treatment		*	*
LSD ($\alpha = 0.05$)		20	20

^zIndicates seed soaked in deionized water, humic acid (HA) or nutrient controls (NC) at the indicated concentration as mg·L⁻¹.

*Significant at $\alpha = 0.05$.

Table 2. Root fresh weight (mg) for cucumber, squash, geranium, and marigold seedlings germinated in substrate drenched with deionized water, humic acid (HA), and nutrient control (NC) solutions.

Treatment ^z	Marigold	Squash	Cucumber	Geranium
Deionized water	21	350	210	31
2500 HA	36	400	300	51
5000 HA	37	400	310	31
2500 NC	23	340	190	31
5000 NC	10	340	190	20
<i>Significance of F</i>				
Treatment	***	**	*	*
LSD ($\alpha = 0.05$)	13	40	40	12

^zIndicates seed drenched with deionized water, humic acid (HA), or nutrient controls (NC) at the indicated concentration as mg·L⁻¹.

***Significant at $\alpha = 0.05$, 0.01, or 0.001, respectively.

Table 3. Root fresh weight and secondary root length for seedlings of cucumber and squash germinated on towels soaked with deionized water, nutrient (NC) solutions, and humic acid (HA).

Treatments ^z	Root fresh wt (mg)		Secondary root length (mm)	
	Cucumber	Squash	Cucumber	Squash
Deionized water	97	240	441	920
2500 HA	155	313	957	1450
5000 HA	175	357	975	1525
2500 NC	120	236	502	875
5000 NC	107	240	484	915
<i>Significance of F</i>				
Treatment	***	***	***	***
LSD ($\alpha = 0.05$)	22	16	220	175

^zIndicates seed germinated on towels soaked with deionized water, humic acid (HA), or nutrient controls (NC) at the indicated concentration as mg·L⁻¹.

***Significant at $\alpha = 0.001$.

Fig. 1. Root growth of *Cucumis sativus* 'Salad Bush' (cucumber) germinated on germination towels drenched with (left to right) deionized water, 2500 mg·L⁻¹ humic acid (HA), 2500 mg·L⁻¹ nutrient control (NC), 5000 mg·L⁻¹ HA, or 5000 mg·L⁻¹ NC.

seedling growth through mechanisms other than nutrient supply.

Length of the secondary roots was significantly increased by application of HA. These results are consistent with the findings of Malik and Azam (1985) and Mylonas and McCants (1980) who reported a 500% increase in root length in wheat and increased secondary root length in tobacco, respectively. Vaughan (1974) suggested that increased secondary root growth resulted from the formation of complexes of HA with iron in plant tissues preventing the cessation of root growth.

The number of secondary roots was not affected by the application of HA; this contrasts with increased secondary root number observed by O'Donnell (1973) on vegetative geranium cuttings and Mylonas and McCants (1980) in tobacco. This discrepancy may have been due to natural differences in growth patterns between species tested or method of application.

Increased root weight and increased lateral root growth are common responses of seedlings to HA. However, there are at least four distinct types of roots including: 1) adventitious roots; 2) taproots; 3) lateral roots; and 4) basal roots (Aung, 1982; Kahn et al., 1985; Leskovar and Stoffella, 1995; Stoffella, et al., 1979, 1988). These different types of roots are under different genetic controls (Zobel, 1986). However, reports on the effect of HA to date have generally either not differentiated between root type or dealt only with roots as either taproots or lateral roots. From our results with cucumber and squash, it is clear that HA did not cause a general increase in root growth or a general increase in growth and elongation of all lateral roots. Instead, HA specifically promoted the growth of the laterals developing from the lower hypocotyl in the

region where basal roots would be expected to form. The origin of these roots has not been determined, and therefore, they cannot be specifically classified as basal roots. However, from these results it is clear that future studies that examine the effects of HA on root systems must differentiate between root types in order to better define the effect of HA on root growth.

Shoot fresh weight was not significantly increased by HA application. This contrasts with the findings of Malik and Azam (1985) and Vaughan and Linehan (1976) who reported increased shoot fresh and dry weight in seedlings grown in liquid media supplemented with HA, and of Tan and Nopamornbodi (1979), who reported increased shoot fresh weight in corn (*Zea mays* L.) seedlings grown in Hoagland's solution amended with HA. These differences in our results as compared to previous reports could be due to several reasons. First, previous research was conducted in nutrient solutions, whereas our studies were conducted using seed or substrate treatments. Secondly, species may differ in response to HA application. Finally, data in our studies were taken shortly after germination, often before true leaves fully developed. If plants had been allowed to develop for a longer period of time, the increased root weight may have resulted in increased shoot fresh weight.

From this research we conclude that HA application as a seed treatment or substrate drench is effective in increasing root fresh weight in cucumber, squash, geranium, and marigold. Therefore, the potential exists for using HA to increase root growth of seedlings and improve stand establishment in commercial applications such as plug production.

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