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EFFECT OF HUMIC ACID SOAKING OF SEEDS ON SEEDLING GROWTH OF WHEAT (*Triticum aestivum* L.) UNDER DIFFERENT CONDITIONS

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Abstract:

Soaking of wheat seeds in humic acid solution resulted in an increase in the seedling growth. Roots were highly developed and extensive development of secondary roots took place. Soaking of seeds in humic acid solution enhanced the uptake of water by roots whereas shoot portion gathered more dry weight. Maximum effect was obtained after 8 hours of seed soaking. Soaking of seeds also favoured the growth of seedlings in the presence of high concentrations of salts in the growth medium.

Introduction

Soil humus which is the end product of degradative and metabolic activities in soil, is a mixture of numerous compounds generally divided into humic acid (HA), fulvic acid (FA) and humins. The three compounds resemble structurally but differ in molecular weight and functional group content (Schnitzer & Khan, 1972).

In a preliminay study, addition of humic acid in culture medium increased the growth of wheat seedlings upto 500%. The presence of nitrogen in the medium suppressed or completely inhibited the growth enhancing property of humic acid. In contrast to the favourable effects of humic acid in the culture medium (Fialova, 1969; Kononova, 1966; Lee & Bartlelt, 1976; Vaughan & Linehan, 1976; Vaughan & Macdonald, 1976) there are reports which show that soaking of seeds or plant parts in humic acid results in a similar effect on plant growth (Smidova, 1960; Schnitzer, 1967; Schnitzer & Poapst, 1967; O'Donnell, 1973; Vaughan & Linehan, 1976). Any effect of humic compounds in counteracting the deleterious effects of salts on seed germination and plant growth is not known. Studies reported here were to look for the possibility of using humic acid soaking of seeds in enhancing plant growth and counteracting salinity.

Materials and Methods

Humic acid used in this study was obtained from a garden soil. One hundred g

sieved and air-dried soil was shaken in one l of extractant prepared by dissolving 4 g NaOH and 46.44 g $Na_4\ O_7.\ 10\ H_2O$ per l of water. The suspension was allowed to stand overnight, centrifuged at 4000 rpm for 1½ hr and supernatent was filtred. The filtrate was acidified to pH 2.0 with conc. H_2SO_4 and left in oven at 90°C for ½ hr to complete the precipitation. The precipitate consisting of humic acid (HA) was isolated by centrifugation and dissolved in just sufficient solution of 0.1N NaOH. This humic acid (HA) solution contained 0.35 mg C and 0.042 mg N/ml determined respectively by colorimetric (Anonymous, 1976) and microkjeldahl method (Bremner, 1965).

In one experiment, wheat seeds were soaked in humic acid solution for 1,2,4 and 5 hrs and then allowed to germinate at 30°C in Petri plates lined with moist filtre paper. Control seeds were transferred to Petri plates at zero hr of soaking treatments. Germinated seedlings with about 1.5-2.0 cm long main root were suspended with the help of a wire loop in 125 ml Erlenmeyer flasks containing 50 ml distilled water. In this arrangement seed alongwith the roots remained immersed in water. All the treatments were in triplicate with 10 seedlings each. Seedlings were removed from the flasks after 2 weeks and data regarding length, fresh and dry weight of both the root and shoot portion were recorded.

In the second experiment soaking time was increased to 4,8,16 and 24 hrs. After each soaking interval seeds were transferred to sand moistened with water or water containing 2560,3840,6080 or 9280 ppm NaCl, $CaCl_2$, $MgCl_2$ and $Na_2\ SO_4$ mixed together in a ratio of 4:5:1:10 as used by Malik *et al.* (1979) for artificial salinization of soil. Seeds were placed about 1 cm below the sand surface. Sand was thoroughly washed with acid and distilled water before use. Ten seeds were transferred to each salt concentration from each soaking treatment. Unsoaked seeds were transferred to differently salinized sand medium at the start of soaking. All the treatments were in triplicate. The experiment was conducted in a green house at a temperature ranging between 22-27°C. Seedlings were removed from the sand medium after 4 weeks, roots washed free of sand particles and growth characteristics were recorded.

Results and Discussions

The results show that soaking of seeds in humic acid for 1,2,4 and 5 hrs. respectively increased the root length by 23.04, 38.49, 48.72 and 48.72% (Table 1). Fresh weight increased respectively by 62.04, 50.42, 59.72 and 72.52% and dry weight by 21.82, 18.18, 34.55 and 40.00%. Roots contained 88.29, 87.86, 86.84 and 87.36% moisture due to soaking for 4,8,16 and 24 hrs. compared to 84.42% in control. Percent dry matter was 11.71, 12.23, 13.16 and 12.64% compared to 15.58% in control. Roots were highly developed and extensive development of secondary roots took place. Similar findings have been reported by Fialova (1969), O'Donnell (1973). When HA was used in the culture medium shoot length (Table 2) increased by 12.50, 12.50, 12.50, and 25.80%; fresh

weight by 13.82, 15.68, 22.14 and 37.73% and dry weight by 9.47, 16.73, 25.25 and 58.54% due to soaking for 4,8,16 and 24 hrs. Moisture content of shoot decreased and percent dry matter yield increased due to soaking.

Table 1. Effect of humic acid soaking of seeds on root growth of wheat seedlings (measurements are for 10 seedlings)

Treatments	Root length (cm)	Fresh weight (cm)	Dry weight (mg)	% Moisture	% Dry matter
H_2O soaking (control)	195	353	55	84.42	15.58
HA soaking 1 hr	240 (23.08)	572 (62.04)	67 (21.82)	88.29	11.71
HA soaking 2 hr	270 (38.46)	531 (50.42)	65 (18.18)	87.76	12.24
HA soaking 4 hr	290 (48.72)	562 (59.21)	74 (34.55)	86.84	13.16
HA soaking 5 hr	290 (48.72)	609 (72.52)	77 (40.00)	87.36	12.64
LSD (P = 0.05)	19.3	32.1	7.8		

Figures in parentheses indicate percent increase over control due to HA soaking of seeds.

The results indicate that soaking of seeds in humic acid for 4-5 hrs significantly enhanced the root and shoot growth. An increase of 48% and 25% in root and shoot length respectively was recorded due to soaking of seeds for 4 and 5 hrs. A general and significant increase in fresh and dry weight of both roots and shoots was observed. Smidova (1960), Schnitzer (1967), Schnitzer and Poapst (1967) also reported that treatment with 0.001% HA or sodium humate increased the yield of rye and uptake of P and K. Yield, survival and root initiation also increased when seeds were soaked in HA solution before sowing.

Unpublished results indicated that additon of humic acid to the growth medium

enhanced the uptake of water by roots similar to the reports made by Fialova (1969) and Cheng (1977). In the present study, soaking of seeds in humic acid also resulted in an increased uptake of water by roots related to more intensive root growth and hence increased surface area. This increased uptake of water may be helpful in a more efficient uptake of nutrients. Cheng (1977) found that HA in small concentrations increased the permeability of the cell membrane and promoted an active uptake of water and nutrients. Conversely, percent dry matter yield of roots decreased due to humic acid soaking although total dry matter increased. This decrease in % dry matter yield can be explained on the basis that fresh weight showed an increase of 70% over control compared to only 40% in dry matter.

Table 2. Effect of humic acid soaking of seeds on shoot growth of wheat seedlings (measurements are for 10 seedlings)

Treatments	Shoot length (cm)	Fresh weight (mg)	Dry weight (mg)	% Moisture	% Dry matter
H_2O soaking (control)	160	1129	176.3	84.38	15.62
HA soaking 1 hr	180 (12.50)	1285 (13.82)	193.0 (9.47)	84.93	15.07
HA soaking 2 hr	180 (12.50)	1306 (15.68)	205.8 (16.73)	84.24	15.76
HA soaking 4 hr	180 (12.50)	1379 (22.14)	210.0 (19.12)	83.97	16.03
HA soaking 5 hr	200 (25.00)	1555 (37.73)	297.5 (68.75)	82.03	17.97
LSD (P = 0.05)	15.8	27.6	13.9		

Figures in parentheses indicate percentage increase over control due to HA soaking of seeds.

In shoot portion, the dry matter yield considerably increased. The results indicate that humic compounds may be helpful in enhanced crop yields. More studies are, however, needed to bring these effects to field conditions.

In the absence of salts, soaking of seeds in humic acid for 4,8,12 and 16 hrs. resulted in an increase of 98,128,121 and 86% respectively in fresh weight of roots (Table 3-5). Presence of salts in the culture medium had no well defined effect on fresh weight of roots. Humic acid significantly increased the fresh weight of roots in the presence of salts also. From 60-90% increase in the fresh weight of roots grown in 2510-9280 ppm salt solution was recorded due to soaking of seeds for 8 hrs. The enhancing effect of humic acid soaking decreased with increase in salt concentration, however, 6880 ppm salts proved to be more detrimental for HA soaking action.

Table 3. Effect of humic acid soaking of seeds on root fresh weight (g/10 seedlings) of wheat seedlings grown for 4 weeks at different salt concentrations.

Treatments	H_2O soaking (control)	HA soaking (hrs)			
		4	8	16	24
Distilled H_2O	1.00	1.98 (98.0)	2.28 (128.0)	2.21 (121.0)	1.86 (86.0)
H_2O+2510 ppm salts	0.95	1.62 (70.5)	1.87 (96.8)	1.70 (79.0)	1.47 (54.7)
H_2O+3840 ppm salts	0.96	1.56 (62.5)	1.80 (87.5)	1.85 (92.7)	1.49 (55.2)
H_2O+6080 ppm salts	1.12	1.78 (58.9)	1.77 (58.0)	1.70 (51.8)	1.60 (42.91)
H_2O+9280 ppm salts	1.01	1.61 (59.4)	1.70 (68.3)	1.64 (62.4)	1.25 (23.8)
LSD (P = 0.05)					
Salts	0.103				
Soaking time	0.103				
Salts x soaking time	0.325				
Overall	0.23				

Figures in presentheses indicate percent increase due to HA soaking of seeds.

Table 4. Effect of humic acid soaking of seeds on root dry weight (mg/10 seedlings) of wheat seedlings grown for 4 weeks at different salt concentrations.

Treatments	H_2O soaking (control)	HA soaking (hrs)			
		4	8	16	24
Distilled H_2O	165.2	211.8 (28.21)	231.3 (40.01	231.3 (40.01)	225.0 (36.20)
H_2O+2510 ppm salts	157.8	214.8 (36.13)	251.8 (59.57)	225.5 (42.90)	200.0 (26.74)
H_2O+3840 ppm salts	158.5	201.3 (27.00)	201.0 (26.81)	221.3 (39.62)	199.8 (26.06)
H_2O+6080 ppm salts	157.5	201.5 (27.94)	216.0 (37.14)	189.0 (20.00)	164.8 (4.63)
H_2O+9280 ppm salts	122.0	151.0 (23.77)	163.0 (33.61)	147.8 (21.15)	127.3 (4.34)
LSD (P = 0.05)					
Salts	17.6				
Soaking time	17.6				
Salts x soaking time	55.5				
Overall	39.3				

Figures in parentheses indicate percent increase due to HA soaking of seeds.

Dry weight of the roots decreased in the presence of salts. Humic acid soaking resulted in increased dry weight in the presence or absence of salts. At lower salt concentration (2510 ppm), humic acid soaking seemed to be more effective than in the absence of salts. With further increase in salt concentration, however, humic acid soaking proved to be less effective.

Compared to roots, shoot portion was more affected by the presence of salts and showed a pronounced decrease with increase in salt concentration. Soaking of seeds enhanced shoot development to a considerable degree. Even at 3840 ppm salts, soaking of

Table 5. Effect of HA soaking of seeds on shoot dry weight (mg/10 seedlings) of wheat seedlings grown for 4 weeks at different salt concentrations.

Treatments	H_2O soaking (control)	HA soaking (hrs)			
		4	8	16	24
Distilled H_2O	539	634 (17.62	650 (20.59)	624 (15.77)	560 (3.90)
H_2O+2510 ppm salts	481	623 (29.52)	633 (31.60)	546 (13.51)	504 (4.78)
H_2O+3840 ppm salts	448	556 (24.10)	549 (22.54)	520 (16.07)	500 (11.61)
H_2O+6080 ppm salts	309	337 (9.06)	401 (29.77)	339 (9.71)	367 (18.77)
H_2O+9280 ppm salts	248	288 (16.12)	299 (20.56)	320 (29.03)	292 (17.74)
LSD (P = 0.05)					
Salts	22.5				
Soaking time	22.5				
Salts x soaking time	71.0				
Overall	50.2				

seeds for 8 hrs. produced shoot weight equal to that in distilled water without salts. At higher salt concentrations, although dry matter yield of shoot decreased, humic acid soaking counteracted fairly the adverse effects of salts.

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