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INHIBITION OF GERMINATION IN ATRIPLEX TRIANGULARIS SEEDS BY APPLICATION OF PHENOLS AND REVERSAL OF INHIBITION BY GROWTH REGULATORS

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Seeds of *Atriplex triangularis* contained phenols: small seeds had gentisic, salicylic, syringic, and chlorogenic acids and catechol; medium seeds, gentisic and salicylic acids and catechol and protocatechol; large seeds, gentisic, caffeic, and 2-hydroxy-5 methoxy benzoic acids and protocatechol. Small seeds had a lower concentration (0.85 mg g^{-1} dry weight [DW]) of total phenols than medium (1.58 mg g^{-1} DW) and large (4.41 mg g^{-1} DW) seeds. However, the presence of endogenous inhibitors—salicylic, syringic, and chlorogenic acids and catechol—in small seeds, but not in large, could account for germination inhibition in these seeds. Inhibition of germination by exogenous applications of all highly active phenols (10^{-2} M) except salicylic acid was alleviated by the application of gibberellic acid and kinetin.

Introduction

Atriplex triangularis Willd., a halophyte in the family Chenopodiaceae, is widely distributed in inland and coastal marshes in North America (OSMOND et al. 1980). Seeds of *A. triangularis* have a morphological and physiological polymorphism (KHAN and UNGAR 1984*a*, 1984*b*). Small seeds of *Atriplex* are more dormant than medium and large seeds, and germination in these seeds can be stimulated by growth regulators (KHAN and UNGAR 1984*a*, 1985).

Abscisic acid is considered to be a germination inhibitor; however, it is thought to be involved in the inhibition of embryo growth rather than in the initiation of germination (KHAN 1982). Phenols in seeds have also been proposed as germination inhibitors (SREERAMULU 1974; HAMILTON and CARPENTER 1976; NAQVI and HANSON 1982; WILLIAMS and HOAGLAND 1982). The loss of germinability in *Voandzeia subterranea* seeds was positively correlated with an increase in total phenols (SREERAMULU 1983). Exogenous applications of phenols (10^{-3} M) to crop and weed seeds delayed but did not substantially inhibit germination (WILLIAMS and HOAGLAND 1982). Few data are available on the effect of growth regulators on reversing the inhibition of germination by phenols. HAMILTON and CARPENTER (1976) did find that the inhibitory effects of the phenol coumarin on seed germination were alleviated by the application of gibberellic acid (GA_3) and kinetin.

Here we report on the role of phenols in the inhibition of germination in *A. triangularis* seeds and its reversal by growth regulators.

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Material and methods

Fruits of *Atriplex triangularis* were collected near Rittman, Wayne County, Ohio, during October 1982. The procedures for handling seeds were described by KHAN and UNGAR (1984*b*). Small seeds were <1.5 mm; medium seeds were 1.5–2.0 mm; large seeds were >2.0 mm in diameter.

Phenols were extracted by the method of HARBORNE (1973). Seed material (1 g) was extracted in 2 M HCl and digested in a boiling water bath for 30 min, cooled, and filtered. Phenols were then extracted with ether and evaporated; the residue was dissolved in 1 mL ether and applied to Whatman no. 1 chromatographic paper. Two-dimensional ascending chromatography was used; benzene:acetic acid:water (60:70:30, v/v/v, upper phase) was the solvent system in the first direction, and sodium formate:formic acid:water (10:1:200, v/v/v) in the second (IBRAHIM and TOWERS 1960). The chromatogram was air-dried and examined with a UV lamp. Duplicate chromatograms were sprayed with either diazotized p-nitroaniline or diazotized sulphanilic acid (AMES and MITCHELL 1952), and individual phenols were identified by Rf values and coloration (IBRAHIM and TOWERS 1960; HARBORNE 1973).

Three replicates of 1 g of seed material were extracted in 2 M HCl and digested as above. The filtered digest was extracted with ether. Residues were dissolved in 2 mL ethanol and diluted to 50 mL with deionized water. Total phenols in the extract were estimated by the method of SWAIN and HILLIS (1959). A 2-mL aliquot was transferred to ca. 6 mL deionized water; 0.5 mL Folin's reagent was added, and the tube was shaken. After 3 min, 1 mL saturated sodium bicarbonate solution was added, and the mixture was diluted to 10 mL with deionized water. The blue color that formed after 1 h was measured at 725 nm with a Perkin-Elmer Model 360 Spectrophotometer. Total phenolic content was expressed as chlorogenic acid equivalents.

Analytical grade phenols (see table 2) were tested

TABLE 1

TOTAL PHENOLIC COMPOUNDS (mg g^{-1} DW) IN THE POLYMORPHIC SEEDS OF ATRIPLEX TRIANGULARIS

TREATMENT	SEED SIZE		
	Small	Medium	Large
Air-dried	.85 ± .04	1.58 ± .24	4.41 ± .12
Soaked in water	.47 ± .02	.47 ± .05	.70 ± .02
Soaked in 3% NaCl	.45 ± .06	.50 ± .04	.71 ± .02

for their ability to inhibit *Atriplex* seed germination because they were reported to be germination inhibitors (LODHI and RICE 1971; RASMUSSEN and EINHELLIG 1979). Each compound was prepared by dissolving an appropriate amount in water with 1 mL Tween 20 to obtain a concentration of 10 mM. This concentration of phenols was used because lower levels were not inhibitory to seed germination.

Seed surfaces were sterilized with 0.1 strength Clorox (0.5% NaOCl) for 1 min and washed three times with distilled water. Germination was carried out in 50 × 9-mm Gelman tight-fitting plastic petri dishes containing 6 mL test solution. Each dish was placed in a 9-cm-diameter glass petri dish to prevent water loss by evaporation. Seeds were germinated in a growth chamber at alternating temperature regimes of 5 C/12 h dark and 25 C/12 h light (8.5 W m^{-2} Sylvania cool-white fluorescent lamps).

Small seeds were germinated in 10 mM of the phenols for 20 days, and the seven most active phenols were selected for further experimentation. Four replicates of 25 seeds from all seed sizes were used for each treatment. Germination was defined as radicle protrusion through the seed coat and was recorded on alternate days for 20 days. The germination rate was calculated by using a modification of the TIMSON (1965) index of germination velocity = G/T, where G = percentage of seeds germinated at 2-day intervals and T = total germination period. The maximum value possible, using this index for our data, was 50 (1,000/20). We used concentrations of 1.5 mM GA_3, 47 μM kinetin, and 10 mM each of *m*-coumaric, *p*-coumaric, ferulic, salicylic, resorcinol, *p*-hydroxybenzoic, and gentisic acids.

Results

Phenolic content of polymorphic seeds was qualitatively determined. Small seeds contained gentisic, salicylic, syringic, and chlorogenic acids and catechol; medium seeds, gentisic and salicylic acids and protocatechol and catechol; large seeds, gentisic, caffeic, and 2-hydroxy-5 methoxy benzoic acids and protocatechol. The seeds did not vary qualitatively in phenolic content even after being soaked for 24 h in water or 3% NaCl.

Total phenols were estimated in dry seeds and in seeds soaked separately for 24 h in water and in 3% (w/v) NaCl (table 1). The total phenols (mg g^{-1} dry weight [DW]) were higher in large seeds than in small and medium seeds. Soaking in either water or 3% NaCl resulted in leaching of significant amounts of phenolic acids from the seeds: 45% from small seeds and 84% from large seeds.

Phenols were grouped into three categories, based on their ability to inhibit germination. High activity included those that inhibited germination by a minimum of 80%: *m*-coumaric, *p*-coumaric, ferulic, ellagic, salicylic, *p*-hydroxybenzoic, and gentisic acids and resorcinol and quercetin. Medium activity included those that inhibited germination from 30% to 70%: vanillic, *t*-cinnamic, caffeic, and

TABLE 2

SEPARATION OF 21 PHENOLS WITH RESPECT TO ACTIVITY BASED ON GERMINATION INHIBITION OF SMALL SEEDS OF ATRIPLEX TRIANGULARIS

Activity and compound (10 mM)	Germination inhibition (%)
High:	
m-Coumaric acid	99 ± 1.9
Quercetin	98 ± 1.2
Ferulic acid	97 ± 1.9
Ellagic acid	96 ± 1.9
Salicylic acid	95 ± 1.9
p-Coumaric acid	94 ± 2.0
Resorcinol	93 ± 1.0
p-Hydroxybenzoic acid	86 ± 4.2
Gentisic acid	80 ± 3.7
Medium:	
Vanillic acid	62 ± 2.0
t-Cinnamic acid	55 ± 5.7
Caffeic acid	52 ± 4.6
Syringic acid	48 ± 3.7
Catechol	31 ± 4.4
Low:	
Sulphanilic acid	27 ± 2.5
Orcinol	25 ± 3.4
Pyrogallol	27 ± 7.0
Chlorogenic acid	24 ± 4.3
Coumarin	24 ± 4.3
Hydroquinone	14 ± 5.7
Gallic acid	19 ± 3.4

TABLE 3

EFFECT OF PHENOLIC COMPOUNDS, KINETIN, AND GA_3 ON THE PERCENTAGE AND VELOCITY OF GERMINATON OF POLYMORPHIC SEEDS OF ATRIPLEX TRIANGULARIS

PHENOLS AND SEED SIZE (10^{-2} M)	TREATMENTS					
	H_2O control		47 μM kinetin		1.5 mM GA_3	
	A	*B*	*A*	*B*	*A*	*B*
Control:						
Small	91.00[a]	32.15	97.00	41.15	94.00	38.30
	±4.12	±2.24	±1.00	±1.02	±1.15	±1.58
Medium	91.00	33.65	93.00	34.25	77.00	28.35
	±3.78	±1.21	±3.00	±1.21	±6.19	±1.78
Large	95.00	41.25	98.00	41.10	94.00	42.35
	±1.91	±.38	±2.00	±1.13	±1.08	±1.08
Salicylic acid:						
Small	6.67	2.60	16.00	5.46	17.00	6.35
	±1.33	±.40	+.00	±.64	±4.43	±1.78
Medium	8.00	2.30	8.00	2.00	10.00	3.10
	±.00	±.26	±.00	±.00	±6.00	±2.10
Large	4.00	1.60	4.00	1.00	4.00	1.40
	±.00	±.20	±.00	+.00	±.00	±.00
Gentisic acid:						
Small	20.00	5.10	52.00	21.75	52.00	19.45
	±3.65	+.97	±6.32	±2.13	±4.32	±1.95
Medium	20.00	7.20	39.00	16.90	58.00	23.35
	±3.65	±.75	±7.55	±3.19	±2.58	±.73
Large	39.00	13.00	53.50	23.00	61.00	29.05
	±5.25	±2.09	±8.06	±2.95	±5.00	±1.93

NOTE.—*A* = percentage of germination after 20 days; *B* = velocity of germination (maximum = 50).

[a] Mean ± SE.

syringic acids and catechol. Low activity included those that inhibited germination less than 30%: sulphanilic, chlorogenic, and gallic acids and orcinol, hydroquinone, and coumarin (table 2). Most of the phenols had little or no inhibitory activity at lower concentrations but showed significant increase in inhibitory activity at 10 mM.

When the high-activity group of phenols was applied to various seeds, they significantly ($P < .0001$) inhibited germination of all seed sizes compared with water treatments. Because the results for gentisic acid were similar to all other phenols tested except salicylic acid, only the data for these two inhibitors are reported (table 3). Phenols were generally more active in inhibiting germination in small seeds than in medium and large seeds. Addition of GA_3 and kinetin to the phenols significantly promoted both the velocity ($P < .0001$) and percentage ($P < .0001$) of germination in all seed sizes and reversed the effect of all phenols except salicylic acid. Dormancy induced by salicylic acid was not alleviated by GA_3 or kinetin (table 3).

Individual effects of phenols, growth regulators, and seed size and their interaction were generally significant on velocity and percentage of germination. The interaction between growth regulators and seed size was not significant (table 3).

Discussion

Dormancy in small seeds could be caused by the presence of the phenolic compounds, catechol, chlorogenic, salicylic, and syringic acids, which were not present in large seeds. During the germination of *Arachis hypogaea* and *Voandzeia subterranea* seeds, SREERAMULU (1974, 1983) found an increase in *p*-coumaric, *p*-hydroxybenzoic, ferulic, and vanillic acids and a decrease in chlorogenic, caffeic, sinapic, and protocatechuic acids. KHAN et al. (1976) found that the total phenol levels in halophytes (*Suaeda fruticosa, Tamarix indica,* and *Atriplex stocksii*) were higher than that of glycophytes (*Withania sominifera, Raphanus sativus,* and *Spinacea oleracea*). No information is available on the phenols of the seeds of other *Atriplex* species; however, the phenols in *Atriplex triangularis* were also reported in seeds and fruits of other genera (DAS et al. 1967; SREERAMULU 1974, 1983; NAQVI and HANSON 1982).

Inhibition of *A. triangularis* seed germination by *m*-coumaric, ferulic, ellagic, salicylic, *p*-coumaric, resorcinol, and gentisic acids agrees with reports for other genera (LODHI and RICE 1971; RASMUSSEN and EINHELLIG 1979). However, coumarin, gallic acid, and pyrogallol were not as effective in

inhibiting *A. triangularis* seed germination as for the seeds of other genera (MAYER and POLJAKOFF-MAYBER 1982).

Germination inhibition by phenols on all seed sizes of *A. triangularis* was alleviated by 1.5 mM GA_3 and 47 μM kinetin. HAMILTON and CARPENTER (1976) also found that the inhibition of germination by coumarin in *Elaeagnus angustifolia* seeds was alleviated by GA_3 and kinetin. In dormant seeds of *Elaeagnus umbellata,* GA_3 stimulated germination, but kinetin did not (HAMILTON and CARPENTER 1975). WAREING (1965) found that salicylic acid was a strong germination inhibitor. We could not reverse the inhibitory effect of salicylic acid with growth regulators.

Our investigation demonstrated the presence of various phenols in *A. triangularis* seeds and indicated that exogenous treatments with phenols inhibited germination. The inhibition of germination induced by most of these phenols could be alleviated by GA_3 and kinetin.

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