The effect of indole acids on the germination of *Mimosa bimucronata*

by

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Abstract: Seeds of *Mimosa bimucronata* (DC) OK have a period of dormancy due to the impermeability of the seed coats which, once broken through, allow a germination rate of about 100%. Therefore, they may respond to the application of indole acids whatever the effect in the regulation of germination. By applying indole-3-acetic acid, indole-3-butiric acid, and indole-3-propionic acid at concentrations of control, 10, 50, 100, 500, and 1000 ppm, inhibition was observed as the concentrations were increased, probably due to a toxic effect. By buffering the medium at pH 6, the possibilitv of acid effect of auxins on germination was discarted; although buffering of the medium slowed down germination processes during the 15 days of testing, it did not reduce them significantly.

The influence of auxins on the germination of seeds is generally regarded as innocuous (Steward & Hamner, 1942; Poljakoff-Mayber, *et al.*, 1957; Hess, 1975) However, some cases of stimulus are accepted (Audus, 1953; Söding & Wagner, 1955), perhaps through induction in the synthesis of ethylene (Marré, 1977) which can act as a promoter of germination (Leopold & Kriedemann, 1955). The stimulus of the synthesis of ethylene would occur at high concentrations of auxins (Marré 1977), but these would have a toxic effect at these concentrations (Audus, 1953). At higher concentrations of auxins, there is the effect of weak acid by lowering the pH (Albaum & Kaiser, 1937). There is a general consensus that auxins regulate cellular growth (Went, 1939; Thimann & Schneider, 1939; Thimann, 1951, 1969; Leopold & Kriedemann, 1975) and other processes. The control of growth consists in regulating the elongation of the cell wall (Masuda, 1977), which occurs in the presence of auxins or when there is a dropping of pH in the cell wall, increasing the acidity which loosens the wall structure (Rayle & Cleland, 1970; Valent & Albersheim, 1974; Jacobs & Ray, 1976; Maclachlan, 1977). The loosening of the cell wall by the auxins is due to the lowering of the pH in the wall, caused by the pumping of H+ (Fischer & Albersheim, 1974; Perley, *et al.*, 1975; Penny, *et al.*, 1975), or by the effect of auxins on enzymatic proteins (Albersheim, *et al.*, 1977; Maclachlan, 1977). Ferreira (1976) showed that, in acid pH, germination was

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inhibited in intact seeds of *Mimosa bimucronata* (DC) OK. These results, as well as the acid character of auxins, suggested that the toxic effect on the germination of seeds, ocasionally noticed when auxins were applied, was due to the acidity of the medium. The present paper aims at clearing up this issue.

MATERIAL AND METHODS

The fruits of *Mimosa bimucronata* (DC) OK ("maricá") were collected in the 1974 fructification from specimens occurring naturally in Porto Alegre, RS, Brazil. The seeds were extracted from fruits (loments), dried in the laboratory for twenty days at room temperature and sterilized in a 1% solution of sodium hypoclorite ten minutes; following this, they were thoroughly washed in distilled water.

The test seeds were placed in petri dishes (20 per dish) lined with two sheets of filter paper. One dish per treatment was used for each test. The tests were repeated 10 times. The differences between repetitions were not considered significant, and the analysis was made in an overall way, taking into account the total of 200 seeds as the sampling size in each treatment.

The treatments were performed with the following auxins:

indole-3-acetic acid (IAA),
indole-3-butiric acid (IBA), and
indole-3-propionic acid (IPA).

Ten mililiters of the test solution were used at the following concentrations: control, 10, 50, 100, 500, and 1,000 ppm. When pH was allowed to vary (not buffered), the following values were obtained, respectively: 6.1; 5.5; 4.5; 4.1; 3.7; 3.5. In order to set pH at 6, the test solution was dissolved in a buffer of potassium hydrogenftalate and sodium hydroxide 0.1 M (Ohlweiler, 1968). This choice was indicated by the good germination achieved by these seeds at this pH (Ferreira, 1976).

The tests were carried out in dark chambers, with temperature ranging between 18 and 25C. Daily readings were made during 15 days. However, because of the shape of the curves of accumulated germination, the 4th, 7th, and 14th days were chosen as the critical points to be examined, thus characterizing the data analysis in three clear stages.

The germination criterion used consisted in the emergence of the radicle.

The data were analysed by means of x^2 tests and by the Student-Newman-Keuls test for multiple range comparisons between average germinations (Zar, 1974), in cases where significantly different effects were evident among the various treatments. Due to the high number of tests carried out, level 0.01 was assumed as a limit for the statement of statistical significance.

RESULTS

Comparison between germination observed in the control and the lowest concentration of the auxins studied: Table 1 shows the number of germinated seeds, at free and adjusted pH, at the lowest concentration of IAA, IBA and IPA and in the control. On the three days studied there was no significant difference in germination in the different solutions (see values of x^2 in Table 3). The same result was obtained in the tests carried out at free and at adjusted pH. Accordingly, the speed of germination and the germinability* are the same in the presence of water as well as in a concentration of 10 ppm of the acids studied.

TABLE 1

Number of seeds of M. bimucronata *among 200 germinated on the 4th, 7th and 14th days at free pH and at pH 6, in the control and in solutions of 10 ppm of IAA. IBA and IPA*

Solution test	free pH			pH 6		
	4th day	7th day	14th day	4th day	7th day	14th day
IAA	32	39	46	21	29	37
IBA	21	27	36	16	26	36
IPA	21	22	30	13	24	38
Control	33	37	48	26	31	41

Comparison of IAA, IBA and IPA at different concentrations: The number of seeds germinated at different concentrations of the three auxins is presented in Table 2, which shows the results on the 4th, 7th and 14th days, at free and adjusted pH (pH 6). Germination is significantly different in the three auxins when it occurs on the 7th and 14th days, at free pH. The lowest rate of germination is observed with indole-propionic acid. However, this effect on the different auxins is not observed on the 4th day of germination at this pH, and not at any time at adjusted pH (Table 3). On the other hand, a significant effect of auxin concentration was noticed in the three stages both at free pH and at pH 6 (see x^2 in Table 3).

The average germinations obtained at the five concentrations are shown in Table 4. It is evident that germination decreases as the concentration of auxins increases. The phenomenon varies a little according to the different stages (days) and pH used. Therefore, there is no clear separation on the 4th day; however, there is a gradual decrease of germination from the lowest to the highest concentration, both at adjusted and at free pH. As early as the 7th day, germination decreases significantly only at a concentration of 1,000 ppm, at free pH; nevertheless, the decrease occurs at 100 ppm, when pH is adjusted. It should be pointed out that at free pH, comparisons among concentrations involve also a pH variation due to the acid character of the auxins, which increases at higher concentrations. On the 14th day, the predominant inhibiting effect on germination at the highest concentration is evident at both pH levels.

The effect of pH buffering: The preceding tables show that there is an effect of pH buffering on the germination of *M. bimucronata* seeds which becomes more evident by computing the difference between the number of seeds germinated at free pH and at pH 6 (Table 5).

* Germination capacity, final germination.

TABLE 2

Number of seeds of M. bimucronata *among 200, germinated at different concentrations of auxins IAA, IBA and IPA, at free pH and at pH 6 (data referring to the 4th, 7th and 14th days of germination)*

		free pH						pH 6					
Germination day	Auxin	10*	50	100	500	1000(ppm)	TOTAL	10	50	100	500	1000(ppm)	TOTAL
4 th day	IAA	32	20	25	18	12	107	21	16	3	9	2	51
	IBA	21	26	20	14	10	91	16	11	8	6	5	46
	IPA	21	22	17	9	6	75	13	11	4	5	5	38
	TOTAL	74	68	62	41	28	273	50	38	15	20	12	135
7th day	IAA	39	34	38	33	20	164	29	41	15	16	6	107
	IBA	27	30	24	29	20	130	26	20	12	18	13	89
	IPA	22	33	24	19	8	106	24	25	17	11	8	85
	TOTAL	88	97	86	81	48	400	79	86	44	45	27	281
14 th day	IAA	46	38	48	47	32	212	37	48	36	35	24	180
	IBA	36	40	36	37	29	178	36	35	29	39	18	157
	IPA	30	38	36	23	9	146	38	43	37	26	18	162
	TOTAL	112	116	120	117	71	536	111	126	102	100	60	499

* There was no significant difference between the controls and 10 ppm of different acids (Table 1) so it was assumed that this concentration would serve as control.

TABLE 3

Values of x^2 obtained in different comparisons at free pH 6, on the 4th 7th and 14th days after germination

pH and comparison	Degrees of freedom	4th day	7th day	14th day
Free pH:				
Auxins (10 ppm) x water	1	1.90	1.39	2.34
Auxins (10ppm)	2	3.74	6.09	4.30
Auxins (all concentrations)	2	6.19	14.73*	14.82*
Concentration (all auxins)	4	30.29*	20.44*	18.95*
pH 6:				
Auxins (10ppm) x water	1	3.28	0.51	0.27
Auxins (10ppm)	2	2.15	0.57	0.07
Auxins (all concentrations)	2	2.00	3.23	2.11
Concentration (all auxins)	4	41.42*	49.61*	28.90*

* Significant at 0.01.

The effect of pH buffering on germination appears on the 4th and 7th days ($x^2 = 61.68$ and 30.39, respectively, $p < 0.001$). The average germination is higher at free pH than at adjusted pH: on the 4th day, there is an average increase of 9 seeds, and on the 7 th day, the average is 8. This effect disappears on the 14th day, when there is no significant difference in the germination rate of the two pH ($x^2 = 0.93$, $p > 0.25$). On the other hand, this effect is the same in any of the 3 days of germination at different treatments, i. e., the control and the five concentrations of each auxin (non significant x^2 for heterogeneity; Table 5).

DISCUSSION

Ferreira (1976), and Ferreira & Callegari-Jacques (1979) demonstrated that in very few seeds of *M. bimucronata* the coat is permeable. The seeds germinate when they are soaked (Ferreira, 1976); accordingly, the application of any exogenous substance which significantly alters the germination speed or the rate expresses the performance of this substance. Tillberg (1977) refers to large increases in the level of endogenous IAA in the early stages of the germination process, not taking into consideration those auxins applied exogenously, although in some cases the external auxins can function as promoters in the germination of photoblastic seeds (Vyas & Shrimal, 1973). The life cycle of auxins *in vivo* is short, since they are soon destroyed by the IAA-oxidase; they can only survive as conjugated or as esterified auxins (Cohen & Bandurski, 1978; Hall & Bandurski, 1978). The acidity of auxins externally applied is soon buffered by the organism cells (Vanderhoef *et al.*, 1977); this must be true at low concentrations, according to the results reported in this paper (Tables 3 and 4).

Apparently, there is no effective buffering by the germinating seeds at high concentrations of auxins and free pH. Inhibition is stronger in IPA, although this is

TABLE 4

Average number of germinated seeds of M. bimucronata *on the 4th, 7th and 14th days, at concentration of 10, 50, 100, 500, and 1000 ppm (comparisons made by the Student-Newman-Keuls test) * for control see footnote Table 2*

pH	Germination day	Concentrations (ppm) 10	50	100	500	1000
free	4th	24.7[a]	22.7[ab]	20.7[ab]	13.7[bc]	9.3[c]
	7th	29.3[a]	32.3[a]	28.7[a]	27.0[a]	16.0[b]
	14th	37.3[a]	38,7[a]	40.0[a]	39.0[a]	23.7[b]
6	4th	16.7[a]	12.7[ab]	5.0[c]	6.7[bc]	4.0[c]
	7th	26.3[a]	28.7[a]	14.7[b]	15.0[b]	9.0[b]
	14th	37.0[a]	42.0[a]	34.0[a]	33.0[a]	20.0[b]

* The same letters indicate non-significant differences (0.01 level). Six sets of multiple comparisons were carried out among averages, one for each day at adjusted pH (6) and free pH.

TABLE 5

Difference between the number of seeds that germinated at free and adjusted pH, at 6 concentrations of different auxins

Germination day	Auxin	Control	Concentrations (ppm) 10	50	100	500	1000
	IAA	-	11	4	22	9	10
4 th	IBA	-	5	15	12	8	5
	IPA	-	8	11	13	4	1
	Average	7.0	8.0	10.0	15.7	7.0	5.3
	IAA	-	10	-7	23	17	14
7 th	**IBA**	-	1	10	12	11	7
	IPA	-	-2	8	7	8	0
	Average	6.0	3.0	3.7	14.0	12.0	7.0
	IAA	-	9	-10	12	12	9
14 th	IBA	-	0	5	7	-2	11
	IPA	-	-8	-5	-1	7	-9
	Average	7.0	0.3	-3.3	6.0	5.7	3.7

x^2 between free and adjusted pH (d.f.=1): 4th day = 61.68; 7th day = 30.39, both significant at the 0.01 level; 14th day = 0.93, non significant.

x^2 for heterogeneity, involving control and the 15 treatments (d.f. = 15): 4 th day = 18.54; 7 th day = 29.74; 14 th day = 30.01 all of them non significant.

a less effective auxin (Thimann & Schneider, 1939; Thimann, 1969). On the other hand, germination is higher in the first stages at free pH, but the final rate does not differ in a significant way between free and buffered pH. This indicates that the acid effect of auxins is negligible, and that the inhibiting effect must be due to the high toxicity of the high concentrations of auxins.

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RESUMEN

Las semillas de *Mimosa bimucronata* (DC) OK poseen un letargo causado por la impermeabilidad de los tegumentos, que puede ser interrumpido permitiendo un 100% de germinación. Luego de la aplicación de ácidos indólicos hay respuesta, sea cual fuere el efecto en la regulación de la germinación. Aplicando ácido 3 indolacético, ácido indolbutírico y ácido indolpropiónico en las concentraciones de control, 10,50,100,500 y 1000 ppm, se verifica un efecto inhibitorio a medida que aumentan las concentraciones, probablemente por efecto tóxico. Con el medio controlado a pH 6 con amortiguador fue descartada la posibilidad de efecto ácido de la auxinas sobre la germinación. Aunque la fijación del pH del medio disminuye la velocidad del proceso germinativo, durante los 15 días de observación no se disminuye significativamente el porcentaje de germinación.

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