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Agronomy

Improved germination of the seeds of whistling pine (*Casuarina equisetifolia*) Forst and Forst (Casuarinaceae) by various presowing treatments

JMO Eze*, MO Ahonsi

Department of Botany, University of Benin, Benin City, Nigeria

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Summary — Various methods were tried for inducing the seeds of *Casuarina equisetifolia* Forst and Forst (Casuarinaceae) to germinate faster or in greater numbers than under natural conditions. Percentage germination in untreated seeds (control) ranged from 7 to 16% over an average of 6 days. Some treatments increased the percentage germination by different values up to 62%. The most effective treatment was application of a 0.1 mM solution of GA_3 followed by that of a 2 500 mg dm^{-3} solution of ascorbic acid, and a 10 mM solution of $NaNO_3$. Other effective treatments included scarification with concentrated H_2SO_4, and application of 0.1 mM IAA solution. Incubating soaked seeds in continuous red light caused the germination to start earlier and also increased the overall germination. Germination was inhibited under continuous blue light, green light and in the dark. Germination was completely prevented in seeds pretreated with a 0.1 mM solution of ABA.

***Casuarina equisetifolia* / germination / GA_3**

Résumé — Amélioration de la germination des graines de *Casuarina equisetifolia* Forst et Forst (Casuarinaceae) par divers traitements avant semis. *Divers traitements ont été essayés pour induire les graines de* Casuarina equisetifolia *à germer plus vite ou en plus grande proportion que dans les conditions naturelles. Le pourcentage de germination des graines non traitées (témoins) variait de 7 à 16%, en 6 j en moyenne. Certains traitements ont amélioré ce pourcentage de valeurs variées, jusqu'à 62%. Le traitement le plus efficace a été l'application d'une solution de GA_3 à 0,1 mM, suivie de celle d'une solution d'acide ascorbique à 2,5 mg dm^{-3} et d'une solution à 10 mM de $NaNO_3$. La scarification par H_2SO_4 concentré et l'application d'une solution de AIA à 0,1 mM a été également efficace. L'incubation des graines imbibées sous lumière rouge continue entraîna une germination plus précoce, et un pourcentage de germination amélioré. La germination a été inhibée en lumière bleue continue, en lumière verte ou à l'obscurité. La germination a été complètement inhibée par un prétraitement par une solution à 0,1 mM d'AIB.*

Casuarina equisetifolia / *germination / GA_3*

* Correspondence and reprints: Enugu State University of Science and Technology, Faculty of Applied Natural Sciences, Adada Campus, PO Box 161, Nsukka, Nigeria

INTRODUCTION

The whistling pine (*Casuarina equisetifolia*) Forst and Forst (Casuarinaceae) (Syn *C litorea* L) is extensively planted as wind breaks to protect crops in agro-forestry. It is also widely used to stabilize coastal sand dunes because of its resistance to salt-laden winds. The wood is resistant to decomposition in soil or salt water, and is often used as round wood for making piles, poles and fences. The bark is used for tanning. However, the uses for which *C equisetifolia* and its hybrids are most commonly known are as ornamental plants for urban decoration, parks and seaside resorts. It has a high calorific value (*ca* 5 000 kCal kg^{-1}) and the wood is therefore an excellent source of fuel wood and charcoal. The young twigs are also used for feeding cattle (Mclean and Ivimey-Cook, 1968; Gerth Van Wijk, 1971; Howes, 1975; Domergues, 1990).

The germination of the seeds of such an economically important tree is of great interest to different groups of plant scientists such as botanists, agro-foresters and silviculturists. This is accentuated as the successful propagation of any *Casuarina* spp by a vegetative method is very limited even with IBA hormone application (Mahmood and Possuswam, 1980; Turnbull *et al*, 1986; Pinyopusarerk and Bolan, 1990). There is a paucity of reports of germination studies on the whistling pine and the present study was undertaken to extend what is already known about its germination.

Germination studies have been carried out in the seeds of several tree species. For instance, Bibbey (1948) worked on woody plant seeds; the US Department of Agriculture (1948) studied seed and its development in 444 species of woody plants; Gill and Bamidele (1981) studied the germination of 3 savanna trees of Nigeria; Etejere *et al* (1982) and Eze and Orole (1987) studied the germination of *Parkia clappertoniana* and *Prosopis africana*, respectively. Williams *et al* (1989) worked on germination and dormancy in *Ptilotus exaltatus.* Many aspects of the findings from these various studies may apply to the germination of other seeds including *C equisetifolia* but this will remain a supposition until the techniques and procedures are actually tried on the experimental material.

MATERIALS AND METHODS

Source of seeds and collection method

To aid the uniformity of the response, ripe green cones (fruits) were plucked from one particular *Casuarina* tree in a row at the Ugbowo Campus of the University of Benin, Benin City. The cones were dried in the sun inside a beaker to prevent the small, papery, light seeds from being blown away by the wind. The seeds that appeared to be filled were selected while empty-looking and wrinkled seeds were discriminated against since such seeds always failed to germinate in preliminary trials.

Pre-sowing treatments

Chemical scarification was carried out by steeping the seeds in concentrated H_2SO_4 for 5, 10 or 20 min and subsequently washing thoroughly in running tap water for 5 min. Both the acid-scarified seeds and unscarified ones (control) were sown on filter paper moistened with 2, 10 or 50 mM solution of sodium nitrate ($NaNO_3$). Seeds were also treated with a 0.1 mM solution of indole-acetic acid (IAA), gibberellic acid (GA_3) or abscisic acid (ABA). The control and scarified seeds were also soaked in 250, 500, 1 000, 2500, 5 000 and 10 000 mg dm^{-3} of ascorbic acid for 24 h, after which they were sown on water-moistened filter papers in Petri dishes.

The effect of heating on the germination of control and acid-scarified seeds was monitored by preheating dry seeds in the oven for 2 or 5 min at temperatures of 40, 55, 70 and 90°C. After the heat treatment the dry seeds were sown on moist filter papers as usual.

The effect of light was also investigated by incubating the control and scarified seeds sown on moist filter papers and exposing them to lighting conditions of alternating day and night, or continuous fluorescent white light. Furthermore, some of the Petri dishes containing the prepared seeds were covered with blue, green, orange or red cellophane filters and placed under continuous white light. These seeds were thus allowed to germinate under the above-mentioned coloured light.

In all experiments 75 seeds were sown in 3 Petri dishes (each containing 25 seeds). A seed was taken to have germinated once the radicle had pushed out from the seed coat. All seeds were sown on filter paper moistened with water or appropriate solution.

RESULTS

Acid scarification

Five minutes was the most effective time of soaking in concentrated H_2SO_4 as a scarification treatment to improve the percentage germination (table I). No seed was found to survive 20 min of soaking in the acid.

Effect of scarification

The results summarized in tables I-VI show that scarification with concentrated H_2SO_4 was a very effective method for improving the germination of *C equisetifolia* seeds.

Effect of other treatments

Several other treatments were, however, more effective than acid scarification in the sense that the unscarified seeds given such treatments alone had better germination than the acid-scarified seeds. These were soaking with 10 mM $NaNO_3$ solution, (table II), 0.1 mM solution of GA_3 (table III) and 2 500 mg dm^{-3} ascorbic acid solution (table IV). In each of these treatments, however, acid scarification pretreatment (B) gave additional enhancement of germination. Other treatments that were also better than the control alone were: soaking with 0.1 mM solution of IAA (table III); heating to 55 or 70°C (table V); and continuous red light (table VI). Continuous light on the whole seemed to advance the time of germination by 1 d in the seed lots tested (table VI). The tests on light effect were repeated 3 times and although the actual results obtained kept varying, the trend was consistent. Germination was drastically reduced in continuous darkness (table VI) while complete inhibition was caused by 0.1 mM ABA (table III).

DISCUSSION

Primary dormancy has to do with incomplete development of the embryo such that the seed cannot germinate despite satisfactory environmental conditions for germination. After full development the seed may revert to secondary dormancy if external conditions are unfavourable for germination (Wilson and Loomis, 1962). In this context, seed dormancy in *Casuarina equisetifolia* does not appear to be of the primary or secondary type. This is implied from the observation that the seeds used in this study were harvested fresh from the tree and germination occurred readily once the external conditions for germination were satisfied. However, the germination percentage seems to be inherently low. Each experiment in this study was repeated at least twice and the results presented

Table I. Total number germinating out of 75 seeds, untreated (control) or scarified with concentrated H_2SO_4 for various periods of time.

*Time (d) from sowing**	*Untreated (control)*	*Duration (min) of scarification with acid* 5	10	20
5	0	0	0	0
6	3	6	3	0
7	5	10	9	0
8	5	11	11	0
9	5	13	12	0
10	5	14	12	0
11	5	15	12	0
12	5	15	12	0

* No germination observed prior to the first day recorded in the table.

Table II. Total number germinating out of 75 control (A) or scarified (B) seeds moistened with different molar concentrations of sodium nitrate ($NaNO_3$).

*Time (d) from sowing**	*Concentration of $NaNO_3$(mM)* 0 (control)		2		10		50	
	A	B	A	B	A	B	A	B
5	0	0	0	0	0	0	0	0
6	3	8	4	10	1	11	1	5
7	5	13	13	20	12	23	4	7
8	5	13	18	24	20	27	7	8
9	5	13	19	24	22	28	9	8
10	5	13	19	24	23	28	9	8
11	5	13	19	24	24	28	9	8
12	5	13	19	24	24	28	9	8

* No germination observed prior to the first day recorded in the table.

Table III. Total number germinating out of 75 control (A) or scarified (B) seeds moistened with water or 0.1 mM of the named phytohormone.

*Time (d) from sowing**	*Water*		*IAA*		*GA_3*		*ABA*			
	A	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A_0***	*B_0***
2	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	1	0
4	0	0	0	0	0	0	0	0	2	1
5	0	0	0	0	0	0	0	0	2	1
6	4	6	9	5	10	17	0	0	2	1
7	10	9	11	18	26	29	0	0	2	1
8	10	10	14	19	28	36	0	0	2	1
9	10	10	14	19	32	37	0	0	2	1
10	10	10	14	19	36	46	0	0	2	1
11	10	10	14	19	38	47	0	0	2	1
12	10	10	14	19	43	47	0	0	2	1
13	10	10	14	19	43	47	0	0	2	1

* No germination observed prior to the first day recorded in the table. ** Seeds presoaked in ABA for 6 d and thoroughly washed with water before sowing.

here are a confirmation of a consistent trend. Waxy and hard seed coat dormancy has been broken with the use of concentrated H_2SO_4 by several workers (Mayer and Poljakoff-Mayber, 1963; Ballard, 1973; Gill and Bamidele, 1981; Etejere *et al*, 1982; Eze and Orole, 1987). The duration of treatment largely depends on the hardness and thickness of the testa. Thus *Dialium guineensis* required 10 min (Gill and Bamidele, 1981), *Parkia clappertoniana* 15 min (Etejere *et al,* 1982), *Brachiara decumbens* 20 min (Whiteman and Mendra, 1982), and *Acacia farnesiana* 30 min (Gill *et al*, 1986). In the case of *Casuarina equisetifolia* in the present study, only 5 min was required, which is not surprising since the seed is very small and its coat is thin. The basis of action by concentrated H_2SO_4 or other strong mineral acids has been discussed by Mayer and Poljakoff-Mayber (1963).

Under favourable external conditions the percentage germination of the *Casuarina* seeds is low (*ca* 10%), although some of the seeds may

Table IV. Total number germination out of 75 control (A) or scarified (B) seeds pre-soaked for 24 h in various concentrations of ascorbic acid.

*Time (d) from sowing**	*Ascorbic acid concentrations (mg dm^{-3})*													
	0		*250*		*500*		*1 000*		*2 500*		*5 000*		*10 000*	
	A	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	1	3	2	3	5	4	4	3	8	5	3	1	5	2
6	7	6	15	11	14	9	19	7	25	14	13	5	19	8
7	11	13	17	19	15	21	19	15	29	26	16	14	24	12
8	11	13	17	19	15	21	19	21	29	31	16	17	24	19
9	11	13	17	19	16	21	19	21	30	32	16	17	24	19
10	11	13	17	19	16	21	19	21	30	32	16	17	24	19
11	11	13	17	19	16	21	19	21	30	32	16	17	24	19
12	11	13	17	19	16	21	19	21	30	32	16	17	24	19

* No germination observed prior to the first day recorded in the table.

Table V. Number of seeds germinating out of 75 contol (A) or scarified (B) seeds pre-heated in the oven for 2 or 5 min at different temperatures.

*Time (d) from sowing**	*Temperature (°C)*																	
	28 ± 2 (room temp)		*40*				*55*				*70*				*90*			
			Duration of heating (min)															
			2		*5*		*2*		*5*		*2*		*5*		*2*		*5*	
	A	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	3	2	4	5	6	4	3	5	3	7	4	6	7	7	5	2	2	6
7	8	11	8	15	7	11	7	10	7	16	11	11	13	14	11	5	5	10
8	11	17	8	16	8	12	8	13	7	16	11	14	14	17	12	7	6	10
9	11	17	8	16	8	12	10	13	8	16	11	16	15	19	12	7	6	10
10	11	17	8	16	8	12	10	13	8	16	11	16	15	19	12	7	6	10
11	11	17	8	16	8	12	10	13	8	16	11	16	15	19	12	7	6	10
12	11	17	8	16	8	12	10	13	8	16	11	16	15	19	12	7	6	10

* No germination observed prior to the first day recorded in the table.

germinate readily. Even with overcoming hard-coat dormancy with scarification the germination improves only to about 20%. This suggests that water impermeability due to seed coat hardness may not be the only reason for the low percentage germination. This is supported by the favourable results obtained with chemical treatment in the absence of scarification, *eg*, the application of IAA, $NaNO_3$, ascorbic acid and particularly GA_3. With *Prosopis africana* Eze and Orole (1987) obtained 40 and 75% germination, respectively, for the unscarified and scarified seeds treated with $NaNo_3$ compared to 30% germination in the untreated control. The basis of $NaNO_3$ effectiveness has been discussed by Hendricks and Taylorson (1972). The results of Shineng (1988) working on *Sesbania* showed a promoting effect on germination when seeds were pretreated by soaking in ascorbic acid solution. The optimum effect was obtained with a concentration of 500 – 1 000 mg dm^{-3}, but there was no discussion on the basis of the ascorbic acid effect. In the

Table VI. Total number germination out of 75 control (A) or scarified (B) seeds sown on moist filter paper under different light conditions.

Time (d) from sowing	*Alternating day and night*		*Darkness*		*Colour of continuous light*									
					White		*Blue*		*Green*		*Orange*		*Red*	
	A	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	2	1	1	2	2	2	2	2	4	3
6	3	2	0	0	13	8	1	2	2	2	13	7	13	11
7	8	11	1	2	13	15	1	2	3	2	13	11	13	20
8	11	17	1	2	13	15	1	2	3	2	13	11	15	20
9	11	17	1	2	13	15	1	2	3	2	13	11	15	20
10	11	17	1	2	13	15	1	2	3	2	13	11	15	20
11	11	17	1	2	13	15	1	2	3	2	13	11	15	20
12	11	17	1	2	13	15	1	2	3	2	13	11	15	20

* No germination observed prior to the first day recorded in the table.

present study with *Casuarina* the promoting effect of ascorbic acid was confirmed but the optimum concentration was found at 2 500 mg dm^{-3} It should be noted that ascorbic acid is a strong reducing agent. Since it further enhances the germination of seeds adequately scarified with concentrated H_2SO_4, its dormancy-breaking mechanism may be metabolic rather than physical. Conflicting results have been obtained with IAA by other workers but according to Mayer and Poljakoff-Mayber (1963) this depends on the species and IAA concentration used. Baskin and Baskin (1974) and several other workers have reported the stimulating effect of GA_3 on seed germination. In the present study this has been the most effective germination promoter, giving up to 57 and 62%, unscarified and scarified seeds, respectively. A well-known effect of this hormone is to break dormancy and promote germination under otherwise unfavourable conditions, *eg*, germination of light-requiring lettuce seeds in the dark (Baskin and Baskin, 1974). Reynolds and Thompson (1971) showed that the maximum temperature for germination of lettuce seeds could be moved upwards or downwards by treating seeds with kinetin or ABA, respectively. In the present study there was no attempt to investigate the interaction of the effects of ABA and temperature on seed germination. Thus only the inhibitory effect of ABA on germination was confirmed. The usual absence of spontaneous emergence of *Casuarina* seedlings in the vicinity of fruiting parent trees, unlike a number of other trees, is consistent with the low percentage germination of untreated seeds as found in this study. Another rational explanation would be the papery light weight of the seeds which are easily blown away from around the parent tree.

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