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FACTORS INFLUENCING GERMINATION AND DORMANCY OF *HONCKENYA PEPLOIDES* (L). EHRH

Part I. Improvement of Germination

S.A.S. Tirmizi

Department of Botany, University of Sind, Jamshoro, Sind

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Dormancy in *Honckenya peploides* L Ehrh. is environmentally induced presumably as an ecological adaptation to protect seed from premature germination in the adverse environmental conditions. Dormant seeds failed to germinate without treatment and germination was accomplished by subjecting seeds to alternating periods of high and low temperatures. Chemical treatments with gibberellic acid (GA_3), kinetin or a combination of both enhanced germination.

Key words: Dormancy, Germination, *Honckenya peploides.*

INTRODUCTION

Honckenya peploides (L) Ehrh. is a species of the family Caryophyllaceae that exhibit seed dormancy. It has circumboreal distribution [1] and occurs commonly in the proximity of sea shores [2]. The ability of *H. peploides* to inhabit foreshore regions where tidal influence and high salinity makes it impossible for other species to survive has been remarked upon by Westhoff [3]. It has also been reported that *H. peploides* stabilizes drifting sand by binding sand particles around its roots [4].

An extensive literature is available on the dormancy and germination of different species, but there is insufficient information available on the dormancy of *H. peploides.* Reports mention the improvement of germination of this species with gibberellic acid (GA_3) treatment [5]. Goas and Guedes [6] suggested that the mechanical resistance of the integument is the cause of dormancy of this species.

Owing to the paucity of information and the importance of this species in stabilizing mobile sand dunes, the factors influencing dormancy and germination of *H. peploides* were felt to justify further investigation. The present work is an attempt to determine which environmental factors exert influence on *H. peploides* and which conditions inhibit germination.

MATERIALS AND METHOD

Dorman seeds were collected from Gibraltar Point, Lincolnshire, Britain in the year 1981 and 83.

Experimental technique. Germination experiments were conducted in 9cm diameter Petridishes. Seeds were germinated on four layers of moist filter papers (Whatman No. 1). Seven ml of distilled deionized water or appropriate test solutions were supplied to the Petridishes (7ml was considered to be suitable, since this did not submerge the seeds nor create abrupt changes of concentration in test solution as a result of evaporation of water from the Petridishes). Solutions were changed on alternate days after washing the Petridishes with distilled water or test solution to prevent the possibilities of contamination from seed exudates. After commencement germination was recorded daily till there was no sign of further germination for three consecutive days. A seed was considered germinated when the radicle attained the length of 0.5 cm. For each concentration there were five replicate and each replicate contained 20 seeds. Therefore the total germination was considered percent germination.

Effect of temperature. Partially ripened (the term partially ripened was used for seeds of a previous collection which showed 50% germination at 30° in laboratory) and dormant (freshly collected) seeds sown at different temperature regimes of 20°, 25°, 30° and 35° to determine the effect of temperature on seeds and to establish which temperature is optimum for germination.

Thermal fluctuations are quite common in nature, especially in intertidal zones the temperature drops many degrees within a matter of seconds each time waves return tocover sun heated beach [7]. Since *H. peploides* inhabit coastal embryo dunes which frequently come across such conditions an experiment was carried out on alternate temperature ranging from 10° (8 h) and 30° (16 h) to observe the effect on breaking of dormancy. However, it requires a series of experiments but owing to the lack of

time the author has performed only one experiment keeping British summer in view.

Stratification. Dormant seeds were stratified at 5° and 10° under moist condition for 15 and 30 days to observe the effect of chilling on germination.

Chemical treatments. The following experiments on chemical treatments were carried out at 30° Since in initial experiment 30° was found optimum for germination (see result table 1)

Giberrellic acid. Gibberellic acid (GA_3) was supplied to break the dormancy of *H. peploides* in concentrations of 5, 10, 25, 50 and 100 p.p.m. A stock solution of GA_3 was prepared by dissolving it in a few drops of ethanol and then diluting it in deionized distilled water. Appropriate concentrations were prepared from the stock solution.

Kinetin. Effect of kinetin (6-furfuryl-aminopurine) was observed on the breaking of dormancy. Kinetin was supplied in 5, 10, 25, 50, and 100 p.p.m concentration. A stock solution was prepared by dissolving it in boiling water (deionized distilled). Further concentrations were made from the stock solution.

Effect of osmotic pressure on germination. In order to differentiate the effect of environmental stress on the seeds of *H. peploides* a few germination experiments were conducted using mannitol, sea water dilutions and sodium chloride solutions. Since the germnation of *H. peploides* was found to be enhanced by GA_3, the seeds were soaked in 25 p.p.m GA_3 solution for 24 h before sowing in appropriate solution.

Effect of mannitol. An experiment was carried out to observe the effect of high osmotic pressure solutions on the germination of *H. peploides.* A non electrolyte (mannitol) was used at different potentials of −2.027 (2 atm), −4.054 (4 atm), 6.081 (6 atm) and −8.21 bar (8 atm).

Effect of sea water. Sea water dilutions (v/v, natural sea water + distilled water) of 20, 40, 60 and 80% were supplied to observe the effect on the germination of *H. peploides.*

Effect of sodium chloride. Seeds of *H. peploides* were germinated in various concentrations of sodium chloride (25, 50, 100 and 200 mM) to observe and differentiate either osmotic or specific ion effect.

Statistical analysis. The analysis of variance (AOV) was carried out for GA_3, kinetin sea water dilutions and sodium chloride treatments following Bishop [8]. For experiments on temperature and mannitol effect only standard deviations were taken, since, the number of treatments where germination occurred were too small to carry out analysis of variance.

RESULTS AND DISCUSSION

Effect of temperature. Temperature of 20°, 25°, 30° and 35° were used on partially ripened seeds to determine the optimum temperature for germination. The seeds did not germinate at 20° and 25°. At 30° and 35° the percentage of germination was 50% and 10% (Table 1). Therefore 30° was considered optimum for germination.

Dormant seeds did not germinate at any of the temperature investigated. Seeds moistened with distilled water were stratified at 5° and 10° for 15 and 30 days and then transferred to 30° these did not germinate. However, when unstratified dormant seeds were kept for germination at alternate temperatures of 10° (8 h) and 30° (16 h) 80% germination was recorded (Table 1). Germination of seeds on alternate temperature treatment commenced after 8 days. It was concluded that cold pre-treatment is not essential for germination of *H. peploides* seeds and that alternate low and high temperatures enhances its germination.

Table 1. The percent germination of partially ripened and dormant seeds of *H. peploides* at various temperature regimes.

	Temperature regimes in °C					
	Control	20°	25°	30°	35°	
Experiment 1						
Partially ripened seeds	0	0	0	51 ± 7	10 ± 5*	
Experiment 2						10°(8h)/30°(16h)
Dormant	0	0	0	0	0	80 ± 10

* No significant test was carried out (± SD of 5 replicate)

Gibberrellic acid treatment. Gibberellic acid (GA_3) treatments were found to be stimulatory to seed germination. Freshly collected (dormant) seeds were sown in different GA_3 concentrations of 5, 10, 25, 50 and 100 p.p.m. The germination commenced after 8 days. The percentage germination decreased with increasing concent-

ration (Table 2). Germinated seeds exuded a mucilaginous substance which adversely affected the seedlings growth by causing detachment of radicle. Therefore another experiment was carried out in which seeds were soaked in GA_3 concentrations of 5, 10, 25, 50 and 100 p.p.m. for 24 h. After this pre-treatemtn seeds were washed in distilled water before keeping for germination. The percentage of germination increased with the increasing concentrations of GA_3 used. However the radicle of germinated seeds soaked at 50 and 100 p.p.m. become flaccid (Table 2). Comparing these results it seems that soaking seeds in GA_3 concentration is advantageous to germination and that 25 p.p.m. is suitable to obtain highest germination without any damage to the radicle.

Kinetin treatment. The germination of seeds enhanced prominently in kinetin concentrations compared to the control, whereas, it was less than the germination in GA_3 concentrations. The higehst germination was recorded in 25 p.p.m. and the lowest in 5 p.p.m. However at 50 and 100 p.p.m. concentrations the radicle was found to be flaccid quite similar to that obtained in GA_3 concentrations. When GA_3 was suplemented with kinetin in isotonic concentrations of 25 p.p.m germination increased upto 90%. A combination of both GA_3 and kinetin enhanced germination more than kinetin alone (Table 2).

Effect of Mannitol solutions. H. peploides seeds (presoaked in GA_3) germinated only in −2.027 bar (approximately equal to 2 atm) mannitol solution but the germination was lower than the control (Fig. 1). The ungerminated seeds when transferred to distilled water did not germinate at all.

Effect of sea water dilutions. H. peploides seeds (presoaked in GA_3) did not germinate at all in any sea water dilutions. However, when the ungerminated seeds were transferred to distilled water they germinated, but with a significatnly lower percentage than the control. High germination percentage in seeds transferred from 80% dilution seems to be the result of priming effect of high salt concentration a common feature in halophytes.

In a similar experiment seeds were transfered to distilled water and then suplemented with kinetin (25 p.p.m), the germination was improved remarkably (Table 3).

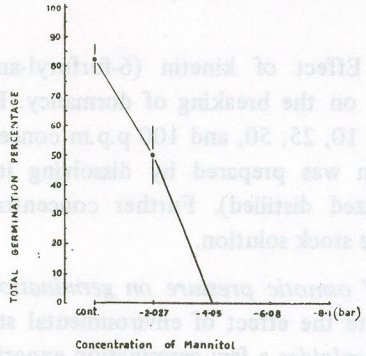

Fig. 1. Indicates the germination of GA_3 treated H. peploides seeds in mannitol solutions of different osmotic pressure (± SD).

Table 2. The germination of *H. peploides* dormant seeds in various gibberellic acid and kinetin treatments.

Concentrations		Total germination percentage					
Experiments	Control	5	10	25	50	100 ppm.	
Gibberellic acid (sowing)	0	81 ± 5.3	81 ± 8.3	76 ± 3	o	o[f]	
Gibberellic acid (soaking)	0	55[a] ± 9.1	65[b] ± 5	76[c] ± 7.9	81[c] ± 2.9	90[d] ± 5.3	ff
							25 ± 25 ppm. GA_3 and K
Kinetin (K)	0	40[a] ± 4.3	50[b] ± 5	71[c] ± 2.9	63[c] ± 10	66[c] ± 9.1	90[d] ± 8

(f) Seedlings become flaccid and died in these concnetrations. The sowing test did not show significant differences on AOV, therefore it is not mentioned here.

(ff) The similar letters indicate the insignificant differences between the means of those treatments, but, they are significant comparative to others having different letters (± SD of 5 replicate P = 0.05, 0.01 and 0.001).

Table 3. The germination of *H. peploides* (presoaked in 25, ppm GA_3) seeds after being transferred from sea water dilutions to (A) distilled water and (B) in kinetin (25 ppm) solution (mean ± SD of 5 replicate)

Sea water dilutions	Total germination percentage				
Experiments	Control	20	40	60	80%
(A) Transferred to distilled water	90^{a} ±12	28^{b} ±5.1	24^{bc} ±5	33^{bcd} ±7.5	39^{d} ±6.1
(B) Transferred to 25 ppm	90^{a} ±12	50^{b} ±5	$55^{b}c$ ±9.1	70^{d} ±9.4	74^{d} ±6.1

* Same control was used for both sets of experiments.

Effect of sodium chlroide. The germination of seeds presoaked in GA_3 irrigated with sodium chloride decreased with increasing concentrations with insignificant differences between control and 25 and 50mM treatments. The significant reduction in germination occurred in 100 and 200 mM sodium chloride solutions compared to the control (Fig. 2).

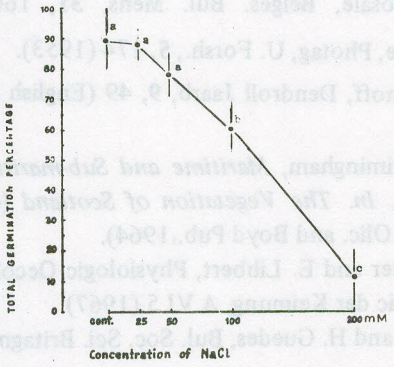

Fig. 2. Indicates the effect of different concentrations of NaCl on the germination of *H. peploids* seeds presoaked in 25 ppm. GA_3 concentrations. (± SD, P = 0.001)

The results suggest that temperature is one of the vital factors in the germination and dormancy of *H. peploides*. In laboratory experiments on partially ripened seeds temperature of 30^{o} was found optimal for germination. However, this temperature seldom occurs in the British climate from where the collection of seeds was made. It is possible that soil temperature is greater than the atmospheric temperature during spring when the germination occurs. Salisbury [9] noted that soil temperature in coastal sand dunes (British) increased upto 30^{o} at this time of year. Therefore, it is possible that the temperature requirment for germination of *H. peploides* could be around 30^{o}.

On the contrary stratification with cold temperatures of 5^{o} and 10^{o} did not favour germination at all. Thus it seems unlikely that cold treatment is necessary before germination. Rather it is possible that *H. peploides* seeds undergo dormancy at winter temperatures below 0^{o} and freezing temperatures may be one of the causes of its dormancy.

Alternating temperatures of 10^{o} (8 h) 30^{o} (16 h) enhanced germination *H. peploides* (80%). It can be presumed that in spring when the seeds pass through several thermal fluctuations they overcome dormancy and start germination. There have been reports on the enhancement of germination of dorment seeds in some species by fluctuating temperatures, for instance, *Cynodon dactylon* [10] *Avium graveolens* [11]; and *Lycopus europaeus* [12-14]. However, the role of fluctuating temperatures is still obscure. A few suggestions have been advanced by Toole *et al.* [15] Cohen [16] and Thompsom [14]. According to Toole *et al.* changes in temperature act biochemically in enzyme thermodynamics to alter the concentration of these reactants on which the germination depends. Cohen's proposal was based on physical changes of state correlated with maximum temperature reached in the higher of the two temperature phases. He proposed that this chang might involve conversion of an active enzyme precurs or to an active stage or result in alteration of membrane permeability, which permit interaction between previously separated compounds. The third assumption put forward by Thompson [14] with special reference to *Lycopus europaeus* is that germination depends on the completion of a minimum of one complete temperature cycly which include two distinct phases and two periods of temperature changes. The experiments so far have been designed to break the dormancy of *H. peploides* and from these results it is not possible to determine the physiological basis of the responses which occur in fluctuating temperatures. Nevertheless, relying on the aforementioned findings it can be presumed that *H. peploides* have a similar pattern of responses. However it requires further study to assess the correct picture of its metabolic changes occurring over a wide range of fluctuating temperatures.

Hormone treatment with GA_3 was found to stimulate germination of *H. peploides*. Kinetin has also stimulated germination but to a lesser extent than GA_3. However kinetin together with GA_3 enhanced germination more than kinetin alone. The stimulatory effect of gibberellins

(GAs) and gibberellic acid (GA_3) in particular for the germination of dormant seeds is an established fact. Amen [17] has postulated that GA_3 plays a universal role in seed germination, Jones and Stoddart [18] summarized the effect of gibberellins that they stimulate germination in seeds where dormancy is imposed by a wide variety of mechanisms e.g. in complete embryo development, mechanically resistant seed coats, presence of germination inhibitors and the factors relating to the physiological competence of the embryo axis. Similarly kinetin (Ctyokinin) has also been reported to release dormancy of seeds [19] Khan [20] postulated the hypothesis of primary, preventive and permissive role of hormones. He emphasized that gibberellins have a primary role in the release of dormancy. Inhibitos (e.g. ABA) and cytokinins have a secondary role i.e. preventive and permissive respectively. He also suggested that combintion of gibbrellins and cytokinins often give better response.

From the present data on GA_3 and kinetin treatment it has become obvious that *H. peploides seeds require some* sort of trigger effect to overcome dormancy. Thus it leads to two possible factors (a) hormone level (gibberellins or cytokinin) is either suppressed or (b) the level is insufficient to initiate germination. However, without biochemical study it is not possible to comment on the hormonal balance, therefore, no attempt has been made to correlate this with the ecological conditions which influence the dormancy in *H. peploides*. The question is 'what could be the ecological conditions which cause either suppression of growth regulators or their insufficient production. The possible answer lies in two ecological factors which are quite common in the British coastal environment 1– increased osmotic pressure (low water potential) 2-increasing salinity. In an experiment in which the osmotic pressure poduced by mannitol had affected germination severly, GA_3 induced seeds germinated only at -2.027 bar (2 atm. p). Similar results were obtained by sea water concentrations where seeds did not germinated at all. When they transferred from sea water concentrations to distilled water after 25 days germination started, although the percentage of germination was very low. In anotehr experiment seeds were transferred from sea water concentrations to distilled water supplemented by kinetin (25 p.p.m.) germination was enhanced more than that of the previous set. Moreover, it was further observed that those seeds which were transferred from 80% sea water dilution (80 parts sea water and 20 parts distilled water) started germination earlier than those in lower concentrations. Therefore it appears likely that osmotic stress and sea water concentrations affect seed germination of *H. peploides* in the coastal environment.

In order to differentiate the osmotic and specific Ionic effect of sea water(or salinity) on the dormancy of this species, an experiment was conducted with sodium chloride concentrations after treating the seeds with GA_3 (25, ppm.). The incidence of Germination resulting decease with increasing concentration. therefore this result rules out the specific ion effect on inducing dormancy. These results leads to the conclusion that (a) high osmotic pressure of the soil substratum causes dormancy and (b) salinity or osmotic stress under field conditions acts as a primming effect which on alleviation or subsidence of stress boosts germination. Similar results have been reported by Binet [21-22] Ungar [23,24] and McKee and Ungar [25]. However, the species studied by them apparently respond to osmotic stress of sodium chloride.

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