

PRESENCE OF AN INHIBITOR IN THE "SEEDCOAT" OF RAGI

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THE experiments of Kidd and West¹ showed that germination of barley and other cereals could be hastened by presoaking. Chippindale² had demonstrated this in many other species of Graminae. During the process of seed-hardening in ragi it was observed in this laboratory that seeds treated with 50% moisture followed by drying at room temperature, germinated earlier and also showed initial vigour.³ Several reports show the presence of a germination inhibitor in the seed-coats of different species of plants.⁴⁻⁶ There are instances as in *Euphorbia* sp., and *Dectis papposa* where the seeds contain a water-soluble germination inhibitor, which can be easily leached with water. When the leached seeds were placed in their own leachate, they did not germinate.⁷ The possibility of the existence of such a germination inhibitor in the 'seedcoat' of ragi was examined, as a possible explanation for the earlier germination and vigour in the treated seeds.

Aqueous leachates from seeds of three varieties of ragi H. 22, Aruna and Annapurna were prepared by soaking 100 mg. of seeds in 100 ml. of distilled water for 24 hr. with occasional stirring. At the end of the 24 hr. period the remaining water was filtered and the filtrate used for test as the leachate. In one variety Annapurna, air was bubbled through the seeds, during the period of soaking. This is referred to as the aerated leachate. The pH of these leachates, immediately after filtering, was 6.2, 4.7, 5.1, 7.4 for H. 22, Aruna, Annapurna and Annapurna (aerated) respectively.

Germination tests were conducted in petri dishes with filter-paper soaked with the test leachate. Each petri dish had 4 ml. of the leachate. The effect of the leachate was compared with water in each variety. The number of seeds germinated (emergence of the radicle was taken as the criterion for germination) was counted at the end of 24 hr. and 48 hr. The data on germination are presented in Table I. It can be seen that germination is inhibited in the initial stages; however, at the end of the 48 hr. period the inhibitory effect is overcome in the variety H. 22. In the varieties Aruna and Annapurna even though there was an increase in the percentage of germination with time, the inhibitory effect is still pronounced.

The seeds of Annapurna seem to be more susceptible.

TABLE I

Effect of the aqueous leachates of different varieties of ragi on the percentage germination of the same variety and on that of other varieties. Data represent average of two experiments for 24 hours and 48 hours

	H ₂₂		Aruna		Annapurna	
	24 hrs.	48 hrs.	24 hrs.	48 hrs.	24 hrs.	48 hrs.
Water	92	94	78	89	55	81
Leachate of H ₂₂	60	93	30	86	27	46
Leachate of Aruna	52	86	39	67	20	61
Leachate of Annapurna	59	90	37	69	12	57
Leachate of Annapurna (aerated)	82	92	62	78	25	48

Regarding the growth of the coleoptile (including the primary leaf) and the seminal root also the leachates had inhibitory effect, as can be seen from the data in Table II. The inhibitory effect is more pronounced in the variety Annapurna with the leachate from

TABLE II

Effect of the aqueous leachates of different varieties of ragi on the growth of the coleoptile and seminal root of the same variety and on that of other varieties of ragi at the end of 48 hours. Data represent the average of three experiments for coleoptile length and two experiments for root length

	H ₂₂		Aruna		Annapurna	
	Length of coleoptile	Length of root	Length of coleoptile	Length of root	Length of coleoptile	Length of root
(mm.)						
Water	10.3	12.0	6.6	13.0	5.3	15.5
Leachate of H ₂₂	6.6	8.5	4.3	7.5	3.0	6.5
Leachate of Aruna	4.3	7.5	4.3	7.5	2.3	8.0
Leachate of Annapurna	6.3	9.5	4.3	7.0	2.3	3.5
Leachate of Annapurna (aerated)	8.0	10.0	4.3	6.0	4.0	7.5

Annapurna only. Even in the other varieties the growth in length of the coleoptile and the seminal root is approximately 50% of the growth in pure water (Fig. 1).

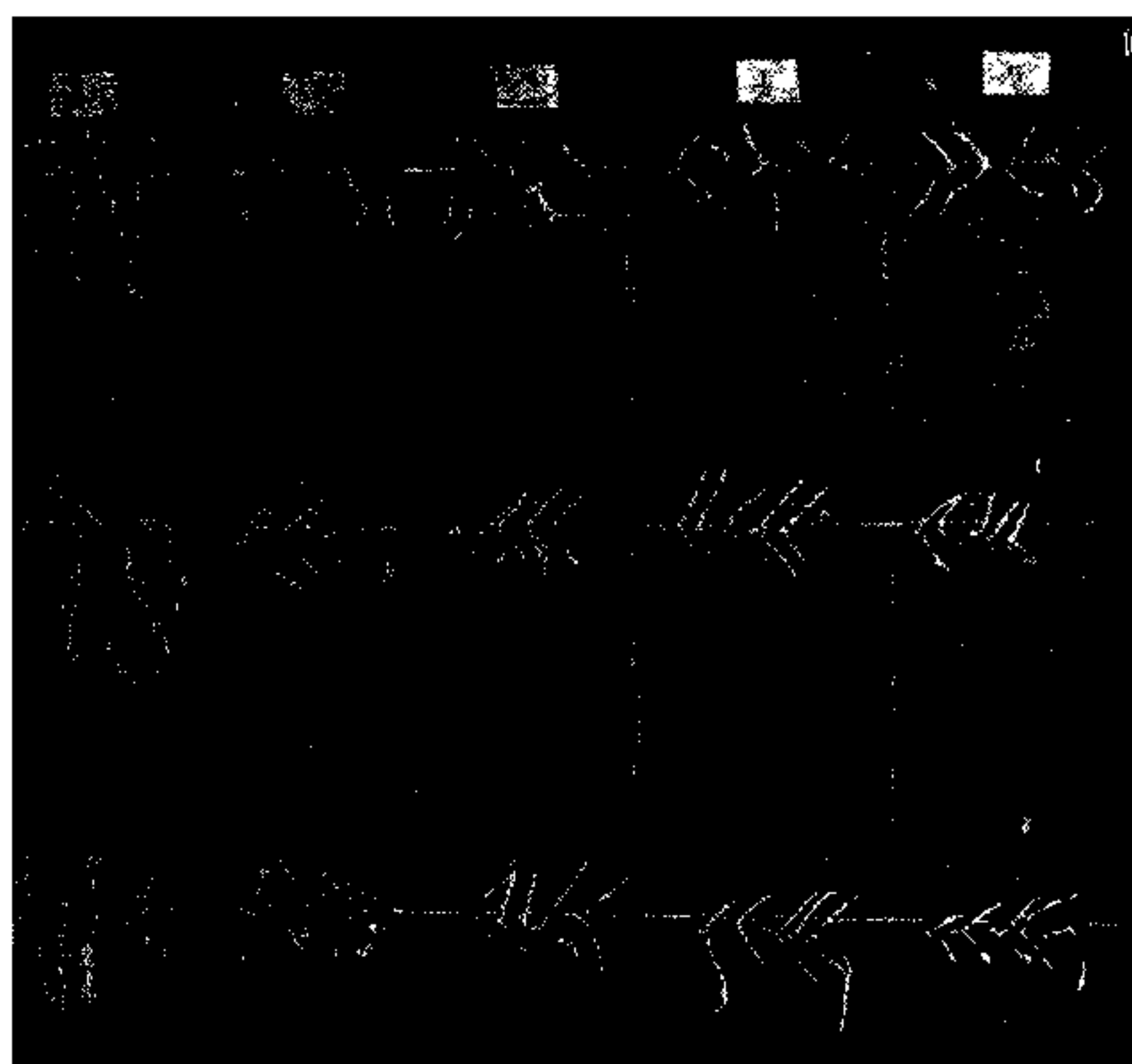


FIG. 1. Showing the effect of water leachates of ragi seeds (1. Water, 2. H. 22, 3. Aruna, 4. Annapurna, 5. Annapurna (aerated) on the germination of ragi (H. 22. top line, Aruna: Centre line, Annapurna: Bottom line).

Paper chromatography of the leachates was conducted to determine the R_f of the inhibitor. Five ml. each of the leachates from three different varieties were reduced to about 0.2 ml and developed in phenol: water (1:4 v/v) solvent system. After washing the paper with absolute ethyl alcohol, the paper was cut into 10 equal strips. Each strip was taken in a petri dish with 1 ml of water for germination test using 10 seeds. In all cases and in three different experiments the R_f of the inhibitor was in the range of 0.6 to 0.8 with a peak at 0.7.

The original leachate is brown in colour. It was decolorised by activated carbon. The coloured fraction was eluted from activated carbon with iso-propanol and water 1:1 (v/v). The eluate was evaporated to dryness at 60° C. and the residue was taken in water, maintaining the original concentration. The coloured and colourless fractions, along with the original leachate, were tested for germination using different concentrations. The results are presented in Table III. It can be seen that the decolorised

TABLE III

Effect of different concentrations of original leachate (O.L.), decolorised fraction (D.F.), and coloured fraction (C.F.), on (a) germination percentage, (b) length of seminal root, (c) length of coleoptile from variety Aruna on variety H₂₂

Dilution (ml. of leachate in 5 ml.)	(a)				(b)				(c)			
	Water	O.L.	D.F.	C.F.	Water	O.L.	D.F.	C.F.	Water	O.L.	D.F.	C.F.
00	93	32	25
0.10	..	98	86	90	..	40	43	34	..	23	26	25
0.25	..	96	80	92	..	46	41	31	..	26	24	25
0.50	..	90	80	92	..	54	55	41	..	30	27	27
1.00	..	88	72	91	..	39	43	27	..	21	24	20
2.50	..	86	44	90	..	37	41	28	..	17	24	21
5.00	..	68	48	96	..	15	40	12	..	13	21	18

fraction shows a marked effect on the germination percentage and with increasing concentration the percentage inhibition is also increased, while in the original leachate the inhibitory effect is less marked. The coloured fraction has little inhibitory effect on germination while on the other hand it shows a slight promotive effect. This perhaps explains the lesser inhibitory effect of the original leachate. In the growth of the coleoptile and the seminal root the coloured fraction shows inhibitory effect even when only 0.1 ml. of the fraction was used in 5 ml. and the dilution is 50 times the original concentration. This inhibitory effect is also seen in the original leachate. The decolorised fraction, however, appears to be free from the inhibitor of the coleoptile or seminal root.

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