SEED DORMANCY AND ITS CONTROL BY GERMINATION INHIBITOR IN *ANAGALLIS ARVENSIS* L. VAR. *CAERULEA* GREN ET GODR.

by K. P. SINGH, *Department of Botany, Banaras Hindu University, Varanasi 5*

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Seeds of *Anagallis arvensis*, a common winter annual of crop-fields, show post-harvest dormancy varying between four months to more than a year. Duration of dormancy depends upon climatic factors like temperature and rainfall during the storage. Seeds contain water-soluble inhibitor, which appears to control the duration of dormancy; higher amount of inhibitor being associated with deeper dormancy. Light seems to be necessary to counteract the effect of inhibitor.

INTRODUCTION

Anagallis arvensis, a member of family *Primulaceae*, is a common winter annual of crop-fields in India. Its seeds mature in the months of March and April, but germinate in nature at the start of winter season in November when temperature is low. Further, the seeds have been shown to be light sensitive (Grant Lipp and Ballard 1963). It was observed that freshly collected seeds gave very low germination percentage, even when moisture, low temperature and light were provided. But when these seeds were washed in running water, under same experimental conditions, more than 80 per cent germination occurred. These observations suggested the occurrence of post-harvest dormancy and the presence of some water-soluble inhibitor in the seeds of *A. arvensis*. This paper presents observations on some factors affecting the duration of post-harvest dormancy and the possible role of inhibitor in controlling the dormancy.

EXPERIMENTAL PROCEDURE

Seeds of *A. arvensis* were collected from wheat-fields in eight districts of Uttar Pradesh in the first fortnight of April 1965. They were stored in bakelite screw stoppered glass jars at laboratory temperature in darkness.

Germination tests were made by placing the seeds between moist folds of filter paper in Petri dishes. Petri dishes were placed in light-proof cardboard boxes, which were placed at 9–12 °C. Germination test was carried out for 15 days and daily the dishes were exposed to white light of about 160–200 lux intensity for 10 minutes. For each treatment three Petri dishes, with 100 seeds in each, were used.

Results

Experiment 1. *Germination of seeds stored at room temperature*

Seeds collected from the university campus were periodically tested for germination. The results are presented in Fig. 1. It is evident that as the seeds age the percentage as well as the rate of germination increases. Fifteen-day-old seeds gave 17 per cent germination in 18 days. After eight months 91·5 per cent germination was obtained within 12 days.

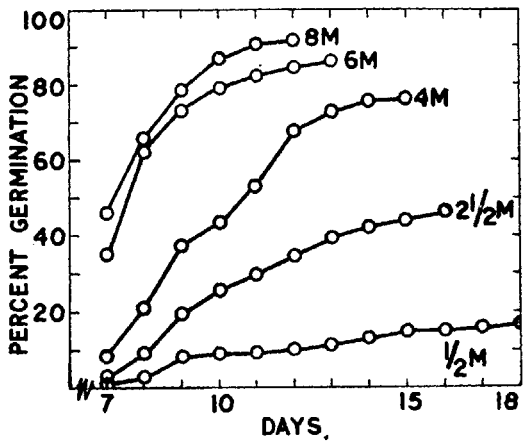

Fig. 1. Effect of age (months) on the rate and the percentage germination of seeds of *Anagallis arvensis.*

Seeds collected from seven other districts of Uttar Pradesh were stored in the laboratory and tested for germination at four-month intervals. It was found (Table I) that in all collections, the percentage germination increased with increasing storage period.

Table I

Percentage germination of seeds from different localities during storage at room temperature

Localities	Storage period in months		
	4	8	12
Agra	.. 56·0±7·0	70·6±4·0	82·3±3·0
Etawah	.. 75·6±3·9	84·0±1·6	90·9±0·9
Jhansi	.. 88·8±2·3	94·3±3·6	96·6±1·8
Gwalior	.. 86·3±2·1	89·0±3·7	95·9±2·1
Kanpur	.. 74·6±2·6	82·0±1·6	89·9±1·1
Banda	.. 49·0±2·4	60·0±2·9	75·3±1·9
Allahabad	.. 85·6±1·6	89·0±1·4	94·6±2·0

(Mean ± standard deviation)

It is also clear that duration of dormancy varies in different seed collections. After four months seeds from Jhansi, Gwalior and Allahabad were almost out of dormancy, while those from Agra and Banda remained dormant to a certain extent, even after a period of eight months in storage.

EXPERIMENT 2. *Germination of seeds stored at different temperatures*

In this experiment seeds collected from university campus were stored at 10, 20, 30 and 40 °C in tightly corked specimen tubes on 29 April 1965. Results of germination tests made after three and eight months are given in Table II.

TABLE II

Percentage germination of seeds stored at different temperatures

Storage temperature °C	Storage period in months	
	3	8
0 ..	63·3±3·7	63·3±3·7
10 ..	81·0±0·8	85·6±2·9
20 ..	83·3±2·7	90·3±1·8
30 ..	86·3±0·8	80·3±1·2
40 ..	92·0±1·6	— —

(Mean ± standard deviation)

It is axiomatic that the removal of dormancy depends on storage temperature. After three months, seeds stored at 40 °C had become non-dormant, whereas those stored at lower temperatures were still partially dormant. After eight months the seeds stored at 20 °C had reached non-dormant state and those stored at 10 °C also showed an increase in germination percentage. Seeds stored at 0 °C still gave only 63 per cent germination.

EXPERIMENT 3. *Germination of seeds stored in soil at different depths*

Seeds from university campus were used in this experiment. Seeds (previously stored at room temperature) were placed in nylon cloth bags (10×5 cm) and specimen tubes (tightly corked and sealed with wax), which were buried in soil at different depths on 12 July 1965. The nylon bags were perforated and hence the seeds were exposed to natural conditions of leaching, etc. Sealed specimen tubes served as controls. At intervals the seeds were removed from the soil and placed for germination in the laboratory. The results are set in Table III.

The data given in Table III show that leaching action of rain-water removes the dormancy of seeds of *A. arvensis*. The seeds lying in the surface

TABLE III

Percentage germination of seeds buried in soil at different depths

Treatment	Depth in cm	Per cent germination after	
		15 days	90 days
Nylon bags	10	99·0±0·0	94·3±4·4
Nylon bags	20	94·6±2·4	92·0±2·9
Nylon bags	30	94·0±2·1	87·0±0·7
Sealed specimen tube ..	10	75·0±2·1	86·0±2·1
Sealed specimen tube ..	20	77·4±2·6	86·3±4·8
Sealed specimen tube ..	30	74·4±1·9	83·0±2·9

(Mean ± standard deviation)

layer of soil (10 cm) become completely non-dormant within 15 days, presumably due to greater leaching activity. On the other hand, seeds buried at greater depths show slightly lower percentage germination. But in general, seeds enclosed in nylon bags gave higher percentage germination than those kept in sealed tubes. During these 15 days total rainfall was 153·4 mm. After three months (end of the rainy season) the percentage germination of seeds enclosed in nylon bags slightly decreased.

EXPERIMENT 4. *Effect of washing on germination*

Seeds from university campus were washed in running water for various durations and then placed for germination. This experiment was done 20 days after seed collection. Results are set in Table IV.

TABLE IV

Effect of washing on germination

Washing duration hours	Per cent germination
0 (control) ..	12·2±2·2
24 ..	80·5±2·4
48 ..	82·0±1·9
72 ..	74·0±3·2

(Mean ± standard deviation)

It is evident from Table IV that washing of seeds increases percentage germination. This observation suggests the presence of water-soluble inhibitor which is removed by washing.

In another experiment (performed six months after collection) seeds from Agra were washed for 48 hours. One lot of seeds was kept for germination immediately. The rest of the seeds were divided into two lots, which were placed in a dry Petri dish in darkness at 10 and 30 °C. Germination tests were made after 2, 20 and 45 days, with portions of seeds from both lots. The data obtained are given in Table V.

TABLE V

Effect of air drying the washed seeds on percentage germination

Storage temperature °C	Storage period in days			
	0	2	20	45
30	94·0±0·8	91·6±1·6	88·6±1·6	85·3±2·7
10		90·0±1·6	86·3±1·5	81·0±1·4

(Mean ± standard deviation)

Table V shows that if washed seeds are allowed to air dry, either at 10 or 30 °C, their percentage germination decreases. This experiment supports the data obtained in Experiment 3.

EXPERIMENT 5. *Effect of aqueous seed-extract on germination*

Extract of two 0·5 g lots of seeds from Agra, one lot, being washed in running water for 48 hours, was prepared as follows. A lot of 0·5 g seeds was crushed with small amount (about 10 ml) of glass distilled water in a glass mortar. The extract was decanted in a 50 ml centrifuge tube. Residue was further extracted thrice with small quantity of water. Combined extract was centrifuged at 2,000 rpm for two minutes and the supernatant was poured in a 50 ml volumetric flask. The residue in the centrifuge tube was suspended in 5 ml water and centrifuged. Supernatant was added to the 50 ml volumetric flask and the volume was made up. Test seeds (university campus collection) were placed for germination between folds of filter paper moistened with 10 ml of seed-extract. In all the subsequent experiments similar procedure was followed.

Data given in Table VI show that extract of unwashed seeds is more inhibitory than the extract of washed seeds. But slight inhibition is caused by extract of washed seeds also. Analysis of variance of data gives F value due to variation between treatments to be 13·24, which is significant at one per cent level. Hence it appears that washing does not completely remove the inhibitor, but decreases its amount to a level, where germination is not impeded.

TABLE VI

Effect of extracts of washed and unwashed seeds (Agra collection) on germination of seeds collected from university campus

Treatment, extract of	Per cent germination on	
	7th day	15th day
Unwashed seeds ..	36·0	82·0
Washed seeds ..	44·0	86·0
Water control ..	45·6	91·6

EXPERIMENT 6. *Inhibition by extract of seeds imbibed for different periods*

0·5 g lots of seeds of Agra collection were placed between moist folds of filter paper at 9–12 °C. After different periods of imbibition extracts of seeds were prepared and 10 ml of it was pipetted into Petri dishes containing seeds from university campus. Percentage germination of seeds from university campus are given in Table VII.

TABLE VII

Effect of extract of seeds imbibed for different durations on germination of university campus seeds

	Water control	Extract of unimbibed seeds	Extract of seeds imbibed for (in days)					
			1	2	3	4	5	6
Percentage germination of seeds from university campus	80·0	76·0	76·3	67·3	50·6	61·0	65·6	70·3

The data show that the extract of unimbibed seeds is slightly inhibitory. Inhibition increases with imbibition up to third day. After third day, inhibition decreases gradually up to sixth day. Analysis of variance of the data yielded F value, due to variation between treatments, to be 48·1, which is significant at 0·1 per cent level. Hence it appears that when *Anagallis arvensis* seeds are placed for germination, there is an increase in the inhibitory activity and only when this activity decreases, germination is possible by sixth or seventh day.

EXPERIMENT 7. *Effect of seed-extract on water uptake of germinating seeds*

100 mg lots of seeds of university campus collection were allowed to imbibe at 9–12 °C in extracts prepared in Experiment 6. After three days seeds were removed, dried between folds of dry filter paper and weighed. Increase in weight is presented in Table VIII.

TABLE VIII

Water uptake by 100 mg seeds (university campus) in different seed-extracts

	Water control	Extract of unimbibed seeds	Extract of seeds imbibed for (in days)		
			2	4	6
Water uptake* in mg	40·2	37·8	39·3	39·7	41·8

* Average of four replicates in each treatment

It is observed that there is no significant difference in water uptake by seeds in extracts of different imbibition periods. Hence it follows that the inhibitory effects of seed extract are not due to osmotic reactions, but are the result of chemical inhibition.

EXPERIMENT 8. *Effect of extract of seeds stored at different temperatures on germination*

Extracts of seeds stored at different temperatures (Experiment 2) were prepared and applied to seeds of university campus collection placed for germination. Results are presented in Table IX.

TABLE IX

Effect of extract of seeds stored at different temperatures on germination of university campus seeds

	Extract of seeds stored at °C				Water control
	0	10	20	30	
Percentage germination of seeds from university campus	82·6	86·0	88·0	92·3	92·6

It is observed that inhibitory activity of extract increases with lowering of storage temperatures. These data have been subjected to analysis of variance and the F value due to variation between treatments is calculated to be 19·3, which shows that variations due to seed-extracts are significant at 1 per cent level.

EXPERIMENT 9. *Effect of extracts of freshly collected and one-year-old seeds on germination*

Extracts of freshly collected dormant seeds (April 1966) and one-year-old (April 1965) non-dormant seeds (both from university campus) were added to Petri dishes containing non-dormant seeds of university campus (April 1965) placed for germination. Data are set in Table X.

TABLE X

Effect of extract of dormant and non-dormant seeds on percentage germination of non-dormant seeds

Treatment, extract of	Per cent germination, after	
	7 days	15 days
Dormant seeds ..	29·3	74·3
Non-dormant seeds ..	39·6	82·0
Water control ..	46·3	90·0

In a parallel experiment the freshly collected seeds gave only eight per cent germination, showing that they are strongly dormant. From this experiment it is clear that the extract of dormant seeds is more inhibitory than that of non-dormant seeds. F value due to variation in treatments, calculated by analysis of variance, is 109·1, which is significant at 0·1 per cent level. Hence it is apparent that the inhibitor content of dormant seeds is greater than that of non-dormant seeds.

EXPERIMENT 10. *Effect of light exposure during imbibition on the inhibitory activity of seed-extract*

Two batches of seed of 0·5 g lots were allowed to imbibe water for three, six and nine days. One batch was kept in complete darkness, while the other was exposed daily to light of 160–200 lux intensity for 10 minutes. Experiment was so arranged that all the imbibition periods ended the same day, when their extracts were prepared and tested with seeds of university campus. Results are given in Table XI.

Data presented above have been subjected to analysis of variance, which shows that the difference, due to effects of extracts of seeds exposed to light or kept in darkness during imbibition period, was significant at 0·1 per cent level. However, the differences due to various imbibition periods were found to be insignificant. Nevertheless, this experiment does show that the extract of seeds exposed to light is less inhibitory.

TABLE XI

Inhibition of germination by extract of seeds imbibed in dark and light (10 minutes daily)

Extract of seeds imbibed in	Per cent germination of test seeds		
	3	6	9
	(imbibition period in days)		
Light	79·6	81·6	76·2
Dark	73·3	72·3	63·0
Water control ..		88·3	

DISCUSSION

Post-harvest dormancy.—The data given above clearly demonstrate that the seeds of *Anagallis arvensis* show post-harvest dormancy. The seeds gradually become non-dormant during dry storage in laboratory. Hence the collections from different places cannot be characterized by their percentage germination, but the period of release from dormancy should be emphasized. In the collections studied this period varies from less than four months to more than 12 months under laboratory storage conditions. Grant Lipp and Ballard (1963) have pointed out that, apart from being genetically controlled, the duration of dormancy in *Anagallis arvensis* depends on the temperature prevailing during seed formation and maturation. They have shown that lower temperatures during seed maturation cause prolonged dormancy.

Climatic factors, like temperature and rainfall, to which the seeds of *Anagallis arvensis* are exposed after maturation, seem to be of great importance in controlling the duration of dormancy. Higher temperature reduces the period of dormancy. Similarly, washing of seeds artificially or by natural rainfall immediately removes dormancy. Under Indian conditions seeds of *A. arvensis* mature in March-April. Afterwards, they are subjected to high temperatures (up to 45 °C) during summer (April–June), which is followed by moderately high (30–35 °C) temperature and plenty of moisture during the rainy season (July–September). Later the temperature drops to about

15–20 °C by the end of October, when germination of *A. arvensis* is observed in the fields.

Water-soluble inhibitor in the seeds.—The observations, that the washing of dormant seeds induces about 90 per cent germination and that seed-extract inhibits the germination of same seeds, clearly establish a water-soluble inhibitor in the seeds of *A. arvensis*. Experiment 7 provides additional evidence for the presence of a chemical inhibitor in the seed-extract. Inhibitor content seems to change when seeds are placed for germination. Grant Lipp and Ballard (1963) have shown that non-dormant seeds of *A. arvensis* start germinating from the first day they are set out and by fourth day maximum germination is attained. In our collections germination starts on the sixth day and continues for about 12 days. Dry seeds contain very small amount of inhibitor. When the seeds are imbibed an increase in inhibitor content takes place. Similarly, in case of *Fraxinus excelsior* dry seeds do not contain any inhibitor but, after soaking, a strong inhibitor develops (Wareing and Villiers 1960). In the case of *A. arvensis* after three days, inhibitor content decreases gradually and germination does not start before the sixth day.

Possible role of inhibitor in seed dormancy.—The general problem of dormancy imposed by growth inhibitors has been reviewed by Evanari (1949), Toole *et al.* (1956) and Wareing (1965). Most of the authors (Barton and Solt 1949; Luckwill 1952; Lasheen and Blackhurst 1956; Flemion and De Silva 1960; Villiers and Wareing 1965) did not find strict relation between decrease of inhibitor and break of dormancy. However, in the case of *A. arvensis*, the decrease in inhibitory activity of seed-extract, when the dormant seeds step out of dormancy, is very clearly demonstrated. Storage of seeds at higher temperatures, which reduce the duration of dormancy, also decreases the inhibitory activity of seed-extract.

It is very likely that the changes in inhibitory activity of seed-extract are due to changes in the amount of inhibitor. The inhibitor is gradually metabolized during storage, particularly at higher temperatures. Further, it seems that even after prolonged storage (more than a year) or washing, the inhibitor is not completely removed. But a reduction in level is sufficient to allow germination, provided light is available. Probably light is required to overcome the inhibitory effects either by reducing the amount of the inhibitor or by inducing the formation of some growth-promoting substance (Wareing 1965).

Another explanation that could be offered is that the seeds of *A. arvensis* contain two inhibitors. One of them, present superficially, is highly water-soluble, while the other is deep-seated (possibly in the embryo). The superficial inhibitor is easily removed by washing or is readily metabolized at high temperatures. But the deep-seated inhibitor is relatively stable and requires

the action of light to counteract its effects. This inhibitor probably persists for a longer period, because the author has observed that even three-year-old seeds of *A. arvensis* require light for germination.

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References

Barton, L. V., and Solt, M. L. (1949). Growth inhibitors in seeds. *Contr. Boyce Thomson Inst. Pl. Res.*, **15**, 259–78.

Evanari, M. (1949). Germination inhibitors. *Bot. Rev.*, **15**, 153–94.

Flemion, F., and De Silva, D. S. (1960). Bioassay and biochemical studies on extracts of peach seeds in various stages of dormancy. *Contr. Boyce Thomson Inst. Pl. Res.*, **20**, 365–79.

Grant Lipp, A. E., and Ballard, L. A. T. (1963). Germination pattern shown by light sensitive seeds of *Anagallis arvensis*. *Aust. J. biol. Sci.*, **16**, 572–84.

Lasheen, A. M., and Blackhurst, H. T. (1956). Biochemical changes associated with dormancy and after ripening of blackberry. *Proc. Am. Soc. hort. Sci.*, **67**, 331–40.

Luckwill, L. C. (1952). Growth-inhibiting and growth-promoting substances in relation to the dormancy and after ripening of apple seeds. *J. hort. Sci.*, **27**, 53–67.

Toole, E. H., Hendricks, S. B., Borthwick, H. A., and Toole, V. K. (1956). Physiology of seed germination. *Ann. Rev. Pl. Physiol.*, **1**, 299.

Villiers, T. A., and Wareing, P. F. (1965). The growth substance content of dormant fruits of *Fraxinus excelsior* L. *J. exp. Bot.*, **16**, 533–44.

Wareing, P. F. (1965). Endogenous inhibitors in seed germination and dormancy. 'Encyclopaedia of Plant Physiology', Springer, Berlin, **15**, 90915, 909–24.

Wareing, P. F., and Villiers, T. A. (1960). Changes in buds and seeds in response to chilling. 'Plant Growth Regulation', Iowa State University Press.