J. Indian bot. Soc. 60 : 51-57, 1981.

AUTECOLOGICAL STUDIES IN *INDIGOFERA LINIFOLIA* (L. f.) RETZ. I. GERMINATION BEHAVIOUR OF THE SEEDS[1]

PIRATLA N. RAO AND B. V. N. REDDY

Department of Botany, Nagarjuna University, Nagarjunanagar-522 510, Guntur Dt., AP.

ABSTRACT

Seeds of *Indigofera linifolia* (L. f.) Retz. possess seedcoat dormancy. Various physical and chemical treatments were tried to induce germination in the freshly collected seeds. Seeds stored for a minimum period of 90 days and those stored up to 280 days germinated without any pretreatment. Mere submergence in water for a period of 2 hours induced germination in 35% seeds. Seeds kept in hot water at 56±2°C for 6 hours showed 64% germination. Direct germination of seeds between 0—45°C revealed that 27±2°C is optimum for imbibition and germination. Exposure to alternate cold and hot temperatures and pretreatment of the seeds with concentrated sulphuric acid for 10 minutes broke dormancy satisfactorily. Yellow, white, red, blue and green lights are favourable for germination in the sequence given. Acetic acid, nitric acid, copper sulphate and coumarin at different ppm concentrations proved unsatisfactory. The optimum pH for germination was found to be 7.5.

INTRODUCTION

Indigofera linifolia is a common member of the weed flora of Guntur mostly during the rainy season inhabiting open places, roadsides, canal bunds, grasslands and cultivated fields. The species shows considerable variation in its habit being pros-, trate and spreading or becoming decumbent in grasslands and the stem may be round or angled with or without grooves. The foliage is polymorphic ranging from linear to linear-lanceolate or short and obovate. The weed is predominantly distributed in the plains throughout India, Ceylon, Upper Burma, Afghanistan, Abyssinia, Malaya and North Australia. The weed is used as fodder to cattle and the seeds as food during famine by inhabitants in parts of Rajasthan deserts and West Indies. Previous works on species of *Indigofera* are those of Rugmini (1960), Chatterji and Baxi (1964), Vyas and Agarwal (1970, 1972) and of Sen (1976). Rugmini (1960 ; also cited in Pandeya *et al.* 1968) provided data about reproductive capacity in *I. linifolia* but none on the germination behaviour of seeds. The present workers recorded tricotyledony during germination studies in *I. linifolia* (Rao and Reddy, 1979) and also studied the aggressive capacity of the species (Reddy and Rao, 1979). This paper summarises the significant results obtained from the various experiments conducted in relation to the germination behaviour of the seeds in this weed species.

OBSERVATIONS

Seeds of *Indigofera linifolia* are round in shape, dark brown to black in colour with

[1] Accepted for publication on June 14, 1980.

We wish to express our thanks to Dr. D. N. Sen of Jodhpur University for encouragement and for providing valuable literature on *Indigofera* and to Prof. YSRK Sarma and Dr. R. S. Ambasht of the BHU for a copy of the BHU Dissertation abstracts, and to Prof. A. S. Rao for facilities. The second author acknowledges the award of a fellowship by the C.S.I.R.

a diameter of 1.29-1.38 mm. (measured with a screw gauge). The average weight of each seed is 1.59±0.022 mg. Seeds are nonendospermic and possess hard seed-coat. Seeds immediately after collection do not germinate well owing to seed-coat dormancy. All the following experiments were conducted in petri-dishes between two moist filter papers at room temperature, in diffuse day light unless otherwise stated. Two replicates of each 50 seeds were maintained in all cases. The detection of radicle protrusion was taken as the criterion for germination. Seeds collected on 10th December, 1977 from the University grasslands and stored in stoppered bottles under dry conditions were used where fresh seed was not tested. Seeds which showed about 50% increase in volume were counted for the value of percentage of imbibition. Recordings were made every 24 hours or as stated in the text. Experiments included a series of pretreatments and the results are presented here. For the determination of the value of standard error in the ensuing experimental results, the respective data shown in percentage was transformed to arcsin $\sqrt{\text{percentage}}$ (Schefler, 1969 ; 112).

1. *Water submergence :* Seeds were submerged in tap water for periods ranging from 2 hours to 144 hours and the results are given in Table I.

It is significant from the Table I that mere water submergence for a period of 2 hours could break the dormancy to an extent of 35%. However, increase in the duration of submergence has not influenced the percentage of either imbibition or germination.

2. *Effect of storage* : Stored seeds germinated to an extent of 85±9.38% after a three month storage when watched for eight weeks. Seeds successfully germinated even after a storage for 280 days. This shows that germinability is naturally restored after 10-12 weeks of seed dispersal, and this capacity persists for a considerably long time thereafter. This, however, is a time consuming technique.

3. *Hot water treatment* : Seeds were kept in a beaker of tap water and maintained in an incubator at a temperature of 55± 2°C for a maximum period of 72 hours. Seeds were picked out from the hot water at varying durations and were germinated. Observations recorded up to the eighth day from the beginning of the experiment are given in Table II.

Seeds showed highest percentage of germination after six hours. Longer periods gradually resulted in a softening of the seeds to the extent of forming a paste.

4. *Temperature treatment* : Fresh seeds were directly maintained to germinate at temperatures ranging from 0 to 45°C and the data are shown in Table III.

There is practically no germination at temperatures other than room temperatures (December-January).

5. *Effect of alternate temperatures* : Fresh seeds were maintained for specific alternating periods at cold (10°C) and hot (55°C) provided by using a refrigerator and an incubator respectively. Seeds so treated were then subjected to germination in diffuse daylight and the results cited in Tbble IV.

While fresh seeds retained their dormancy, seeds treated this way showed appreciable germination, maximum percentage being at higher durations of treatment.

6. *Sulphuric acid treatment* : Seeds pretreated with sulphuric acid (96% w/w) for various durations and after thorough washing were kept for germination, observed for five weeks and the results listed in Table V.

Hundred per cent germination was

TABLE I

EFFECT OF WATER SUBMERGENCE ON GERMINATION

	Control	Duration of submergence in hours				
		2	24	48	72	96	144
Percentage of germination ..	7 ±1.51	26 ±2.93	29 ±3.53	34 ±0.83	35 ±2.09	11 ±1.34	27 ±1.81
Percentage of imbibition ..	13 ±3.66	32 ±1.76	36 ±0.77	38 ±0.77	38 ±1.67	16 ±2.19	33 ±4.31
Number of days watched ..	22	33	27	29	27	31	28

TABLE II

EFFECT OF HOT WATER TREATMENT ON GERMINATION

Duration of the treatment in hours ..	Control	3	6	24	28	50
Percentage of germination	6.5 ±0.89	42 ±8.20	64 ±0.77	32 ±2.62	9 ±1.41	8 ±3.04

TABLE III

EFFECT OF TEMPERATURE ON GERMINATION

Temperature in °C	0	5	10	28±2	45
Percentage of germination	0	0	0	7±1.55	0

TABLE IV

EFFECT OF ALTERNATE TEMPERATURES ON GERMINATION

Duration in hours	Percentage of germination	Percentage of imbibition	Number of days watched
Control ..	8 ±1.71	13 ±3.66	20
12 hours hot & 12 hours cold ..	32 ±2.60	41 ±4.94	21
36 hours hot & 36 hours cold ..	51 ±1.64	77 ±2.91	21
48 hours hot & .. 48 hours cold ..	56 ±4.87	80 ±10.46	22
72 hours hot & .. 72 hours cold ..	63 ±2.54	81 ±5.88	22
72 hours cold & .. 200 hours hot ..	53 ±2.48	74 ±3.67	21

observed in seeds pretreated for 10 minutes which thus proved to be an effective dormancy breaker. The experiment was conducted using the one seeded pods also of the species and the results are in Table VI.

Results with fruits indicate that fruit wall does not permit quick acid action. Treatment of seeds for 20 minutes inhibited germination percentage to eight while the same treatment with one seeded pods induced germination up to 51%. This is because the pod treatment affects the scarification of seeds more slowly than when the seeds are directly exposed to this concentration.

7. *Effect of different light conditions on seeds treated with sulphuric acid and alternate temperatures* : Because these two treat-

TABLE V

EFFECT OF SULPHURIC ACID TREATMENT ON GERMINATION

Duration of the treatment in minutes ..	Control	5	10	15	20
Percentage of germination	7 ±3.25	94 ±7.28	100 ±0	46 ±4.87	8 ±0
Percentage of imbibition	13 ±3.67	94 ±7.28	100 ±0	46 ±4.87	8 ±0

TABLE VI

EFFECT OF SULPHURIC ACID ON GERMINATION USING PODS

Duration of the treatment in minutes ..	Control	10	20
Percentage of germination ..	0 ±5.31	31 ±8.19	51
Number of days watched ..	15	15	15

ments tamed the dormancy considerably, experiments were extended to examine the germination behaviour in different light conditions of the pretreated seeds and the results are given in Table VII and Table VIII.

The germination and imbibition percentages were thus found to be higher in continuous light in both these experiments.

8. *Effect of different wave lengths of light on germination* : On 20-3-1978, three months old seeds made permeable with concentrated sulphuric acid were kept exposed to different colours of light for germination. The different wave lengths were supplied from a 40 watt HMT bulb by wrapping the petri dishes with cellophane paper of different colours. The results of the experiment as on 14-4-1978 are given in Table IX.

With yellow and red lights, there was hundred per cent imbibition on the same day and 98 and 96 percentage germination on 22-3-1978, i.e. within two days. But, in dark, white and green lights, though the percentage of imbibition was about 95% within two days the germination was a little less. It was 6% in dark, 3% in white and 4% in green. But in blue light, on 22-3-1978, there was 100% imbibition but only 70% germination. On 23-3-1978 the germination percentages in darkness, green, white, blue, red and yellow were 6, 15, 27, 73, 90 and 98 per cents respectively. Yellow light stimulated the germination to the maximum followed by red and blue. Germination appeared to be less affected in the presence of green and white, while darkness seems to have suppressed it. If the duration of the experiment was prolonged to four weeks, the variation in the percentages of imbibition and germination gradually became insignificant. The untreated seeds remained unaffected by any wavelength changes and remained passive.

9. *Effect of pH on germination* : Seeds were germinated in petri dishes between filter papers moistened with solutions having different pH values. The optimum pH was found to be 7.5. Acidic or alkaline tendencies reduced imbibition and germination. This may be the cause for the dense appearance of seedlings in the localities studied which showed a pH range of 7.1 to 7.8.

The results using acetic acid and nitric acid for pretreatment of seeds

TABLE VII

EXPERIMENT USING SULPHURIC ACID PRETREATED SEEDS

Light condition	Diffuse day light		Continuous light		Continuous darkness	
Control (untreated-UT) or treated (T)	UT	T	UT	T	TU	T
Percentage of germination	7 ±1.51	85 ±5.80	9 ±1.37	92 ±9.83	4 ±1.12	67 ±6.08
Percentage of imbibition	13 ±3.66	94 ±14.35	17 ±2.12	96 ±11.59	10 ±2.66	78 ±9.64
Number of days watched	27	27	27	27	27	27

TABLE VIII

EXPERIMENT USING ALTERNATE COLD (50 HRS.) AND HOT (200 HRS.) PRETREATED SEEDS

Light condition	Diffuse day light		Continuous light		Continuous darkness	
Control (Untreated-UT), or untreated (T)	UT	T	UT	T	UT	T
Percentage of germination	7 +1.51	72 +4.08	9 +2.89	75 +5.65	4 +1.04	67 +6.0
Percentage of imbibition	13 +3.66	83 +8.16	17 +3.27	89 +17.74	10 +1.37	71 +3.19
Number of days watched	27	5	27	5	27	5

TABLE IX

EFFECT OF DIFFERENT WAVELENGTHS ON GERMINATION

Colour of the light	Percentage of germination		Percentage of imbibition	
	Untreated with acid	Treated	Untreated with acid	Treated
Control	7±1.51	91±17.74	10±1.41	100±0
Black	4±0	85±23.47	4±1.04	100±0
White	8±1.71	97±10.67	13±1.09	100±0
Green	0	88±10.04	1±0	94±14.35
Yellow	7±1.55	98±8.12	12±2.54	100±0
Red	8±3.40	96±11.57	11±1.30	100±0
Blue	4±1.02	88±10.71	5±1.71	100±0

proved to be unsatisfactory since only a negligible percentage of germination was observed. Results of direct germination using different ppm. solutions of copper sulphate and coumarin also were unyielding.

DISCUSSION

Rugmini (1960) and Sant (1972) did not come across any dormancy in *I. linifolia*. But in the present study, seeds of *I. linifolia* dispersed in December cannot germinate until the onset of next monsoon due to the presence of dormancy. The seeds fall into the category of hard seeds. Nitsch (1971) commented that seeds of legumes are notorious for having hard coats that are impermeable for water. Possession of this feature ensures greater survival value and biological advantage in adopting the growth cycles of the plant to the seasonal and fortutious variation in the environmental conditions (Villiers, 1972 ; also see Mall, 1954 ; Toole *et al.*, 1956 ; Amen, 1968 ; Nitsch, 1971 ; Vyas and Agarwal, 1972 ; Roberts, 1972). This also incidentally offers a resistant barrier to measures of weed control (Villiers, 1972 : p. 230). This is true of several weeds (Barton, 1965 ; Singh, 1968; Choudhary, 1975, 1976 ; Vyas and Agarwal, 1970, 1972 ; Sen, 1976). A number of natural factors that relieve dormancy were cited (Thurston, 1960 ; Villiers, 1972) but attempts to overcome this feature are legion in literature. It is this dormancy that accounts for the poor germination of freshly harvested seeds in *Indigofera linifolia* in a period of 27 days. Among the various treatments cited and tried to circumvent dormancy, pretreatment with concentrated sulphuric acid for 10 minutes proved very satisfactory. With sulphuric acid, 10 minutes was found to be optimum for *I. cordifolia* (Vyas and Agarwal, 1972) and 30 minutes for *I. astragalina* (Vyas and Agarwal, 1970). In *I. cordifolia*, Sen (1976) found that ten minutes pretreatment with sulphuric acid was sufficient for seeds collected in 1974 but only 5 minutes for those collected in 1975. In the same species germination percentage was 90 in freshly harvested seeds in 15 days while in the previous year it was only 10-20%. Different seed batches are prone to react differently to the treatment given, a feature cited by Villiers (1972). The situation in another leguminous plant may be recalled here. The seeds of yellow form of European lupine are dormant while those of white forms are not (Nikolaeva, 1969). Similar situation was noticed in *Amaranthus retroflexus* (McWilliams *et al.*, 1968). Agarwal (1971) and Kaul (1974) used thiourea for breaking dormancy. Went (1957) demonstrated the usefulness of alternating temperatures on plant growth. This was tried with benefit in several cases but in *I. linifolia*, an exposure to alternate cold (50 hours) and hot (200 hours) yielded a maximum of 65% germination alone in that experiment which makes it less effective as a dormancy breaker. Red and yellow lights stimulated germination in treated seeds better in a shorter time—an observation in conformity with that of Vyas and Agarwal (1972). The percentage of germination was more in continuous light in the present study. In another legume, *Desmodium gangeticum* (Ramakrishnan, 1964), it was found that germination was maximun in continuous darkness and minimum in continuous light. The reduced percentage of imbibition and germination of *Indigofera linifolia* in blue and green lights tends to endorse the views expressed by Mayer and Mayber (1963) that both these wavelengths are likely to exert an inhibitory effect on germination.

King (1974) mentioned that individual

weed species are not entirely reliable as indicators of narrow ranges of soil pH values. But germination of seeds of *Indigofera linifolia* was observed to be maximum in near neutral soils and the optimum pH range for this species is between 7.1 and 7.8. On the other hand, Vyas and Agarwal (1972) reported poor germination at pH 7 but good germination at 6.5 and again 7.5. This shows that slightly acidic or slightly alkaline soils are suitable for the growth of *Indigofera cordifolia*. These examples illustrate a fair degree of reliability as indicators of soil pH in their respective areas of study.

REFERENCES

AGARWAL, S. K. 1971. Effect of thiourea and ascorbic acid on seed germination. *J. Indian bot. Soc.* **50 :** 374-376.

AMEN, R. D. 1968. A model of seed dormancy. *Bot. Rev.* **34 :** 1.

BARTON, L. V. 1965. Dormancy in seed imposed by seed coat. *Encycl. Pl. Physiol.* **45 :** 727-745.

CHATTERJI, U. N. AND D. BAXI. 1964. A preliminary and comparative study of germination behaviour of seeds of five species of *Indigofera* Linn. Part II. Proc. Symp.: *Problems of Indian Arid Zone.* UNESCO, Jodhpur, India.

CHOUDHARY, R. L. 1975. Biological observations on *Andrographis paniculata* Nees. *Botanique* **6 :** 103-108.

CHOUDHARY, R. L. 1976. Seed germination of *Triumfetta rhomboidea* Jacq. *Botanique* **7 :** 9-14.

KAUL, R. 1974. Some aspects of seed germination in *Hemigraphis dura*. *J. Indian bot. Soc.* **53 :** 71-75.

KING, L. J. 1974. *Weeds of the World.* Wiley Eastern Rep. New Delhi.

MALL, L. P. 1954. Germination studies of three common weeds of dry phase of low lying lands. *Proc. Nat. Acad. Sci.* (India) **24 :** 197-204.

MAYER, A. M. AND A. P. MAYBER. 1963. *The germination of seeds.* Pergamon Press. New York.

MCWILLIAMS, E. L., R. Q. LANDERS AND J. P. MAHLSTEDE. 1968. Variation in seed weight and germination in populations of *Amaranthus retroflexus*. *Ecology.* **49 :** 290-296.

NIKOLAEVA, M. G. 1969. *Physiology of deep dormancy in seeds.* Nat. Sci. Found. Washington, D.C.

NITSCH, J. P. 1971. Perennation through seeds and other structures : Fruit development. In : F. C. Steward (Ed.) *Plant Physiology* VI A. Academic Press.

PANDEYA, S. C., G. S. PURI AND J. S. SINGH. 1968. *Research methods in Plant Ecology.* Asia Publishing House.

RAMAKRISHNAN, P. S. 1964. Notes on the Ecology of *Desmodium gangeticum* DC. *Proc. Nat. Acad. Sci.*, (India). **30 :** 339-347.

RAO, P. N. AND B. V. N. REDDY. 1979. Tricotyledony in *Indigofera linifolia*. *Geobios.* **61 :** 29-30.

REDDY, B. V. N. AND P. N. RAO. 1979. Aggressive capacity of *Indigofera linifolia* (L. f.) Retz. *Geobios* **6 :** 336-337.

ROBERTS, E. H. 1972. Dormancy—a factor affecting seed survival in the soil. In : *Viability of Seeds.* E. H. Roberts (Ed.) Chapman & Hall.

RUGMINI, C. R. 1960. Investigations on the autecology of some weeds. *BHU Dissertation abstracts.* 1965.

SANT, H. R. 1972. Ecological studies with special reference to the reproductive capacity in relation to grazing of *Indigofera linifolia* Linn. *Botanique.* **3 :** 107-116.

SCHEFLER, W. C. 1969. *Statistics for the Biological Sciences.* Addison-Wesley. London.

SEN, D. N. 1976. *Ecophysiological studies on weeds of cultivated fields with special reference to Bajra and Til.* 2nd. progress report of US PL-480 Project No. A7-CR-425, Jodhpur.

SINGH, J. S. 1968. Comparison of growth performance and germination behaviour of seeds of *Cassia tora* L. and *C. obtusifolia* L. *Trop. Ecol.* **9 :** 64-71.

THURSTON, J. M. 1960. *Dormancy in weed species.* Blackwell Sci. Pub. Oxford.

TOOLE, E. H., S. B. HENDRIKS, H. A. BORTHWICK AND V. K. TOOLE. 1956. Physiology of seed germination. *Ann. Rev. Pl. Physiol.* **7 :** 299-324.

VILLIERS, T. A. 1972. Seed Dormancy. In : T. T. Kozlowski (Ed.) *Seed Biology*, Vol. II : 219-281.

VYAS, L. N. AND S. K. AGARWAL. 1970. Studies in the extent and role of dormancy in the seeds of *Indigofera astragalina*. *J. Indian bot. Soc.* **49 :** 158-163.

VYAS, L. N. AND S. K. AGARWAL. 1972. Studies in the germination behaviour of *Indigofera cordifolia* Heyne ex Roth. *Jap. J. Ecol.* **22 :** 162-169.

WENT, F. W. 1957. *The experimental control of Plant Growth.* Chron. Botan., Waltham, Mass., USA.