ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. 52, nr 1: 35-44
1983

Diversity in the seeds of *Trigonella occulta* Delile

RAMESH C. BHATIA

Department of Botany, Government Science College, Gwalior 474009, India

(Received: October 22, 1980)

Abstract

Dimorphism in the seeds of *Trigonella occulta* Delile of 4 studied seed provenances has been observed. The seeds of various origin show also diversity in their germination and imbibition behaviour, however low temperature of nearly 10°C is essential for germination in all cases. There is the correlation between morphological and physiological characteristics of some seed lots.

INTRODUCTION

Trigonella occulta Delile is an annual weed of specialized habitat, appearing on drying temporary ponds at the end of summer rainy season. It is widely distributed in Europe, Nubia, Sind and a large part of India. It generally forms a carpet type pure stands in association with *Crypsis aculeata* (L.) Cut.

Its wide but specialized distribution has attracted the author's attention. The aim of the present work is therefore to find out the morphological and physiological variations in the seeds of various provenance in India.

MATERIAL AND METHODS

The seeds were collected in Viz. Bhopal, Gwalior, Rewa and Ujjain from 23.05.64 to 10.06.64. Five localities in each district were randomly selected and the fruits were mixed together, dried under bright sunlight, stored in corked glass bottles, and considered as one sample. All the localities were drying temporary pond beds. The temperature of the storage ranged from 25 to 37°C. The morphological analysis of the seeds was based on weight, size index (lenght × breadth), shape index (lenght/breadth), and seed coat pattern, of a 100 seeds in each sample. The quality of the experimental seeds was good and they were healthy.

The percentage of viable seeds varied from 98.8 to 100% in all the four samples. This was tested by T.T.C. (2,3,5-triphenyl tetrazolium chloride) treatment. The seeds longitudinally cut into two halves were kept in 0.1% aqueous solution of T.T.C. at 10°C. The seeds where embryos were stained red were considered viable and the rest non-viable (Roberts 1972).

The physiological analysis of the seeds was based on the germination capacity both of untreated and treated seeds and the percentage water imbibition. The seeds used for germination studies were stored for at least 6 weeks, as they required an after-ripening period (Bhatia 1965). The temperatures under which the seeds were kept for germination were T_1 — normal laboratory conditions where the temperature ranged from 24 to 32°C; T_2 — the constant temperature of 35 ± 2°C; T_3 — the constant temperature of 10 ± 2°C; T_4 — the temperature of 35 ± 2°C and 10 ± 2°C applied for 12 h alternatively. The seeds in all the samples did not germinate under T_1 and T_2. Better germination was observed under T_3 and T_4 conditions, hence they were considered as suitable temperatures for germination. The mud treatment was applied by tying up the seeds in muslin cloth, waterlogging in mud, in laboratory at the temperature ranging from 25 to 35°C. The concentrated sulphuric acid (sp. gr. 1.84) treatment was given in the usual way. The imbibition of water by the seeds (42 weeks old) was determined by measuring the percentage of distilled water imbided during their immersion for 24 h or 48 h in distilled water under T_3. The imbibition percentage was calculated on the basis of initial weight of the seeds before the experiment and percentage increment in the final weight after the experiment.

All the germination studies were carried out in sterilised Petri-dishes lined with moist filter papers with a 100 seeds in each. The analyses were repeated three times. The emergence of radicle was considered as the criterion for germination. The germination was observed for 90 days in each sample till complete germination was obtained. The statistical analysis of the data was done (Snedecor and Cochran 1968, Panse and Sukhatme 1978). On account of the inequality in the variance in germination, the values were transformed into degrees on a suitable scale. The angular transformation was used for the variance analysis.

RESULTS

Morphological studies. The seed is exalbuminous, oblong, smooth, mottled or unmottled and green, greenish yellow or yellow. Two forms of seeds were noted on the ground of seed coat pattern, the mottled

form and unmottled form. The first one was green and found at Gwalior while the second was yellow and found at Rewa. The average values of seed weight, size index, shape index, and seed coat pattern with their statistical characteristics as mean, standard deviation, coefficient of variation and fold variation are recorded in Table 1.

Table 1

Variation in the morphological features of seeds

Parameters	Seed provenence				Mean ±S.D.	C.V., %	Fold variation
	Bhopal	Gwalior	Rewa	Ujjain			
Av. wt., mg	0.78	0.93	0.89	0.92	0.88 ±0.07	7.82	1.19
Av. size index, mm	1.41	1.65	1.53	1.61	1.55 ±0.11	6.83	1.17
Av. shape index	1.39	1.36	1.36	1.46	1.39 ±0.05	3.38	1.07
Seed coat pattern	MGY	MG	UY	MGY			

Analysis of variance

Parameter	S.V.	D.F.	S.S.	M.S.S.	C.D.	F.
Av. wt.	seed provenence	3	0.04	0.01	0.22	0.58 NS
Av. size index	,,	3	0.1	0.03	1.73	0.6 NS
Av. shape index	,,	3	0.02	0.01	0.23	0.23 NS

NS — non-significant at 0.05 P; Av. — average; C. D. — critical difference (±) or least significant difference (LSD) at 0.05 P; C. V. — coefficient of variation;; D. F. — degrees of freedom; F — variance ratio observed; M. S. S. — mean sum of squares; MG — mottled green; MGY — mottled greenish yellow; UY — unmottled yellow; S. D. — standard deviation; S. S. — sum of squares; and S. V. — source of variation.

It was noted that standard deviation and coefficient of variation which are measures of dispersion and variation independent of the unit of measurement respectively, were low for seed weight, size index and shape index. The fold variation of these parameters was never more than 2 times in any case. The analysis of variance has shown that the morphological characteristics like seed weight, size index and shape index of the seed of various origin did not differ significantly. The variation in the seed coat pattern was quite apparent. The values of critical difference also showed that all the mean values were not significantly different from each other at 5% probability.

Physiological analysis. The germination capacity was observed in untreated seeds which were previously dry stored for 6 weeks and in treated seeds (mud and sulphuric acid treated) which were dry stored for 10 and 12 weeks respectively. The mud treatment was given for 19 days and sulphuric acid treatment for 1 to 60 minutes. Both un-

treated and treated seeds were kept for germination under T_3 and/or T_4 temperatures. The results are shown in Tables 2, 3 and 4.

The seeds of Ujjain showed the highest germination capacity and of Gwalior the lowest. The T_3 was more suitable temperature than T_4. The variations in the germination capacity of the seeds of various origin were significant. The critical difference (C.D.) or least significant difference (L.S.D.) of 3, 6, and 12 values i.e. interaction (S $\times$T), seed

Table 2

Variation in the germination capacity of untreated seeds

Temperature	Seed provenance				Mean of 12 values
	Bhopal	Gwalior	Rewa	Ujjain	
T_3	21.3 (27.37)	10.7 (19.01)	50.7 (45.38)	51.3 (45.77)	33.5 (34.38)
T_4	6.3 (14.43)	7.7 (16.02)	20.3 (26.69)	43.7 (41.35)	19.5 (24.62)
Mean of 6 values	13.83 (20.9)	9.17 (17.52)	35.5 (36.03)	47.5 (43.56)	

Analysis of variance

S.V.	D.F.	S.S.	M.S.S.	C.D.	F.
Replication	2	64.28	32.14	1.68	6.51 *
Temperature (T)	1	571.06	571.06	1.37	115.6 *
Seed provenance (S)	3	2747.81	915.94	1.94	185.41 *
T$\times$S	3	246.57	82.19	2.75	16.64 *
Error	14	69.13	4.94		
Total	23	3698.85			

Values in parentheses are angular transformations. * — significant at 0.05 P; other abbreviations same as in Table 1.

provenance (S), and temperature (T) showed that all the means were significantly different from each other and the percent seed germination. Capacity depended upon the seed provenance and germination temperature.

The seeds treated with H_2SO_4 showed maximum germination capacity in Ujjain seeds and minimum in Bhopal seeds. The treatment was helpful in increasing the germination capacity in all the cases. The optimum for germination duration of the treatment varied in the seeds of different origin. The mud treated seeds also showed significant variations in germination capacity. The Gwalior seeds showed the highest germination value while Ujjain seeds showed the lowest. The overall effects of the seed provenance the treatments (mud and H_2SO_4) on germination capacity were found significant and the results of analysis of critical differences between means different values differed from

each other, indicating thereby that the significant variations in the germination capacity were due to seed provenance.

The rate of imbibition of distilled water under T_3, by the seeds of different provenance has been shown in Table 5. The seeds of Rawa and Ujjain imbibed maximum and minimum water respectively. The percentage of imbibition significantly varied in the seeds of different origin.

Table 3

Variation in the germination capacity of H_2SO_4 treated seeds placed under T_3

Seed provenance	Duration of treatment in minutes							Mean of 21 values
	1	2	5	10	30	60	control	
Bhopal	5.7 (13.76)	27.3 (31.48)	51 (45.57)	95 (77.35)	92.7 (74.51)	78 (62.06)	4.3 (11.9)	50.57 (45.23)
Gwalior	11 (19.12)	26.7 (31.07)	68.3 (55.77)	72 (58.1)	93.7 (75.56)	53 (46.72)	10 (18.3)	47.81 (43.52)
Rewa	13 (21.10)	30 (33.18)	95.7 (78)	98.7 (83.46)	58 (49.64)	38.7 (38.44)	11.7 (19.96)	49.4 (46.26)
Ujjain	30.3 (33.40)	52 (46.15)	88.3 (70.06)	99 (85.38)	98.7 (83.46)	93 (74.82)	27.3 (31.51)	69.8 (60.68)
Mean of 12 values	15 (21.84)	34 (35.47)	75.83 (62.35)	91.17 (76.07)	85.75 (70.79)	65.67 (55.61)	13.33 (20.42)	

Analysis of variance

S.V.	D.F.	S.S.	M.S.S.	C.D.	F.
Replication	2	36.49	18.25	2.02	3.42 *
Seed provenance (S)	3	3952.25	1317.42	2.33	246.71 *
Duration of treatment (D)	6	37991.58	6331.93	3.09	1185.75 *
S×D	18	5304.86	294.71	6.17	55.19 *
Error	54	288.20	5.34		
Total	83	47573.38			

Values in parentheses are angular transformations. Abbreviations same as in Tables 1 and 2.

Correlation analysis. The coefficient of correlation "r" between morphological (seed weight, size index, shape index, and seed coat pattern) and physiological characteristics (imbibition for 24 h and germination capacity of treated seeds under T_3) was determined for the seeds of 4 provenances three replications each (n=12). The results are shown in Table 6. The values of correlation coefficient (r) between seed weight, size index or shape index and germination capacity were positive and lower than 1, which indicated that a strictly functional relationship did not exist although there was a trend between them.

The increasing seed weight, size index and shape index tended to be associated with the increasing germination capacity. The values of "r" between seed coat pattern and germination capacity, and seed weight, size index, shape index or seed coat pattern and imbibition were negative which revealed a perfect negative functional relationship. The increasing seed coat pattern i.e. mottled greenish seeds were associated with the decreasing germination while the increasing seed weight, size index, shape index or seed coat pattern were associated with decreasing imbibition and vice-versa.

Table 4

Variation in the germination capacity of mud treated seeds

Temperature	Seed provenance				Mean of 12 values
	Bhopal	Gwalior	Rewa	Ujjain	
T_3	87.7 (69.49)	96.7 (79.66)	88.3 (70.45)	66 (54.38)	84.68 (68.5)
T_4	81.3 (64.49)	94 (76.23)	73.7 (59.16)	63 (52.56)	78 (63.11)
Mean of 6 values	84.5 (66.99)	95.35 (77.95)	81 (64.81)	64.5 (53.47)	

Analysis of variance

S.V.	D.F.	S.S.	M.S.S.	C.D.	F	
Replication	2	53.14	26.57	2.68	2.13	NS
Temperature (T)	1	171.09	171.09	2.19	13.73	*
Seed provenance (S)	3	1812.81	604.27	3.09	48.50	*
T×S	3	77.63	25.88	4.37	2.08	NS
Error	14	174.47	12.46			
Total	23	2289.14				

Values in parentheses are angular transformations. Abbreviations same as in Tables 1 and 2.

The seeds of Gwalior were mottled green and had the lowest seed shape index and germination capacity, higher imbibition and the highest seed weight and seed size index. The seeds of Rewa were unmottled yellow, having lower seed weight, seed size index and seed shape index, higher germination capacity and the highest imbibition. The seeds of Bhopal were mottled greenish yellow, having lower seed shape index and germination capacity, the lowest seed weight and seed size index, and higher imbibition. The seeds of Ujjain were mottled greenish yellow, having the lowest imbibition, higher seed weight and seed size index, and the highest seed shape index and germination capacity.

Table 5

Variation in the imbibition % of untreated seeds under T_3

Duration of imbibition, h	Seed provenance				Mean of 12 values
	Bhopal	Gwalior	Rewa	Ujjain	
24	139.5	139.08	142.76	86.26	126.9
48	167.62	185.16	218.63	130.18	175.4
Mean of 6 values	153.56	162.12	180.7	108.22	

Analysis of variance

S.V.	D.F.	S.S.	M.S.S.	C.D.	F
Replication	2	93.04	46.52	6.41	0.65 NS
Seed provenance (S)	3	17052.22	5684.07	7.4	79.6 *
Duration of imbibition (D)	1	14112.04	14112.04	5.23	197.62 *
S×D	3	1786.94	595.65	10.47	8.34 *
Error	14	999.73	71.41		
Total	23	34043.97			

Abbreviations same as in Tables 1 and 2.

Table 6

Correlation coefficient between morphological and physiological characteristics of seeds

Physiological characteristics	Morphological characteristics			
	seed weight	seed shape index	seed size index	seed coat+ pattern
Germination	0.27 NS	0.44 NS	0.03 NS	−0.7 NS
Imbibition	−0.45 NS	−0.96 NS	−0.41 NS	−0.25 NS

n = 12.

+ classified on the basis of score points. MG — 100, MGY — 75 and UY — 2.

Other abbreviations same as in Tables 1 and 2.

DISCUSSION

The study of morphological and physiological features of seeds is helpful in understanding the pattern of seasonal distribution of species. The study of the variability among the seeds of *Trigonella occulta* Delile of different seed provenances has revealed the occurrence of two forms of seeds, the mottled form and unmottled form, on the ground of their seed coat pattern. The former was green or greenish yellow found at Bhopal, Gwalior and Ujjain and the later was completely yellow found at Rewa only. The variations in other morphological

characteristics viz. seed weight, size and shape indexes were also found, although they were of no significant value. The variability of seed size and weight which may be controlled by specific genetic constitution, is often influenced by interspecies competition for food during embryo development and seed maturation.

The study of the physiological behaviour of seeds based on germination capacity and imbibition percentage, has shown the occurrence of significant variations among them. Such variations occured both in untreated and treated seeds. Low temperature of nearly 10°C was found essential for germination in all the cases, as the seeds showed maximum germination under constant temperature of 10°C (T_3), while the temperature of 35 and 10°C applied for 12 h alternatively (T_4) was favourable. The constant temperature of 35°C (T_2) and the normal laboratory temperature ranging from 24 to 32°C (T_1) were unfavourable conditions. The seeds under T_1 could not germinate as the requirement of low temperature of nearly 10°C could not by fulfilled there. The untreated seeds of Ujjain showed the highest and those of Gwalior the lowest germination capacity. The seeds pretreated with mud and sulphuric acid showed significant variations in their germination capicity. The sulphuric acid treatment was proved to increase the germination capacity, the optimum duration of which for germination was 10 minutes in all the cases except Gwalior seeds where it was 30 minutes, the reason of which may be harder seed coat. The percentage of imbibition of water significantly varied in the seeds of different origin. The imbibition is actually a process of water uptake indicating thereby the degree of permeability of the seed coat to water.

The analysis of coefficient of correlation between morphological and physiological characteristics of seeds of different provenance has revealed correlations, although they were of non-significant value. The difference in size and weight of seeds responsible for more efficient segregation in their dispersal (Salisbury 1942), is therefore of great ecological significance in the distribution of many plant species. The seed potential to germinate in different environmental conditions or habitats ensure perpetuation and occupation by plants of various ecological niches and thus direct the species evolution. Sen and Chatterji (1968), Chawan and Sen (1973a,b), Bohra and Sen (1974), Bhati and Sen (1978) and Bhatia (1980, 1981) made studies of similar type. Cavers and Harper (1966), Harper et al. (1970), Pathak et al. (1974) and Tanwar and Sen (1980) have discussed seed polymorphism connected with differences in germination behaviour. Sen (1977) was of the opinion that variations of results of the ecological studies done so far on seed germination are

probably due to the fact that the pattern of seed collection is based on Mendelian population. For a correct and uniform conclusion the seeds of a particular species must be collected from pure live stock.

Acknowledgments

The author is thankful to Professor L. P. Mall, Head, School of Studies in Botany, Vikram University, Ujjain, India for valuable quidance and also to Ministry of Education, Government of India for awarding the scholarship.

REFERENCES

Bhatia R. C., 1965. Autecological study of some weed Flora at Gwalior and suburbs. Ph. D. Thesis, Vikram University, Ujjain.

Bhatia R. C., 1980. Seed variability in *Glinus lotoides* L. Seed Res. 8: 156-162.

Bhatia R. C., 1981. Seed variability in *Heliotropium supinum* Linn. Geobios 8: 112-115.

Bhati P. R., Sen D. N., 1978. Adaptive polymorphism in *Ipomoea pes-tigridis* (*Convolvulaceae*), a common rainy season weed of the Indian arid Zone. Plant Syst. Evol. 129: 111-117.

Bohra P. N., Sen D. N., 1974. Seed patterns and germination behaviour in *Crotalaria medicaginea* Lamk. growing in Indian arid Zone. Curr. Sci. 43: 591-592.

Cavers P. B., Harper J. L., 1966. Germination polymorphism in *Rumex obtusifolius*. J. Ecol. 54: 367-382.

Chawan D. D., Sen D. N., 1973a. Diversity in germination behaviour and high temperature tolerance in the seeds of *Corchorus aestuans* Linn. Ann. Arid Zone 12: 23-32.

Chawan D. D., Sen D. N., 1973b. Diversity in germination behaviour and chemical scarification for hard seed coat in *Corchorus aestuans* Linn. Broteria 42: 19-24.

Harper J. L., Lovell P. H., Moore K. G., 1970. The shape and size of seeds. Ann. Rev. Ecol. Syst. 1: 327-356.

Panse V. G., Sukhatme P. V., 1978. Statistical methods for Agricultural workers. I.C.A.R., New Delhi.

Pathak P. S., Devroy R., Rai P., 1974. Autecology of *Leucaena leuco-cephala* (Lam.) Dewit. I. Seed polymorphism and germination. Trop. Ecol. 15: 1-10.

Roberts E. H., 1972. Viability of seed. Chapman and Hall, London.

Salisbury E. J., 1942. The Reproductive capacity of plants. G. Bell and Sons, London.

Sen D. N., 1977. Environment and seed germination in Indian plants. The Chronica Botanica Co., New Delhi. p. 84.

Sen D. N., Chatterji U. N., 1968. Diversity in germination of seeds in *Calotropis procera* R. Br. populations. Osterr. Bot. Zeit. 115: 6-17.

Snedecor G. W., Cochran W. G., 1968. Statistical methods. Oxford and I.B.H. Publishing Co., New Delhi.

Tanwar G. S., Sen D. N., 1980. Germination behaviour in dimorphic seeds of *Melothria maderaspatana* L. Flora 170: 351-353.

Zróżnicowanie nasion Trigonella occulta Delile

Streszczenie

Obserwowano dymorfizm nasion *Trigonella occulta* Delile pochodzących z 4 miejsc. Nasiona różniące się pochodzeniem odmiennie kiełkowały i pęczniały w różnych temperaturach; jednak niska temperatura około 10°C okazała się być optymalną do kiełkowania we wszystkich przypadkach. Wykazano korelację między cechami morfologicznymi i fizjologicznymi dla niektórych prób nasion.