Arunachalam, V., Murty, B. R. and Anand, I. J.
Subspecific Differentiation in Nicotiana Rustica.
Indian Journal of Genetics and Plant Breeding,
25 (1965): 217-223.

9-2

SUBSPECIFIC DIFFERENTIATION IN *NICOTIANA RUSTICA* L.

B. R. Murty, I. J. Anand and V. Arunachalam

Division of Botany, Indian Agricultural Research Institute, New Delhi

(Accepted: 25-v-1965)

Among the two most important cultivated species of *Nicotiana*, *rustica* is comparatively restricted in its distribution to cooler climates, with ample sunshine and well drained soils. In the genus *Nicotiana*, the variability in this species is next only to that of *N. tabacum*, although, it is not found in wild state, except the one case *N. rustica* var. *Pavonii* reported by Goodspeed (1954). In India, it is grown mostly in Punjab, Uttar Pradesh, Bihar, Assam and the northern parts of West Bengal. However, wide variation could have accumulated in this species since it is grown for diverse uses such as hookah, chewing and snuff. The classification of this species by Howards (1910) into four groups was mostly based on the nature of inflorescence, internode length and clustering of flowers, since they found only a limited range of variation for the other characters. The disposition of the stamens in relation to stigma was also used as another criterion. Variable amount of outbreeding (upto even 50 per cent.) was reported in this species by Breese (1959). The work of Mather and his colleagues, during the past fifteen years, in this species revealed considerable non-allelic interactions and genotype-environmental interactions, although additive variance was predominant for a majority of the characters. Since *N. rustica* as grown in India is subjected to limited human selection, the operation of genetic drift (the plants are mostly topped) could be important in the divergence within the species and would be an interesting study. A set of fifteen varieties in the predominantly *rustica* region (Bihar) from which varieties adapted to the adjacent States were evolved were examined for the genetic divergence between them in respect of four characters influencing yield.

Materials and Methods

Fifteen varieties representing the spectrum of farmers' bulks of *rustica* were included in the present study. They were grown during the years 1962 and 1963. The plants were not topped to permit normal development of the plant and to study the variation in panicle characters. Four characters, height (X_1), height excluding panicles (X_2), number of leaves (X_3) and size of leaves (X_4) were studied on individual plants. The sample size was fifteen plants per variety. The spacing within and between the rows was two feet.

RESULTS

The analyses of plot means have revealed significant differences between varieties for each of the characters except for number of leaves, indicating variability even within the limited samples examined (Table 1). The correlations between pairs of characters did not reveal any strong association between any two of the four characters indicating that recombinations with optima for each would be possible. The test by Wilks' Λ criterion has indicated significant differences between the varieties when all the four characters were considered together (χ^2 for d.f. 56=786·19).

TABLE 1

ANOVA for some vegetative and reproductive characters in fifteen varieties of Nicotiana rustica

Source	DF.	Height (cm.)		Height upto panicle (cm.)		No. of leaves		Leaf size (sq. cm.)	
		M.S.S.	F	M.S.S.	F	M.S.S.	F	M.S.S.	F
Blocks	2	976·1		946·3		101·4	N.S	40531·5	
Varieties	14	2381·2	2·57*	1233·9	4·19**	1376·4	1·19	396009·9	8·17**
Error	28	927·9		294·1		1161·4		48464·5	

*Significant at 5% level **Significant at 1% level

The transformation of the plot means into uncorrelated variables was done by the pivotal condensation method described by Rao (1952) (Table 2). The 105 possible comparisons between pairs of varieties are presented in Appendix. Variety No. 8 was unique in being the most divergent from the rest.

Leaf size and height excluding panicle contributed most to the divergence. Number of leaves was the least useful in discriminating between varieties for divergence.

The populations could be grouped into four clusters (Table 3, Fig. 1), with 7, 5, 2 and 1 varieties in the first, second, third and fourth clusters, respectively. The intra-cluster divergence was similar in all the groups. Clusters I, II and III were equidistant from each other. An examination of the means of these clusters (see Table 4) revealed that the members of the first cluster were the tallest with comparatively longer internodes and smaller leaves. The members of the second cluster were medium tall but with leaves of intermediate size. Members of cluster III were of medium height but with smaller and fewer leaves. Cluster IV, which had only one variety (No. 8), was unique with

TABLE 2

Original means (X) for four characters and the mean values of transformed variables (Y)

Var. No.	Name of the variety	Height X_1 (cm.)	Height upto panicle X_2 (cm.)	No. of leaves X_3	Leaf size X_4 (sq. cm.)	Y_1	Y_2	Y_3	Y_4
1.	Belsandi	55·2	35·9	29·1	83·6	9·1	6·3	4·6	−4·4
2.	Chatwana	56·5	37·9	28·1	158·2	9·3	7·0	4·4	−2·2
3.	Diakharswar	55·7	38·6	32·6	101·8	9·1	7·6	5·1	−3·8
4.	Ghasrama	47·3	28·6	19·7	177·4	7·8	4·2	3·2	−0·7
5.	Harpursadi	60·9	39·5	29·9	207·3	10·0	6·9	4·7	−1·4
6.	Hirawata	40·8	26·3	21·1	51·3	6·7	4·5	3·3	−3·6
7.	Kharga	54·7	33·7	31·8	97·8	9·0	5·3	5·0	−4·2
8.	Kharagpur	57·9	36·6	31·5	349·9	9·5	6·0	4·9	+3·0
9.	Kursaila	48·3	29·6	32·0	173·2	7·9	4·5	5·0	−1·2
10.	Mandhatakhaga	49·0	31·9	28·4	123·1	8·0	5·6	4·4	−2·5
11.	Meerganj	54·6	34·6	25·0	116·7	9·0	5·7	4·0	−3·3
12.	Motihari	57·5	35·5	22·1	212·3	9·4	5·6	3·5	−0·9
13.	Pakur	50·2	30·7	26·5	127·6	8·2	4·7	4·2	−2·8
14.	Patrahi	49·3	31·9	28·0	90·3	8·1	5·5	4·4	−3·5
15.	Santiniya Parihar	61·2	38·7	33·1	169·0	10·0	6·4	5·2	−2·8

$Y_1 = 0{\cdot}0328\,X_1$
$Y_2 = -0{\cdot}0458\,X_1 + 0{\cdot}1054\,X_2$
$Y_3 = 0{\cdot}0018\,X_1 - 0{\cdot}0012\,X_2 + 0{\cdot}0294\,X_3$
$Y_4 = -0{\cdot}0351\,X_1 + 0{\cdot}0200\,X_2 - 0{\cdot}0052\,X_3 + 0{\cdot}0059\,X_4$

TABLE 3

Average intra- and inter-group distances $[D^2/(D)]$ among some varieties of N. rustica

Cluster	I	II	III	IV
I	0·04/(0·20)	0·09/(0·30)	0·10/(0·32)	0·46/(0·68)
II		0·05/(0·22)	0·13/(0·36)	0·29/(0·54)
III			0·04/(0·20)	0·22/(0·47)
IV				—

Varieties included I—1,6,7,10,11,13,14; II—2,3,5,12,15; III—4,9; IV—8

the largest leaf size, medium height and close internodes, indicating the predominant role of leaf size in the intraspecific divergence.

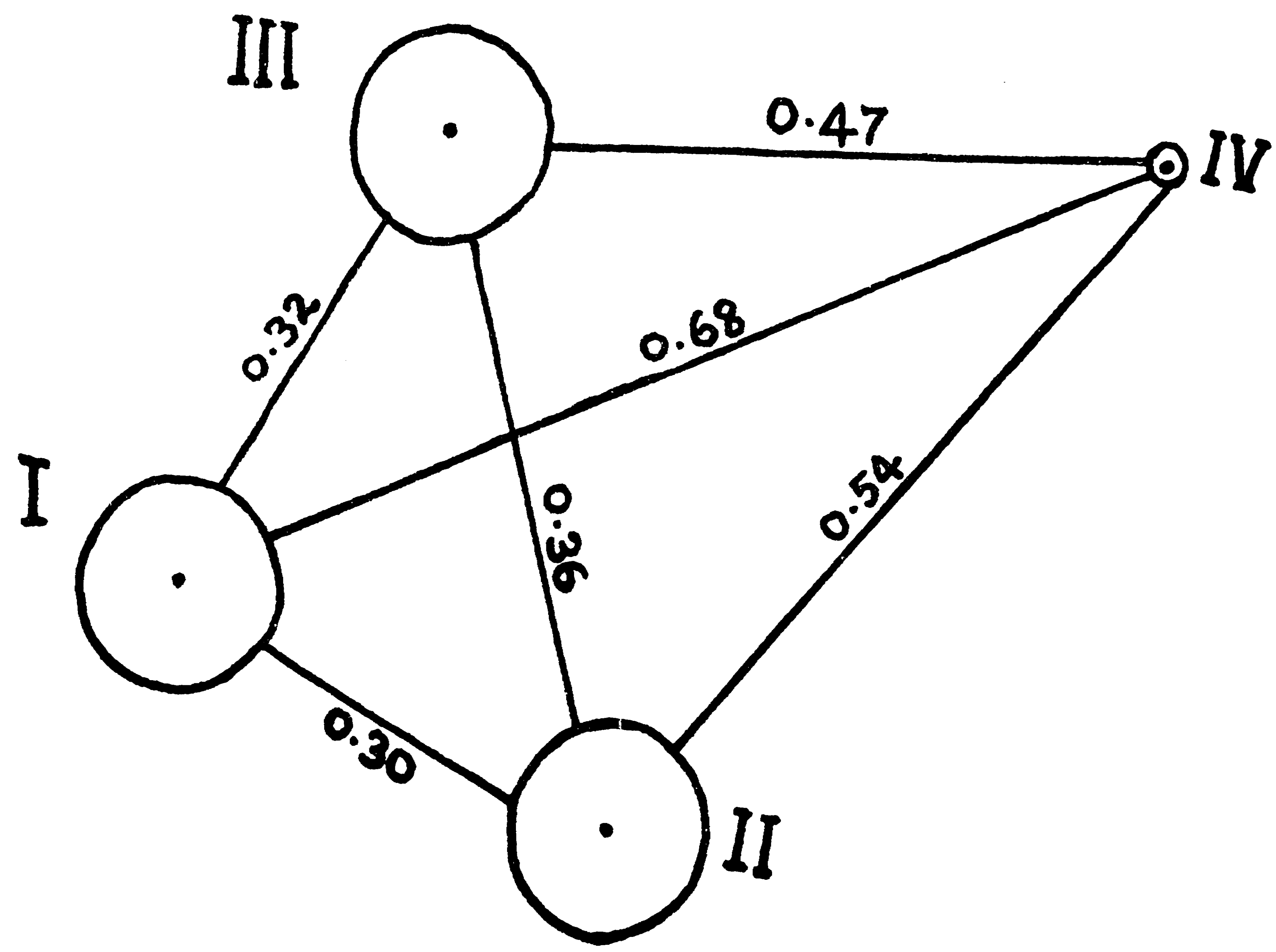

FIG. 1 Genetic Divergence in *Nicotiana rustica*

Groups	*Varieties included*
I	1,6,7,10,11,13,14
II	2,3,5,12,15
III	4,9
IV	8

TABLE 4

Group means for four characters in N. rustica

Cluster	Height (cm.)	Height excluding panicle (cm.)	No. of leaves	Leaf-size (sq.cm.)
I	79·64	31·43	28·13	106·70
II	58·36	38·04	29·16	169·72
III	50·95	31·60	22·35	147·05
IV	57·90	36·60	31·50	349·90

The geographical distribution of the varieties and their genetic divergence could not be correlated. Moreover, type 8 from Kharagpur is from the Monghyr district while Motihari in the second group is closer to the Himalayan range. Similarly, Pakur is in the eastern most part adjacent to West Bengal but is associated with Mandhata and Meergunj varieties. The Kharagpur variety is from a hilly area interspersed with the plains of Ganges. This region is hotter as compared to northern Bihar. The survival value of this large-leaved type would be low in northern Bihar because of the occurrence of frost.

From the canonical analysis of the data, the values of λ_1, λ_2 and $\lambda_3+\lambda_4$ were 50·1, 24·9 and 6·0 respectively accounting for 61·9 per cent., 30·7 per cent. and 0·4 per cent. of the total. An examination of the first and second canonical vectors confirmed the potent role of leaf size in the differentiation between the varieties as seen below :

	X_1	X_2	X_3	X_4
Z_1	0·1303	–0·0158	0·0063	0·9911
Z_2	0·6186	0·7052	0·3372	–0·0763

Discussion

The present study revealed considerable divergence even within a limited number of varieties of *N. rustica* chosen from Bihar. The retention of variability even within this restricted area could be due to more than one cause. The most important of the factors for the conservation of variability would appear to be the high degree of out–crossing, large reproductive capacity, genetic drift due to the retention of only a few plants for seed purposes after topping the rest of the crop, small holdings in which each farmer retains his own seed, and the diverse range of diurnal variation in temperature during the crop season in North Bihar as compared to the southern and eastern parts. It is significant that leaf size and relative proportion of panicle to the total plant height were potent differentiators; these could have been obliterated if the material was topped.

The uniqueness of population 8 appears to be due to the warm climate in the area, proximity to the coal-fields with a considerable change in the micro-flora and fauna of the region as compared to the other tobacco growing tracts of Bihar. Smith (1952) observed that the heritability for leaf size was very low, being 11·2 per cent. only, although the number of effective factors was low. Jinks (1954) reported that incomplete dominance and non-allelic interactions play significant roles in the control of this character. Therefore, it would appear that the variable outbreeding system in this crop which increased with changes in temperature could have been responsible for maintaining heterozygosity and for permitting considerable non-allelic interaction for this character. It is proposed to verify this phenomenon in crosses between Kharagpur and other varieties. It, therefore, would appear that the classification by the Howards could be revised by assigning appropriate roles to leaf size and relative proportion of panicle to height.

Summary

A statistical analysis of genetic divergence, as measured by D^2 statistic, in fifteen populations of *Nicotiana rustica*, was attempted. Among the four

characters chosen, namely, total height, height excluding panicle, number of leaves, and leaf size, leaf size was the predominant factor contributing maximum to the inter-varietal diversity. The results were confirmed by canonical analysis.

One of the varieties was unique and was the most divergent from the rest. The causes for the observed diversity in a limited material were discussed in relation to the role of genetic drift and diverse ecological conditions. The use of leaf size and proportion of panicle to plant height as classificatory criteria is suggested.

REFERENCES

Anderson, T. W. (1958). *Introduction to Multivariate Statistical Analysis.* John Wiley & Sons, New York.

Breese, E. N. (1959). Selection for different degrees of outbreeding in *Nicotiana rustica*. *Ann. Bot.*, **23:** 331-40.

Goodspeed, T. H. (1954). *The Genus Nicotiana.* Chronica Botanica Co. Waltham, Mass., U.S.A.

Howard, A. and G.L.C. Howard (1910). Studies in Indian Tobaccos–I. The types of *Nicotiana rustica* L. Yellow flowered tobacco. *Mem. Dept. Agric., India Bot. Ser.*, **3:** 59-176.

Jinks, J. L. (1954). The analysis of continuous variation in a diallel cross of *Nicotiana rustica* varieties *Genetics*, **39:** 767-88.

Rao, C. R. (1952). *Advanced Statistical Methods in Biometric Research.* John Wiley & Sons, New York.

Smith, H. H. (1952). Fixing transgressive vigour in *Nicotiana rustica.* In *Heterosis*, Edited by Gowen, University of Iowa, Ames, pp. 161-74.

APPENDIX

Total D^2 values between fifteen varieties of N. rustica

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	—	5·34	2·26	21·34	10·13	11·05	1·29	55·05	14·45	5·05	1·81	14·18	5·89	2·33	4·03
2		—	3·21	13·67	1·26	16·02	7·29	28·38	9·12	3·69	3·20	4·68	7·04	5·48	1·92
3			—	25·95	6·90	18·63	5·64	48·30	17·12	7·22	4·90	14·95	11·04	6·03	3·38
4				—	14·86	9·36	17·65	23·23	3·64	6·70	11·12	4·71	5·95	11·09	17·96
5					—	23·25	11·35	20·49	9·87	6·89	6·69	3·74	10·39	10·22	2·32
6						—	8·82	56·10	9·66	5·29	7·12	16·08	3·66	4·06	18·85
7							—	52·30	10·19	3·94	1·92	13·24	3·27	1·56	4·39
8								—	20·70	33·18	41·50	17·08	38·28	45·42	33·65
9									—	3·01	7·88	5·47	3·33	6·58	10·14
10										—	1·74	5·51	1·03	1·04	5·27
11											—	6·55	1·95	1·13	3·42
12												—	6·57	9·68	7·30
13													—	1·23	7·19
14														—	5·83
15															—