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DIALLEL ANALYSIS FOR NICOTINE CONTENT AND LEAF THICKNESS IN HOOKAH AND CHEWING TOBACCO (*NICOTIANA RUSTICA* L.)

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ABSTRACT

A ten-parent diallel study conducted on the genetic architecture of nicotine content and leaf thickness in hookah and chewing tobacco (*Nicotiana rustica* L.) showed that mean squares due to general and specific combining ability were significant, suggesting the importance of both additive and dominance components. The line Farrukhabad was identified as best general combiner for simultaneous improvement in both characters. The crosses T 238×Tangua, Hazro×Tangua, and Farrukhabad×NP 222 for nicotine content and Chaithar×M_4, Farrukhabad×Tangua, and Farrukhabad×Peshawar Snuff for leaf thickness were the best specific combinations for nonfixable components. Low narrow sense heritability was observed for both characters. Numerical analysis for leaf thickness revealed asymmetrical gene distribution and unequal allele frequency. Reciprocal recurrent selection or diallel selective mating, which exploits both fixable and nonfixable components, has been suggested for the improvement of these traits.

Key words: Hookah and chewing tobacco, *rustica* tobacco, genetic analysis, combining ability, nicotine content, leaf thickness.

Nicotiana rustica L., popularly known as Motihari tobacco, is an important cash crop in West Bengal, Uttar Pradesh, Bihar and Gujarat. While top grade leaves are used for chewing, the lower grade leaves are utilised for hookah purposes. Although the demand for quality is not very high in this case as compared to *Nicotiana tabacum*, there is a need to develop strains with broad, thick leaves rich in nicotine, strong in aroma, which develop white incrustation of calcium salts during fermentation. Generally a thick leaf with high nicotine content is preferred by the consumer because when mixed with lime, such leaf gives graininess while high nicotine content gives the desired kick and satisfaction. Sometimes it is also used in cigarette and bidi blends. With the cost of flue cured Virginia tobacco soaring high, there is a frantic search for a cheaper tobacco that can be blended without impairing the cigarette quality considerably. Hookah and chewing tobacco might fit in ideally to reduce cost of cigarette production. Very little information on genetic improvement of its yield and quality traits is available [1–4]. A systematic production breeding programme has been initiated at this Research Station, and it was thought necessary to simultaneously obtain information on the nature and magnitude of gene action and identify elite parents and appropriate cross combinations for improving nicotine content and leaf thickness along with higher cured leaf yield in this crop.

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MATERIALS AND METHODS

The experimental material consisted of 10 lines of hookah and chewing tobacco and their all possible crosses, excluding reciprocals. The 10 parents and their 45 F_1 were planted in November, 1984 at the Central Tobacco Research Station, West Bengal. Each entry was represented by a single row of 15 plants with 60×45 cm spacing. The experiment was planted in randomized block design with two replications. The crop received basal dose of 15 tonnes farm yard manure/ha and 112 kg N, P and K each/ha as urea, DAP and K_2SO_4, respectively. Crop cultivation was done as per the usual practice. Gap filling was done within a week to ensure uniform stand. All the plants were topped 3 leaves below the bald head. A light irrigation was given after topping. The plants were harvested in 3–4 primings depending on maturity. The harvested leaves were sun-dried for whole day, and transferred in bundles of 10–12 leaves to a *kutcha* (thatched) curing barn for a month. After complete drying, the leaves in each bundle were spread on top of each other and put in a heap for fermentation. The heap was turned when the temperature reached 55°C. Complete fermentation took 45 days. Two sets of samples were drawn. From each plot 30 leaves per sample were drawn at random for quality analysis. For leaf thickness, uniform vertical discs of 1.4 cm diameter were cut out by a cork borer. From each leaf 3 discs were cut from the central portions of the top, middle and bottom segments. The discs were exposed to 60% relative humidity for 72 h, weighed and expressed as mg/cm^2. From the second set, the leaves were freed from midrib and lamina, dried in oven at 72°C for 48 h, and powdered to pass through a 40-mesh sieve. Nicotine estimation was done as suggested by [5]. The results were analysed according to [6]. Estimates of combining ability [7], genetic analysis [8] and narrow sense heritability [9] were calculated.

RESULTS AND DISCUSSION

The analysis of variance indicated significant differences between treatments for both characters (Table 1). Numerical analysis [8] was carried out only for leaf thickness, as the basic assumptions underlying diallel analysis were not fulfilled in case of nicotine content. For leaf thickness, the estimates of additive (D) and

Table 1. Analysis of variance (M.S.S.) for combining ability, nicotine content and leaf thickness in hookah and chewing tobacco

Source	d.f.	Nicotine content	Leaf thickness
Replications	1	0.04	0.93
Treatments	54	1.96**	12.14**
gca	9	1.01**	7.51**
sca	45	0.97**	5.78**
Error	54	0.01	0.44
gca/sca		10.15	13.22
CV (%)		8.36	5.76

**P = 0.01.

nonadditive (H_1 and H_2); variances were significant. The H_1 (22.3) and H_2 (20.1) components were higher than D (3.7), suggesting the importance of dominance. The magnitude of H_1 and H_2 suggests unequal allele frequency. Preponderance of dominance and asymmetrical gene distribution for cured leaf yield and its components have been reported in *rustica* tobacco [4]. The KD/KR ratio of 1.4 indicated 1–2 gene complexes for every single recessive gene in the inbreds. Narrow sense heritability for leaf thickness (19.7%) and nicotine content (16.9%) was low, suggesting rather limited scope for improving these characters through straight selection.

COMBINING ABILITY ANALYSIS

Analysis of variance for combinig ability (Table 1) showed highly significant differences due to general (gca) and specific combining ability (sca), the magnitude of former being larger for both traits. The gca effects for nicotine content (Table 2) revealed Hemti to be the best general combiner. Similar observation was reported earlier [2]. The lines Farrukhabad and M_4 also showed positive gca effects for this

Table 2. Estimates of gca effects and parental means (in parentheses) for nicotine content and leaf thickness in hookah and chewing tobacco

Parent	Nicotine content (%)	Leaf thickness (mg/cm^2)
White Pattar	−0.07	0.33
	(5.80)	(17.67)
T 238	−0.39**	−0.84**
	(5.11)	(16.79)
Hazro	−0.13	−0.57**
	(5.32)	(18.84)
Chaithar	−0.25**	−0.19
	(4.32)	(15.60)
Farrukhabad	0.37**	1.51**
	(4.23)	(20.94)
NP 222	−0.32**	−1.28**
	(3.92)	(15.29)
M_4	0.19*	0.22
	(7.10)	(17.89)
Peshawar Snuff	−0.01	0.65**
	(5.71)	(21.08)
Hemti	0.46**	0.22
	(6.35)	(18.29)
Tangua	0.16	−0.04
	(5.54)	(16.04)
SE (gi)	0.08	0.18
SE (gi - gj)	0.13	0.27
CD_{gi-gi} (0.05)	0.03	0.14
Rank correlation (r_s)	0.54	0.71*

*P = 0.05; **P = 0.01.

character. Rank correlation coefficient (r_s) between gca effects and parental means for leaf thickness was significant, suggesting that the parents can be selected on the basis of their mean values. Lines Farrukhabad and Peshawar Snuff gave best gca effects for leaf thickness. While nicotine content has positive effect on chewing and smoking strength, a thick leaf imparts body to the leaf [10]. Hence line Farrukhabad appears to be the best choice for improving both the traits simultaneously.

The sca effects (Table 3) for nicotine content ranged from −1.38 in cross White Pattar × Tangua to 2.71 in cross Farrukhabad × Hemti. The crosses T 238 × Tangua, Hazro × Tangua, and Farrukhabad × NP 222 also gave high sca effects. For leaf thickness, the crosses showing high sca effects were Chaithar × M_4 (8.11), Farrukhabad × Tangua (7.16), and Farrukhabad × Peshawar Snuff (6.04), indicating their usefulness in recombination breeding programme. In general, most of the crosses with high sca effects had at least one parent with good gca effect, indicating dominant × recessive interaction. For leaf thickness, cross Farrukhabad × Peshawar Snuff gave high sca effects. Both the parents involved in this cross had high gca (Table 2), showing additive or additive × additive type of interaction. Due to epistatic interaction, crosses like T 238 × Hazro and White Pattar × Peshawar Snuff gave high sca effects for nicotine content.

Table 3. Estimates of specific combining effects for nicotine content (above diagonal) and leaf thickness (below diagonal) in hookah and chewing tobacco

Parents	White Patter	T 238	Hazro	Chaithar	Farrukhabad	NP 222	M^4	Peshawar Snuff	Hemti	Tangua
White Patter	—	0.72	0.81	1.15	0.55	0.72	1.73	1.99	0.58	−1.38
T 238	3.25	—	1.21	0.57	1.60	0.85	0.16	0.16	−1.13	2.36
Hazro	0.35	3.58	—	0.04	0.64	0.09	0.35	0.76	1.03	2.28
Chaithar	2.58	2.18	−0.43	—	0.12	1.59	−0.01	1.19	1.48	1.97
Farrukhabad	1.41	1.99	4.27	4.42	—	1.85	1.77	1.46	2.71	−0.06
NP 222	5.24	0.18	2.60	2.67	2.08	—	1.36	0.55	1.93	0.51
M_4	0.50	5.01	2.13	8.11	0.88	3.09	—	0.98	−0.35	0.18
Peshawar Snuff	3.52	1.33	2.61	2.11	6.04	−3.60	−1.06	—	0.49	0.76
Hemti	6.12	−0.05	3.59	2.94	−0.78	1.50	−3.24	1.30	—	1.52
Tangua	2.15	4.12	1.23	3.19	7.16	−3.13	−2.93	−0.47	3.09	—

Standard error:	Nicotine content:	Leaf thickness:
SE (ij)	0.26	0.55
SE (ij−ik)	0.42	0.89
SE (ij−lk)	0.40	0.85

Previous studies in this crop [3, 4] revealed that both additive and dominance components are important for cured leaf yield and its component characters. Good gca for leaf length, width and area in line Farrukhabad, and high gca for green leaf yield in Hemti have been reported earlier [3]. In the present study, both these lines have shown good gca for quality traits, hence can be utilized in breeding programme for yield. Since additive and nonadditive components were important for both the traits, recurrent selection [11], diallel selective mating [12], or any other method, which capitalises on both additive and nonadditive effects, could be used to effect the desired yield and quality improvement in this crop.

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