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GENETIC DIVERSITY AMONG MUTANT GERMPLASM ACCESSIONS OF *NICOTIANA TABACUM* AS DETERMINED BY MORPHOLOGICAL PARAMETERS

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Low levels of variability prevailing in the cultivated genotypes of crops leads to genetic vulnerability and lower/marginal yield gains. Creating variation using mutation breeding is one of the options to overcome this problem. ICAR-CTRI is maintaining 35 mutant lines (including natural mutants) which is harboring a vast genetic variability. The diversity of 29 agro-morphologcal characters was studied in these lines for assessing the suitability of these lines for tobacco breeding programme. Principal component analysis (PCA) of the recorded characters indicated that, out of seven components obtained, PCA1 PCA2 are contributing major variability (about 41%). Grouping of the genotypes based on UPGMA produced four distinct clusters with 6.5 distance. The dissimilarity index (DI) showed that the mutants RT 63-2 and CM 3-1 were distantly related with the maximum value of 0,867 and similarly the mutants R90-1 and R91 1 were closely related with low DI value of 0.125 The genotypes were varying twenty six characters and such genotypes with diverse morphological traits can be utilized in tobacco breeding for yield and quality improvement.

INTRODUCTION

Tobacco (*Nicotiana tabacum*, 2n=4X=48) is an important commercial crop grown mainly in United States, China, Brazil and India. It is native to America and later introduced to other parts of the world where it is mainly cultivated for its narcotics properties. Tobacco is generally used in the manufacture of different smoking products, chewing products and snuff. The crop is also exploited for the photochemical and their value added products like solanesol, seed oil, malic acid, citric acid, proteins etc. Tobacco is one of the important high value commercial crops In India and grown on 0.47 million hectares in India. India occupies second place in world tobacco production (800 M kg) after China (2392 M kg) during the year 2017 and is one of the dominant exporter of tobacco leaves (FAOSTAT, 2018). Tobacco and tobacco products are earning approx Rs.20,000 Cr. to the national exchequer by way of excise duty and approx.Rs.5000 Cr. by way of foreign exchange every year (Tobacco Board, 2018). Looking at the steady growing demand of tobacco leaves and limited scope for expanding the area of its cultivation, there is need for enhancing the of the crop. In order to increase the productivity levels of tobacco, breeding high yielding and stress tolerant varieties is essential, which in turn depends on the available variation.

Even though tobacco is often cross pollinated crop, the variability in the cultivated lines has been eroded in course of time in domestication process. Number of studies indicated lack of diversity in the cultivated tobacco genotypes (Goodspeed 1954; Chase *et al.,* 2003; Knapp *et al.,* 2004, Sarala and Rao, 2008; Baghyalakshmi *et al.*, 2018). Earlier reports also stated that a high degree of genetic relatedness exists among modern varieties in the different tobacco types (Garner *et al.* 1936; Murphy *et al.* 1987; Bindler *et al.* 2005; Sarala *et al.*, 2008). Utilization of existing variation in the germplasm is an important step in increasing the variability of the varieties for avoiding genetic vulnerability. It remains to be seen, however, whether or not theoretically low levels of genetic variability within tobacco germplasm pools (Garner *et al.* 1936;

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Murphy *et al.* 1987) will become an obstacle to continued cultivar improvement. In order to overcome this issue, there is a need to create variability artificially through mutations and isolation of rare genetic variants in the established pure lines. Mutation could be created with either physical or chemical mutagens. Once after the genotypes are mutated, it is essential to evaluate the diversity present within them to select diverse parents for making crosses. The diversity is usually studied by agro morphological characters due to its easiness, even though the variability in germplasm is difficult to distinguish due to the overlapping variations (Ahsyee, 2013). The information generated by morphological traits on diversity in tobacco has been studied vastly. ICAR-CTRI, as a National Active Germplasm Site, maintains 35 mutant including genetic variant (natural mutants) lines in its genebank. The variability existing in these lines has been studied to make these diverse lines available for crop improvement programme.

MATERIALS AND METHODS

The present study was undertaken at Katheru farm, ICAR- CTRI, Rajahmundry, during Rabi 2017-18 using 35 mutant/genetic variant (natural mutants) tobacco genotypes from gene bank maintained at the institute (Table 1). Preparatory cultivations such as deep ploughing in summer and 2-3 ploughing between July to September were carried out to make the field free of weeds. The nursery bed was raised during September and the seedlings were transplanted to the main field in first fortnight of November 2017. The spacing adopted was 70 x 60 cm and ten plants per entry were maintained. Recommended crop production and protection practices were followed to raise a healthy crop. Morphological observations were recorded in three plants after confirming the uniformity within the row. About 29 morphological observations were recorded in 35 entries to evaluate the diversity available within the entries.

Statistical analysis: All the morphological observations were first converted into scoring pattern to resemble qualitative characters. Principal component analysis (PCA) was performed using SPSS 16.0 version to obtain the pattern of data matrix for determining the selection criteria and identification of morphological characters highly responsible for diversity. Those PCs with Eigen values greater than one were selected (Jeffers, 1967) for further analysis. Morphological characters that were not exhibiting variation or highly correlated to another character were excluded from further analysis.

Cluster analysis: The diversity prevailing among the burley genotypes was computed using

Table 1: Mutants / Genetic Variants used in the study

Mutant No.	Entry details
Mut 1	A. Special
Mut 2	CM 1-1
Mut 3	CM 3-1
Mut 4	CM 5-1 (324C)
Mut 5	CM 15-1
Mut 6	GS-1
Mut 7	R10-1
Mut 8	R11-1
Mut 9	R20-1
Mut 10	R59-3
Mut 11	R90-1
Mut 12	R91-1
Mut 13	R92-1
Mut 14	R123-1
Mut 15	R124-1
Mut 16	R 77-B
Mut 17	RT 24-1
Mut 18	RT143-2
Mut 19	RT151-1
Mut 20	RT 6-1
Mut 21	RT 65-1
Mut 22	RT 44-2
Mut 23	RT 63-2
Mut 24	RT Late
Mut 26	RT76-1
Mut 27	TBST95-M1
Mut	ISH-27-M1
Mut	SG 27—M1
Mut	SG-48-M1
Mut	SG-47-M1
Mut	ISH-39-M1
Mut	R699-M1
Mut	ISH-22-M1
Mut	ISH-38-M1
Mut	NP-4-M1

Computer Software Program–DARwin (Perrier and Jacquemond-Collet 2006). Dissimilarly matrix for morphological observation was constructed using Rogers-Tanimoto coefficient of associations to find out genetic relationships. These data were subjected to unweighted pair groups method with arithmetic mean (UPGMA) analysis to generate dendrogram using DARwin 5.0 and dissimilarity was estimated based on the respective morphological scoring.

RESULTS AND DISCUSSION

About 29 morphological observations consisting of five plant characters, 13 leaf characters and 11 flower/fruit/testa characters were recorded on all the entries (Table 2). The genotypes found to differ for all the traits except for the traits like color of midrib, inflorescence position relative to upper leaves (IPRUL) and development of stamens (FDS). For the other traits,

Table 2: Morphological observations recorded with number of plants under each category

S.No	Characters (Code)	Character category(Genotype Number in brackets)
	Plant	
1	Shape (PS)	Conical (19), Cylindrical (16)
2	Height (PHT)	Very Short (3), Short (6), Medium (9), Tall (13), Very Tall (4)
3	Habit (PH)	Open (13), Erect (14), Semi Erect (8)
4	Internodal Length (PIL)	< 4 cm (13), <6 cm (21), >6cm (1)
5	Number of Leaves (PNL)	Very Few (2), Few (11), Medium (7), Many (10), Very Many (5)
	Leaf	
6	Type (LT)	Sessile (34), petiolate(1)
7	Angle of Insertion (LAI)	Very Acute (14), Moderately Acute (20), Right Angle (1)
8	length (LL)	Short (4), Medium (12), Long (16), Very Long (3)
9	Width of Blade (WB)	Very Narrow (8), Narrow (17), Medium (8), Broad (2)
10	Midrib (LM)	Thin (12), Medium (13), Thick (10)
11	Veins-thickness and angle (LV-T&A)	Thin (17), Medium (12), Thick (6)
12	Blade Shape (LBS)	Lanceolate (9), Narrow Elliptic (15), Broad Elliptic (11)
13	Tip Shape (LTS)	Medium Pointed (18), Strongly Pointed (17)
14	Blistering of Blade (puckering) (LBB)	Absent or Very Weak (1), Weak (18), Medium (16
15	Undulations of Margin (LUM)	Absent or Very Weak (1), Weak (18), Medium (14), Strong (2)
16	Development of Auricles (LDA)	Absent or Very Weak (1), Weak (21), Medium (13)
17	Colour of Blade (LCB)	Light Green (1), Medium Green (17), Dark Green (17)
18	Color of Midrib (LCM)	White Greenish (35)
	Flower	
19	Time of Flowering (TF) (50% of plants with at least one corolla open)	Very Early (4), Early (17), Medium (14)
20	Inflorescence Shape (IS)	Spherical (25), Flattened Spherical (2), Inverted Conical (1), Double Conical (7)
21	Inflorescence Compactness (IC)	Loose (12), Medium (21), Dense (2)
22	Inflorescence Position Relative to Upper Leaves (IPRUL)	Above (35)
23	Length /Size (FL)	Medium (24), Long (11)
24	Expression of Tips of Corolla (FTC)	Medium (4), Strong (29)
25	Colour of Corolla (FCC)	White (5), Light Pink (25), Medium Pink (3), Variegated (1)
26	Length of Pistil Relative to Stamens (FLPS)	Shorter (14), Equal Length (15), Longer (5)
27	Development of Stamens (FDS)	Full (35)
28	Fruit Form (FF)	Rounded (2), Ovate (17), Conical (16)
29	Testa Colour (TC)	Light Brown (19), Dark Brown (11), Cream (4)

Table 3: Total Variance explained through principal component analysis

Principal Component	Initial Eigen values		
	Total	% of Variance	Cumulative %
1	6.540	26.161	26.161
2	3.566	14.266	40.427
3	2.469	9.874	50.301
4	2.143	8.574	58.875
5	2.025	8.100	66.974
6	1.570	6.278	73.252
7	1.134	4.534	77.787
8	.926	3.703	81.489
9	.817	3.268	84.757
10	.777	3.108	87.865
11	.693	2.771	90.636
12	.411	1.645	92.281
13	.399	1.596	93.877
14	.343	1.370	95.247
15	.257	1.030	96.277
16	.234	.936	97.213
17	.218	.871	98.083
18	.173	.690	98.774
19	.108	.431	99.205
20	.089	.356	99.561
21	.054	.215	99.776
22	.036	.145	99.921
23	.020	.079	100.000
24	6.753E-6	2.701E-5	100.000
25	1.124E-6	4.495E-6	100.000

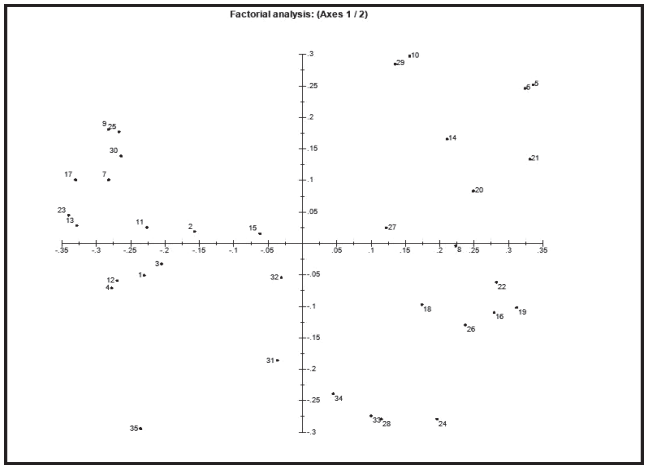

Fig. 1: Two-dimensional plot of principal coordinate analysis of tobacco mutant accessions based on morphological traits

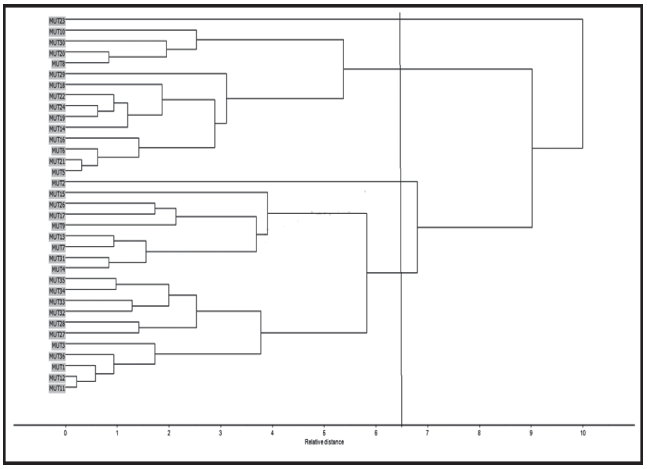

Fig. 2: Dendrogram of mutant tobacco accessions constructed based on UPGMA method.

Table 4: Eigen value ("Load") of the correlation matrix and its contribution to total variation of mutant accessions

Traits	Component						
	PCA1	**PCA 2**	**PCA 3**	**PCA 4**	**PCA 5**	**PCA 6**	**PCA 7**
Plant							
Shape (PS)	-0.804	-0.049	-0.166	-0.128	-0.138	0.22	0.105
Height (PHT)	0.492	0.415	0.167	0.361	-0.002	-0.039	0.003
Habit (PH)	0.409	-0.355	0.454	0.377	-0.267	0.205	-0.056
Internodal Length (PIL)	0.229	0.235	0.281	-0.019	0.123	0.713	-0.27
Number of Leaves (PNL)	**0.780**	0.211	0.006	0.321	0.13	-0.029	0.052
Leaf							
Angle of Insertion (LAI)	-0.485	0.097	-0.319	-0.04	-0.024	0.453	-0.145
length (LL)	**0.747**	0.155	0.206	0.211	0.019	-0.08	0.131
Width of Blade (WB)	**0.814**	-0.034	0.213	-0.289	0.043	0.161	0.081
Midrib (LM)	**0.608**	0.288	-0.236	0.023	0.403	0.338	0.01
Veins-thickness (LVT	**0.721**	0.317	-0.118	-0.059	0.327	0.11	0.21
Veins- angle (LVA)	0.468	0.214	0.001	-0.554	0.107	0.16	0.241
Blade Shape (LBS)	**0.724**	-0.256	0.369	-0.291	-0.16	0.08	0.025
Tip Shape (LTS)	-0.387	0.407	-0.386	0.525	0.258	-0.127	0.223
Blistering of Blade (puckering) (LBB)	-0.109	0.282	0.031	-0.408	0.517	-0.112	-0.128
Undulations of Margin (LUM)	-0.077	0.225	0.031	-0.021	0.63	-0.252	-0.105
Development of Auricles (LDA)	**0.636**	0.247	-0.160	-0.218	-0.258	-0.418	0.224
Colour of Blade (LCB)	-0.547	-0.093	0.222	0.151	0.389	0.139	0.557
Flower							
Time of Flowering (TF)	0.0	**0.752**	-0.418	0.061	0.012	0.082	-0.046
Inflorescence Compactness (IC)	0.445	0.154	-0.482	-0.212	-0.06	-0.269	-0.554
Length /Size (FL)	0.394	0.046	0.020	0.775	-0.084	-0.068	-0.158
Expression of Tips of Corolla (FTC)	-0.399	**0.720**	0.516	-0.05	-0.142	-0.067	-0.07
Colour of Corolla (FCC)	-0.399	**0.720**	0.517	-0.053	-0.14	-0.068	-0.069
Length of Pistil Relative to Stamens (FLPS)	-0.402	**0.717**	0.518	-0.051	-0.14	-0.067	-0.07
Fruit Form (FF)	0.153	0.411	-0.286	-0.197	-0.65	-0.052	0.325
Testa Colour (TC)	-0.089	-0.400	0.465	-0.126	0.391	-0.43	-0.068

the entries were distributed in different categories, indicating the existence of variability in the mutant lines studied. Mutation creates heritable variations and that can be used to improve a crop where the variability has reached a plateau. The developed mutant lines are to be documented before using it in the breeding programme. The variables that are strongly associated in the same group may share some underlying biological relationship, and these associations are often useful for generating hypothesis for better understanding of behaviour of complex traits that would allow breeders to maximize their knowledge (Maji and Shaibu, 2012). The variability studied through qualitative trait was reported to be equally effective in diversity analysis as compared with molecular markers in crops (Rukhsar *et al.,* 2017).

Initially, PC analysis for determining the selection criteria and identification of morphological characters highly responsible for diversity was conducted on all the morphological traits recording variability except color of midrib, inflorescence position relative to upper leaves (IPRUL) and development of stamens (FDS) that showed no variation. Majority of the observed variation (91%) in the agro-morphological traits found to be explained by eleven PCA components

(Table 3). The half of the total variability (50%) found to be explained by PCA1, PCA2 and PCA3 components (Table 3). PCA1 explained 26% of the variation and was loaded mainly on width of leaf base, plant leaf number, leaf length, leaf blade shape, leaf veins thickness and leaf development of auricles (Table 4). PCA2 elucidated 14% of variation with four main characters viz., time of flowering, expression of tips of corolla, color of flower corolla and length of pistil relative to stamens.

The biplot graph showed that the genotypes were found to be highly variable (Fig 1.). To study genetic diversity a range of algorithms have been used of which, UPGMA and Ward's methods are the most popular approaches. Of the algorithms, the UPGMA is the most valid method in accordance with the relationship of family based on their genetic material (Mohammadi and Prasanna, 2003). The UPGMA analysis grouped the accessions into 4 groups in the distance of 6.5 from the origin (Fig 2). Based on the distance, one accession was found in cluster I and III, 14 accessions were grouped in cluster II while cluster IV accommodated 19 accessions. The dissimilarity index (DI) showed that the mutants RT 63-2 and CM 3-1 were distantanly related with the maximum value of 0,867 and similarly the mutants R90-1 and R91-1 were closely related with low DI value of 0.125.

The present study indicated that ample amount of variability existed in the mutations studied. In order to understand the wholesome picture of genetic variability within these genotypes, molecular markers based diversity can also be studied. After variability analyses, breeding programme can be formulated to breed genetically diverse high yielding varieties to overcome the genetic vulnerability to biotic and abiotic stresses caused by uniformity within the cultivars.

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