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MORPHOLOGICAL DIVERSITY AND RELATIONSHIPS PATTERNS AMONG A SET OF AIR-CURED EXOTIC GERMPLASM ACCESSIONS OF *NICOTIANA TABACUM*

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The genetic variability in the germplasm is always essential in breeding programme to improve the yield and adaptability, impart resistance to biotic and abiotic factors etc. Low degrees of variability captured in the cultivated genotypes leads to genetic vulnerability and lower marginal yield gains. At ICAR-CTRI a study was conducted to investigate the genetic diversity of 53 exotic air-cured lines collected from Japan using morphological traits. Thirty morphological observations were recorded and among them 26 characters with diversity were subjected to principal component analysis (PCA). Ten PCA components were formed based on the eigen values. Further, the dissimilarity index and relationship between the genotypes were analysed. Clustering through unweighted pair groups defined four distinct clusters in 0 to 0.5 distance. The dissimilarity matrix was worked out between 53 genotypes and the maximum dissimilarity value was found between genotypes Aizu Katai Kari and Connecticut-7-D (0.894) and the minimum dissimilarity value was between Rengeha and Tennessee Red (0.143). The morphological diversity studied indicates that the exotic germplasm collected and maintained at ICAR-CTRI, serves as a variability hub for tobacco improvement.

INTRODUCTION

The *Nicotiana* species is well known for its narcotics properties and rarely for other phyto-chemicals. The tobacco is mainly classified as Flue cured Virginia (FCV) and non-FCV types based on its type of curing. The FCV tobaccos are primarily used for cigarette making as the leaves contain a higher amount of sugars and low nicotine as they are cured under controlled condition in barn through flue curing. Among the non-FCV types, the air cured types are mostly used in blending and as fillers in cigarettes due to its higher nicotine concentration. The air-curing process involves in naturally drying the leaves at lower temperature that allows respiration process in the leaves consuming sugars. This will results in negligible sucrose and reducing sugars in the cured leaf and the higher levels of nicotine than the Virginia types. The air-cured types are more alkaline than FCV tobacco with a slight acidic taste. ICAR-Central Tobacco Research Institute, Rajahmundry, as a National Active Germplasm Site, maintains around 3300 accessions of various tobacco types including wild species. There are about 53 exotic collection of air-cured tobacco lines maintained at the genebank, collected from Japan.

There is always a higher demand for both FCV and non-FCV tobacco types and the improvement of these types mainly depends on the amount of variability available in the germplasm. The parents for the crop improvement programme can be selected based on genetic divergence analysis of the genotypes. Majorly in tobacco, the yields along with the required quality are the criteria in the breeding programme (Sarala *et al.*, 2018). Thus, the crosses thus attempted should be able to produce promising segregants to increase the leaf yield and improve the quality. However, satisfactory results are obtained only if the germplasm employed in the crosess also present high values for the traits of interest (Fu and Somers, 2009). The present study was undertaken to visualize variability present in the exotic

Key words: Air-cured, diversity, Germplasm, Morphological traits, Genetic variability.

collection of air-cured tobacco germplasm for understanding and documenting the diversity present within germplasm for further utilization.

MATERIALS AND METHODS

The present study was conducted at Katheru farm of ICAR- CTRI, Rajahmundry, during Rabi 2017-18. About 53 air-cured genotypes collected from Japan and maintained at the tobacco gene bank at the institute were used for the study (Table 1). Preparatory cultivations such as deep summer ploughing and 2-3 ploughings between July to September were carried out to make the field free of weeds. The nursery of the entries 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.

Table 1: Japan air cured tobacco collections used in the present study

S. No.	Entry Name
1	Aizu Katai Kari
2	Akatsuka Katai Kori
3	Basle
4	Bingoito
5	Blue Pryor
6	Bungo Maruho
7	Connecticut-7-D
8	Duqueshe
9	Florida Round Tip
10	Florida-301
11	Florida-Cuban
12	Forcheimer Havana –2c
13	Hatch
14	Hatononakashu
15	Higoha Takachiho
16	Hinoha Koha
17	Hinohakoha
18	Hinohakoha
19	Ibusuki Komuhei
20	Ibusuki Wild Type
21	Ikvsakaha Katai Kari
22	Isabella-T
23	Izumi
24	Katsuy
25	Kokubu Kohoro
26	Kuofan
27	Kurokamiyama
28	Long Leaf Gooch
29	Madras-T
30	Magnolia-62
31	Maruha Riuo
32	Md-20
33	Miharaha
34	Miurahazamond
35	Nanbukohi
36	Notop
37	Nc39385
38	Okinava-6
39	Okusaha Hanken
40	Rengeha
41	Rk-70
42	Rk-70
43	Sakushuha Binju Huku
44	Tarumizu
45	Tennessee Red
46	Tozan Kataikari
47	Tozan Kataikori
48	Tsuruki Matukawa
49	Uejiha
50	Vsp-26
51	Yellow Butt Pryor
52	Yoshimoha
53	Yonezawaha

Morphological observations were recorded in three plants after confirming the uniformity within the row. Thirty morphological observations were recorded in 53 entries to see 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) analysis was performed using SPSS 16.0 version to analyze 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 highly correlated to another character were excluded from further analysis.

Cluster analysis: The diversity prevailing among the exotic air-cured genotypes was computed using

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 6.0 and dissimilarity was estimated based on the respective morphological scoring.

RESULTS AND DISCUSSION

Crop improvement is directly proportional to the genetic variability available in the germplasm in order to increase the yield, quality, resistance to pest and disease, improve the adaptability etc. Here in this study 53 exotic genotypes of a set of air-cured tobacco germplasm were characterized based on 30 morphological traits. Variability existed for 26 traits in the entries studied (Table 2). The genotypes were found to be diverse in nature for each trait, but for three traits namely color of leaf midrib, inflorescence position relative to upper leaves, development of floral stamens and seed testa colour variability did not exist. Sarala *et al.*, 2018 reported lack of variability in mutant tobacco lines maintained at the Institute for color of leaf midrib, inflorescence position relative to upper leaves and development of stamens.

The characters with nil variations were removed before further analyzing. Further the 26 morphological traits were subjected to principal component analysis (PCA) to analyze the pattern of data matrix for determining the selection criteria and identification of morphological characters highly contributing for diversity. Ten components were formed based on the eigen value among which PCA 1 and PCA 2 accumulates 25.78% of variability and were explaining most of the variability captured (Table 3 and 4). First five components explained 50% of the variation present in the genotypes. Leaf midrib thickness,

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

SN	Characters	No of genotypes observed under each category (in brackets)
1	Plant shape	Conical (14), Cylindrical (39)
2	Plant height	Very Short (2), Short (21), Medium (25) Tall (5)
3	Plant habit	Open (39), Erect (2), Semi Erect (12)
4	Internodal length	< 4 cm (21), 4-6 cm (27), >6cm (5)
5	Number of Leaves	Very Few (1), Few (33), Medium (19)
6	Leaf type	Sessile (52), Petiolate (1)
7	Angle of Leaf Insertion	Very Acute (1), Moderately Acute (30), Right Angle (22)
8	Leaf length	Medium (12), Long (30), Very Long (11)
9	Leaf width	Very Narrow (19), Narrow (32), Medium (2)
10	Leaf midrib	Thin (4), Medium (34), Thick (15)
11	Leaf Veins-thickness	Thin (28), Medium (16), Thick (9)
12	Leaf vein angle	Moderately Acute (49), Right angle (4)
13	Leaf blade shape	Narrow Elliptic (4), Broad Elliptic (28), Ovate (5), Rounded (16)
14	Leaf tip shape	Obtude (7), Slightly Pointed (8), Medium Pointed (36), Strongly Pointed (2)
15	Blistering of Leaf Blade (puckering)	Absent or Very Weak (22), Weak (24), Medium (5), Strong (2)
16	Undulations of Leaf Margin	Absent or Very Weak (39), Weak (11), Medium (3)
17	Development of Leaf Auricles	Medium (27), Strong (25), Very Strong (1)
18	Colour of Leaf Blade	Light Green (2), Medium Green (28), Dark Green (23)
19	Time of 50% Flowering	Very Early (2), Early (51)
20	Inflorescence shape	Spherical (9), Flattened Spherical (9), Inverted Conical (35)
21	Inflorescence compactness	Loose (5), Medium (38), Dense (10)
22	Flower length /size	Medium (41), Long (12)
23	Expression of Tips of Flower Corolla	Medium (37), Strong (16)
24	Colour of Flower Corolla	White (39), Light Pink (6), Medium Pink (1), Variegated (7)
25	Length of Pistil Relative to Stamens	Shorter (28), Equal Length (20), Longer (5)
26	Fruit shape	Intermediate (5), Ovate (25), Conical 23)

Table 3: Eigen value ("Load") of the correlation matrix and its contribution to total variation of exotic air-cured entries

Traits*	Component									
	PCA1	**PCA 2**	**PCA 3**	**PCA 4**	**PCA 5**	**PCA 6**	**PCA 7**	**PCA 8**	**PCA 9**	**PCA10**
PS	-0.446	-0.022	**0.552**	0.395	0.139	0.135	0.156	0.145	0.082	-0.085
PHT	0.299	**0.484**	**0.492**	0.073	-0.347	-0.11	-0.067	-0.286	-0.051	-0.13
PH	0.401	0.313	-0.476	-0.359	0.064	-0.305	0.099	0.048	-0.044	0.325
PIL	-0.271	0.179	0.32	0.3	0.075	-0.516	-0.225	0.001	-0.165	0.183
PNL	0.15	**0.672**	0.152	-0.168	-0.303	0.08	-0.013	0.139	0.28	-0.188
LT	-0.059	0.245	-0.186	-0.493	0.023	-0.022	0.454	-0.328	-0.248	-0.241
LAI	0.083	-0.528	0.275	-0.098	0.166	0.178	-0.267	-0.196	-0.123	-0.233
LL	**0.697**	0.253	-0.147	0.25	0.118	0.105	0.041	-0.251	0.142	-0.036
WLB	0.35	0.462	0.239	0.458	-0.081	0.199	-0.059	-0.081	-0.028	-0.156
LMT	**0.710**	-0.079	0.165	-0.13	0.034	-0.218	0.037	0.26	-0.243	0.067
LVT	**0.755**	-0.181	0.26	-0.154	-0.029	-0.339	-0.118	0.159	-0.024	0.034
LVA	0.219	0.061	0.096	-0.335	-0.313	0.397	-0.273	0.404	-0.161	-0.227
LBS	-0.496	0.299	0.415	-0.356	-0.084	-0.107	0.179	-0.139	0.011	0.042
LTS	0.22	-0.165	0.388	-0.246	0.579	0.208	-0.151	-0.241	-0.096	-0.092
BLB	0.272	-0.17	0.483	-0.15	-0.286	-0.099	0.47	0.099	0.103	-0.154
ULM	**0.654**	-0.123	0.238	0.049	0.048	-0.052	0.365	0.153	-0.011	0.123
DLA	**0.524**	-0.128	-0.178	0.394	-0.02	0.301	-0.017	0.097	-0.453	0.083
LBC	-0.092	0.338	0.288	0.133	0.249	0.322	0.477	-0.049	-0.321	0.337
TF	-0.277	**0.591**	-0.019	-0.065	0.258	-0.064	-0.166	0.477	0.006	-0.04
IS	-0.089	-0.198	0.567	0.025	-0.172	-0.245	-0.23	-0.146	0.063	0.372
IC	0.358	0.127	0.403	-0.234	0.271	0.216	-0.15	0.058	0.232	0.12
FL	0.336	0.097	-0.235	0.529	-0.107	-0.365	0.012	-0.195	0.028	-0.307
ETFC	0.343	0.108	-0.085	0.068	0.623	-0.026	0.107	0.02	0.551	-0.034
CFC	0.138	**-0.687**	0.001	-0.153	-0.287	0.013	0.156	-0.049	0.275	0.003
LPS	0.14	0.081	-0.13	0.121	-0.443	0.477	-0.043	-0.128	0.278	0.462
FS	-0.272	-0.333	0.055	0.385	0.001	-0.035	0.394	0.368	0.084	-0.102

* Plant shape (PS), Plant Height (PHT), Plant Habit (PH), Plant Internodal Length (PIL), Plant Number of Leaves (PNL), Leaf Type (LT), Leaf Angle of Insertion (LAI), Leaf length (LL), Width of Leaf Blade (WLB), Leaf Midrib Thickness (LMT), Leaf Veins-thickness (LVT), Leaf Veins angle(LVA), Leaf Blade Shape (LBS), Leaf Tip Shape (LTS), Blistering of Leaf Blade (puckering) (BLB), Undulations of Leaf Margin (ULM), Development of Leaf Auricles (DLA), Leaf Blade Color (LBC), Time of 50% Flowering (TF), Inflorescence Shape (IS), Inflorescence Compactness (IC), Flower Length (FL), Expression of Tips of Flower Corolla (ETFC), Colour of Flower Corolla (CFC), Length of Pistil Relative to Stamens (LPS) and Fruit Shape (FS)

Table 4: Total Variance explained by the principal components

Compo	Initial Eigenvalues		
nent	**Total**	**% of Variance**	**Cumulative %**
1	3.922	15.085	15.085
2	2.781	10.697	25.782
3	2.487	9.564	35.345
4	2.003	7.702	43.048
5	1.726	6.640	49.687
6	1.535	5.904	55.591
7	1.395	5.366	60.957
8	1.163	4.472	65.429
9	1.151	4.425	69.854
10	1.037	3.989	73.843
11	0.878	3.375	77.218
12	0.795	3.060	80.278
13	0.728	2.801	83.079
14	0.637	2.449	85.528
15	0.597	2.295	87.822
16	0.532	2.046	89.869
17	0.498	1.914	91.783
18	0.395	1.520	93.302
19	0.389	1.498	94.800
20	0.323	1.243	96.044
21	0.293	1.125	97.169
22	0.227	0.875	98.044
23	0.165	0.634	98.678
24	0.151	0.580	99.258
25	0.103	0.396	99.654
26	0.090	0.346	100.000

leaf length, undulations of leaf margins and leaf auricles development were the traits with high variability. Sarala *et al.*, 2019 found that colour of leaf blade, angle of leaf insertion, inflorescence shape, leaf colour of leaf mid-rib, width of leaf blade, plant shape, flower development of stamens and leaf length contributed for variation in burley tobacco genotypes. Characters namely plant height, plant shape, leaf number, time of 50% flowering and flower corolla colour were the other traits which also contribute to the variability. The above characters with high variability may provide for the required gene pool if used in breeding programs (Baghyalakshmi *et al.*, 2018; Gana, 2006; Aliyu *et al.*, 2000). Two-dimensional plot of principal coordinate analysis of exotic air-cured genotype based on morphological traits showed clustering and even distribution of entries (Fig. 1) in clusters indicating diversity.

Further in order to know the dissimilarity index and relationship between the genotypes, data was analyzed in DarWin 6.0.21. Clustering through unweighted pair groups produced grouping that defined four distinct clusters in 0 to 0.5 distance (Fig. 2). There were enormous diversity that resulted in genotypes falling in different clusters with larger distance and within the clusters/sub-clusters, the diversity was minimum. The dissimilarity matrix was worked out between 53 exotic air-cured genotypes and the maximum dissimilarity value was found between Aizu Katai Kari and Connecticut-7-D (0.894). In principle if the divergence is large between the parents are recommend for crossing, since such pairs of varieties brings in maximum heterosis and increase possibility of segregants in advanced generations (Cruz et al., 2012). The minimum dissimilarity value was between Rengeha and Tennessee Red (0.143). Such pairs are not recommended for use in breeding programs in hybridization since they are genetically close and derail the gains due to selection (Cruz *et al.*, 2004). There are various reports which states 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). A solution to this is utilization of all the gene pools via pre breeding and making the material readily available for further broadening the genetic base of tobacco breeding materials. The morphological diversity observed in this study indicates that the exotic air-cured tobacco germplasm maintained at ICAR-CTRI can

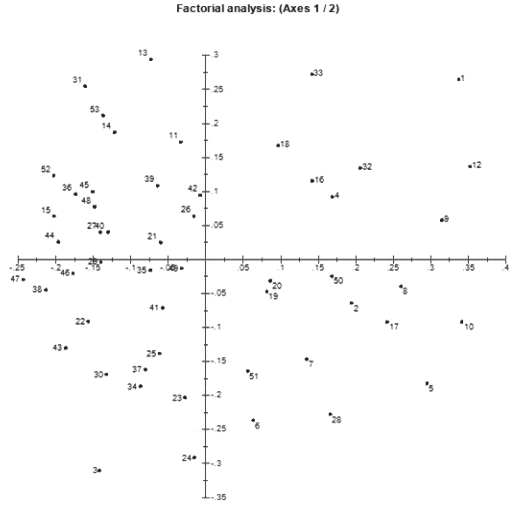

Fig. 1: Two-dimensional plot of principal coordinate analysis of exotic air-cured genotype based on morphological traits

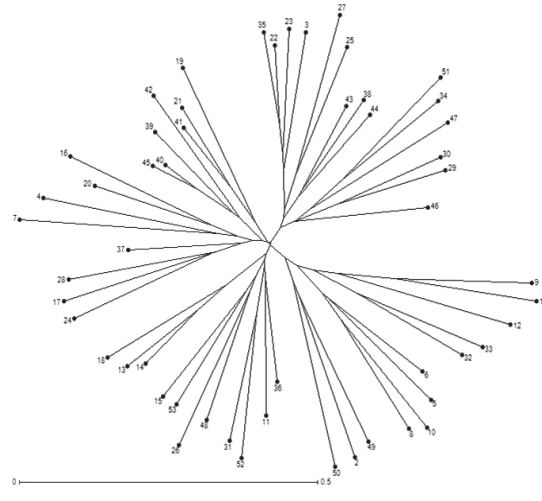

Fig. 2: Dendrogram of air-cured genotypes constructed depending upon morphological traits using Eucledean's distance coefficient and UPGMA method.

serves as source of variation for genotype improvement.

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