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MORPHOLOGICAL DIVERSITY IN BURLEY TOBACCO GERMPLASM

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ICAR-CTRI, Rajahmundry, as a National Active Germplasm Site (NAGS) for tobacco germplasm, maintaining 138 burley tobacco germplasm. The morphological diversity present in this germplasm was investigated for estimating the existing variability for its further utilization. Out of 29 characters studied, the genotypes were found to be diverse for 26 traits and no variation observed for three traits viz., leaf type, inflorescence position and seed testa colour. Principal component analysis (PCA) with 26 diverse characters indicated that majority of the observed variation (91%) in the agro-morphological traits found to be explained by eighteen PCA components. PCA1 alone explained 16% of the variation and was loaded mainly on eight traits viz., colour of leaf blade, leaf angle of insertion, inflorescence shape, leaf colour of mid-rib, width of leaf blade, plant shape, flower development of stamens and leaf length. The biplot of principal coordinates shows that the genotypes are scattered throughout the plot indicating the presence of morphological diversity among the entries. Clustering through unweighted pair groups produced grouping that defined four distinct clusters in 0 to 8 distance. The dissimilarity matrix worked out between 138 burley genotypes ranged from maximum value of 0.99 between YB-24 and Ky-171 to minimum value of 0.065 between Ky-160 and Ky-171. The morphological diversity observed in the study indicates that the burley germplasm maintained at ICAR-CTRI is having sufficient diversity and can serve as a valuable source for burley genotype improvement.

INTRODUCTION

Burley tobacco is grown in an area of 30,000 ha in India with an annual production of about 32 million kg. The burley tobacco is mainly grown during monsoon season in Guntur, East Godavari, West Godavari, Visakhapatnam and Vizianagaram districts of Andhra Pradesh, Warangal district of Telengana, and Rayagada and Koraput districts of Orissa. Burley tobacco leaves are primarily used for cigarette manufacturing. Banket A-1 is the only variety grown at present in various burley growing areas of India. There is a need to develop burley tobacco cultivars for increasing the yield levels and to avoid genetic vulnerability due to the cultivation of a single variety. In breeding improved cultivars with higher yield and quality, selection of parents having desirable trait combinations based on divergence analysis would be more promising (Singh *et al.*, 2013) and satisfactory results can be obtained if the germplasm employed in the cross also present high values for the traits of interest (Fu and Somers, 2009). Hence, surveying the variability in the germplasm assumes significance in initiating breeding programmes.

ICAR-CTRI, Rajahmundry as a National Active Germplasm Site (NAGS) for tobacco germplasm, maintaining around 3300 tobacco accessions including 138 burley tobacco germplasm collected from exotic and indigenous sources. The variability available in the burley germplasm maintained at the Institute was not studied till date. Hence, a study was undertaken to envision the diversity present within burley germplasm for its further utilization in development of burley varieties suitable to Indian conditions.

MATERIALS AND METHODS

One hundred thirty eight burley tobacco accessions maintained at the gene bank of ICAR-

Key words: Burley, diversity, germplasm, morphological traits, Genetic variability.

CTRI, Rajahmundry were used in the study (Table 1). Seedlings were raised on nursery beds during mid September-mid November, 2018 and healthy seedlings were transplanted to the main field in second fortnight of November 2018 at a spacing of 70 x 60 cm in a row trial. Recommended crop production and protection practices were followed to raise a healthy crop. Observations were recorded on 29 morphological characters (Table 2) in three plants after confirming the uniformity within the row.

Statistical analysis: Initially, all the morphological observations were converted into scores so as to resemble as qualitative characters as per the defined notes. The number of entries falling under different categories in each trait was compiled. Morphological characters lacking variability were identified and excluded from further analysis. Principal component analysis (PCA) analysis was performed on the characters showing variability using Unscrambler 2018 version for the identification of morphological characters highly responsible for diversity. The resultant PCs with Eigen values greater than one were selected (Jeffers, 1967) for further analysis.

The diversity prevailing among the burley 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 5.0 and dissimilarity was estimated based on the respective morphological scoring.

RESULTS AND DISCUSSION

One hundred thirty eight burley genotypes were characterized based on five plant characters, 13 leaf characters and 11 flower/fruit/testa characters (Table 2). The genotypes were found to be diverse in nature for most of these traits except for three viz., leaf type, inflorescence position and seed testa colour. Burley genotypes characterized to have sessile leaves with inflorescence position above upper leaves and light brown seed testa colour. Majority of the plants were conical in shape with erect habit and medium to few leaves (Table. 1). The flower colour in most of the entries was mainly pink followed by white with spherical inflorescence. For the other traits, the entries were found to distribute in different categories. These observations indicate the existence of variability for various characters including economic characters like leaf number in burley germplasm.

PC analysis was conducted on 26 morphological traits recording variability for determining the selection criteria and identification of morphological characters highly responsible for diversity. The traits viz., leaf type, inflorescence position and seed testa colour were excluded in PCA analysis as no variation existed for these traits. Out of 26 components formed based on the eigen values, majority of the observed variation (91%) in the agro-morphological traits found to be explained by eighteen PCA components (Table 3). The half of the total variability (53%) found to be explained by six (PCA1 to PCA6) components. PCA 1 and PCA 2 together found to explain 27% of the variability captured. PCA1 alone explained 16% of the variation and was loaded mainly on eight traits (Table 4) viz., colour of leaf blade (0.735), leaf angle of insertion (0.66), inflorescence shape (0.624), leaf colour of mid-rib (0.564), width of leaf blade (0.548), plant shape (0.534), flower development of stamens (0.523), leaf length (0.519). These characters with high variability are expected to provide high level of gene transfer if used in breeding programs (Baghyalakshmi *et al.*, 2019; Aliyu *et al.*, 2000).

The biplot of principal coordinates (Fig 1) shows that the genotypes are scattered throughout the plot indicating that the genotypes are morphologically different from each other and the germplasm as whole is harboring larger variability. Further in order to know the dissimilarity index and relationship between the genotypes, data was analyzed in DarWin 5.0. Clustering through unweighted pair groups produced grouping that defined four distinct clusters in 0 to 8 distance (Fig. 2). The genotypes falling in different clusters with larger distances may be having huge diversity and genotypes within the clusters/sub-clusters with less diversity. The dissimilarity matrix worked out between 138 burley genotypes ranged

Table 1: Burley lines utilised in the study

S.No.	BY No.	Entry name	S.No.	BY No.	Entry name
1.	BY 1.	Aurelius	42.	BY 42.	Gold-G
2.	BY 2.	Apia	43.	BY 43.	Harwin
3.	BY 3.	B-103	44.	BY 44.	Heterosis Burley
4.	BY 4.	B-104	45.	BY 45.	Halleys Special
5.	BY 5.	B-105	46.	BY 46.	Haronic
6.	BY 6.	By-49	47.	BY 47.	Harrow Velvet
7.	BY 7.	By- 53	48.	BY 48.	Harmony
8.	BY 8.	By-64	49.	BY 49.	H-37
9.	BY 9.	Burley-1	50.	BY 50.	H-254-A
10.	BY 10.	Burley S-3	51.	BY 51.	Hy-Burely
11.	BY 11.	Burley-5	52.	BY 52.	HDBRG-CTRI
12.	BY 12.	Burley-21	53.	BY 53.	HDBRG-ITC
13.	BY 13.	Burley-37	54.	BY 54.	HDBRG-LP-2
14.	BY 14.	Burley-49	55.	BY 55.	HDBRG-Guntur
15.	BY 15.	Burely-64	56.	BY 56.	IGB
16.	BY 16.	Burely-C-22-1	57.	BY 57.	Kanazawa
17.	BY 17.	Burely CR-101	58.	BY 58.	Kelley
18.	BY 18.	Burely CR-179	59.	BY 59.	Kentucky
19.	BY 19.	Burley-181	60.	BY 60.	Kentucky-14
20.	BY 20.	Burley Sota-49	61.	BY 61.	Kentucky-15
21.	BY 21.	By-Sota-51	62.	BY 62.	KY-10
22.	BY 22.	By Sota- 62	63.	BY 63.	KY-14
23.	BY 23.	Burely Giuseppina	64.	BY 64.	KY-15
24.	BY 24.	BSRB-1	65.	BY 65.	KY-16
25.	BY 25.	BSRB-2	66.	BY 66.	KY-17
26.	BY 26.	Briarvet	67.	BY 67.	KY-19
27.	BY 27.	Banket A1	68.	BY 68.	KY-21
28.	BY 28.	Banket A 10	69.	BY 69.	KY-41 A
29.	BY 29.	Banket 21	70.	BY 70.	KY-42
30.	BY 30.	Banket 102	71.	BY 71.	KY-5
31.	BY 31.	Banket 127	72.	BY 72.	K-907
32.	BY 32.	Burely 100 A	73.	BY 73.	K-8959
33.	BY 33.	Bolsunvowika	74.	BY 74.	Lec-27-2-B
34.	BY 34.	Bursanica	75.	BY 75.	Leca-10
35.	BY 35.	Burlina	76.	BY 76.	Momi-2
36.	BY 36.	Burley Spartan	77.	BY 77.	N-501
37.	BY 37.	Burley Resistant	78.	BY 78.	N-502
38.	BY 38.	Burley MB-2	79.	BY 79.	N-503
39.	BY 39.	Burley Maruhatayo	80.	BY 80.	N-506
40.	BY 40.	Burley Naga Hatayo	81.	BY 81.	N-508
41.	BY 41.	Burely Granreditto	82.	BY 82.	NC-55

83.	BY 83.	Red Burley	111.	BY 112.	Kentucky -42
84.	BY 84.	R-610	112.	BY 113.	Burley -53
85.	BY 85.	Station Stand Up	113.	BY 114.	324c
86.	BY 86.	Sota 6504	114.	BY 115.	YB21
87.	BY 88.	Sota 6506	115.	BY 116.	YB22
88.	BY 89.	Sota 7303	116.	BY 117.	YB23
89.	BY 90.	Spanish Burley	117.	BY 118.	YB24
90.	BY 91.	Thesues	118.	BY 119.	YB25
91.	BY 92.	T-117	119.	BY 120.	YB4
92.	BY 93.	TN-86	120.	BY 121.	Banket A1
93.	BY 94.	TN-90	121.	BY 122.	Ky 160
94.	BY 95.	VA-163	122.	BY 123.	Ky 171
95.	BY 96.	VA-110	123.	BY 124.	Li Burley
96.	BY 97.	VA-510	124.	BY 125.	Hi Burley
97.	BY 98.	VA-528	125.	BY 126.	TN-90
98.	BY 99.	Vam	126.	BY 127.	KTH 2802
99.	BY 100.	Virginia Zamoska	127.	BY 128.	KTH 2803
100.	BY 101.	WBPR-9	128.	BY 129.	NC-7
101.	BY 102.	WBPR-10	129.	BY 130.	KT-200
102.	BY 103.	WBPR-11	130.	BY 131.	KT-204
103.	BY 104.	Zlatolist	131.	BY 132.	KT-206
104.	BY 105.	Zlatolist	132.	BY 133.	KT-209
105.	BY 106.	Banket 102 x BSRB-2	133.	BY 134.	KT-210
106.	BY 107.	BSRB-2 x Burley 21	134.	BY 135.	KT-212
107.	BY 108.	TN-86	135.	BY 136.	BRK-1
108.	BY 109.	TN-90	136.	BY 137.	BRK-2
109.	BY 110.	Ky-907	137.	BY 138.	BRK-3
110.	BY 111.	Ky-8959	138.	BY 139.	BRK-5

Table 2: Morphological variation recorded in the burley germplasm

S.No	Character (Code)	Character category*
	Plant	
1	Shape (PS)	Conical (112), Cylindrical (26)
2	Height (PHT)	Very Short (1), Short (29), Medium (86), Tall (22)
3	Habit (PH)	Open (17), Erect (71), Semi Erect (50)
4	Internodal Length (PIL)	< 4 cm (71), <6 cm (63), >6cm (3)
5	Number of Leaves (PNL)	Very Few (15), Few (105), Medium (17)
	Leaf	
6	Type (LT)	Sessile (138)
7	Angle of Insertion (LAI)	Very Acute (70), Moderately Acute (65), Right Angle (3)
8	Length (LL)	Short (2), Medium (39), Long (86), Very Long (11)
9	Width of Blade (WB)	Very Narrow (1), Narrow (66), Medium (65), Broad (6)
10	Midrib (LM)	Medium (32), Thick (106)
11	Veins-thickness and angle(LV-T&A)	Thin (55), Medium (64), Thick (19)
12	Blade Shape (LBS)	Narrow Elliptic (57), Broad Elliptic (80), Ovate (1), Rounded (1)
13	Tip Shape (LTS)	Slightly Pointed (6), Medium Pointed (104), Strongly Pointed (28)
14	Blistering of Blade (puckering) (LBB)	Absent or Very Weak (77), Weak (44), Medium (15), Strong (2)
15	Undulations of Margin (LUM)	Absent or Very Weak (86), Weak (37), Medium (12), Strong (3)
16	Development of Auricles (LDA)	Weak (9), Medium (101), Strong (27), Very Strong (1)
17	Colour of Blade (LCB)	Light Green (40), Medium Green (76), Dark Green (22)
18	Color of Midrib (LCM)	Whitish (80), White Greenish (27), Greenish (31)
	Flower	
19	Time of Flowering (TF) (50% of plants with at least one corolla open)	Very Early (4), Early (119), Medium (14)
20	Inflorescence Shape (IS)	Spherical (88), Flattened Spherical (13), Inverted Conical (36)
21	Inflorescence Compactness (IC)	Very Loose (1), Loose (8), Medium (118), Dense (10)
22	Inflorescence Position Relative to Upper Leaves (IPRUL)	Above (138)
23	Flower Length /Size (FL)	Medium (80), Long (57)
24	Flower Expression of Tips of Corolla (FTC)	Weak (95), Medium (40), Strong (2)
25	Flower Colour of Corolla (FCC)	White (1), Light Pink (104), Medium Pink (28), Variegated (4)
26	Flower Length of Pistil Relative to Stamens (FLPS)	Shorter (83), Equal Length (50), Longer (4)
27	Flower Development of Stamens (FDS)	Absent or Rudimentary (16), Full (122)
28	Fruit Form (FF)	Intermediate (16), Ovate (70), Conical (52)
29	Testa Colour (TC)	Light Brown (138)

* Note: The figures in the parenthesis are the number of genotypes under that category

Table 3: Total Variance in burley germplasm explained through principal component analysis Component Initial Eigen values

	Total	% of Variance	Cumulative %
1	4.288	16.492	16.492
2	2.710	10.423	26.916
3	2.103	8.087	35.002
4	1.813	6.975	41.977
5	1.432	5.508	47.485
6	1.337	5.143	52.628
7	1.249	4.803	57.431
8	1.137	4.371	61.802
9	1.060	4.076	65.878
10	0.994	3.823	69.701
11	0.896	3.446	73.147
12	0.820	3.154	76.300
13	0.735	2.829	79.129
14	0.710	2.729	81.858
15	0.665	2.559	84.418
16	0.604	2.322	86.739
17	0.519	1.997	88.736
18	0.494	1.898	90.635
19	0.458	1.762	92.397
20	0.381	1.467	93.864
21	0.361	1.388	95.251
22	0.334	1.286	96.537
23	0.292	1.123	97.660
24	0.222	0.856	98.516
25	0.214	0.825	99.340
26	0.172	0.660	100.000

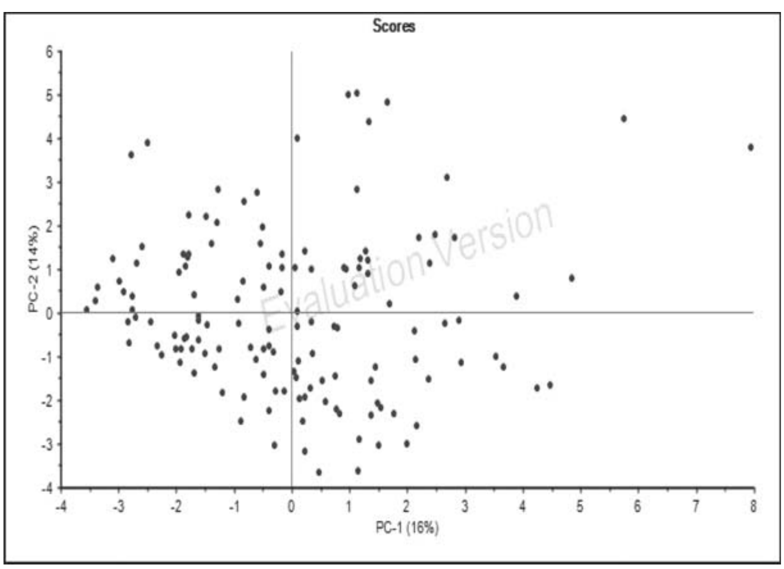

Fig. 1: Two-dimensional plot of principal coordinates of burley genotypes

Table 4: Eigen value ("Load") of the correlation matrix and its contribution to total variation of Burley entries.

Traits	Principal Component								
	PCA1	**PCA 2**	**PCA 3**	**PCA 4**	**PCA 5**	**PCA 6**	**PCA 7**	**PCA 8**	**PCA 9**
PS	0.534	-0.441	0.353	0.187	0.162	-0.047	-0.268	0.109	0.22
PH	0.4	0.475	0.121	0.352	-0.158	-0.002	-0.316	-0.279	0.176
PHT	0.044	0.121	-0.354	0.001	-0.112	0.654	-0.025	-0.25	-0.044
PIL	0.383	0.075	0.374	0.325	0.07	-0.111	0.289	-0.322	-0.326
PNL	-0.017	0.407	-0.405	0.262	-0.229	0.057	-0.162	0.029	0.483
LAI	0.66	-0.333	0.185	0.07	0.073	0.38	0.042	-0.154	0.034
LL	0.519	0.338	0.041	-0.003	-0.384	0.111	-0.008	0.042	-0.08
WB	0.548	0.316	0.295	-0.032	-0.379	0.016	-0.025	-0.168	-0.081
LM	0.285	0.558	-0.121	-0.382	0.084	-0.082	-0.041	0.042	-0.177
LV	0.128	0.451	0.094	-0.551	0.013	0.007	-0.068	0.181	0.067
LBS	0.44	0.298	0.216	-0.111	0.219	-0.408	-0.24	-0.232	0.172
LTS	0.342	0.002	-0.376	0.233	0.04	-0.049	-0.109	0.369	0.018
LBB	-0.193	0.296	0.675	0.048	0.143	0.176	0.136	0.071	0.283
LUM	-0.14	0.322	0.225	-0.466	0.232	0.157	0.22	-0.049	0.398
LDA	0.376	0.208	-0.106	-0.339	-0.374	0.056	0.305	0.201	0.014
LCB	0.735	-0.362	0.051	-0.163	0.047	-0.111	0.099	0.235	0.05
LCM	0.564	-0.412	0.057	-0.071	-0.172	-0.064	0.113	0.331	0.205
TFF	-0.403	0.328	0.245	0.394	0.011	-0.114	0.093	0.287	0.095
IS	0.624	-0.05	0.244	0.014	0.197	0.281	-0.148	0.055	-0.093
IC	0.216	0.164	-0.045	0.546	-0.197	0.082	0.429	0.191	0.131
FL	0.347	0.312	-0.379	0.208	0.264	0.014	-0.007	-0.016	-0.082
FTC	0.354	0.322	-0.276	0.098	0.36	-0.057	-0.35	0.236	-0.128
FCC	0.065	0.425	0.164	0.084	0.353	0.29	0.188	0.326	-0.342
FLPS	0.143	0.103	-0.291	0.156	0.532	-0.003	0.407	-0.179	0.264
FDS	0.523	-0.254	-0.458	-0.229	0.134	0.011	0.209	-0.24	0.147
FF	0.282	0.212	-0.116	0.032	-0.094	-0.545	0.353	-0.124	-0.096

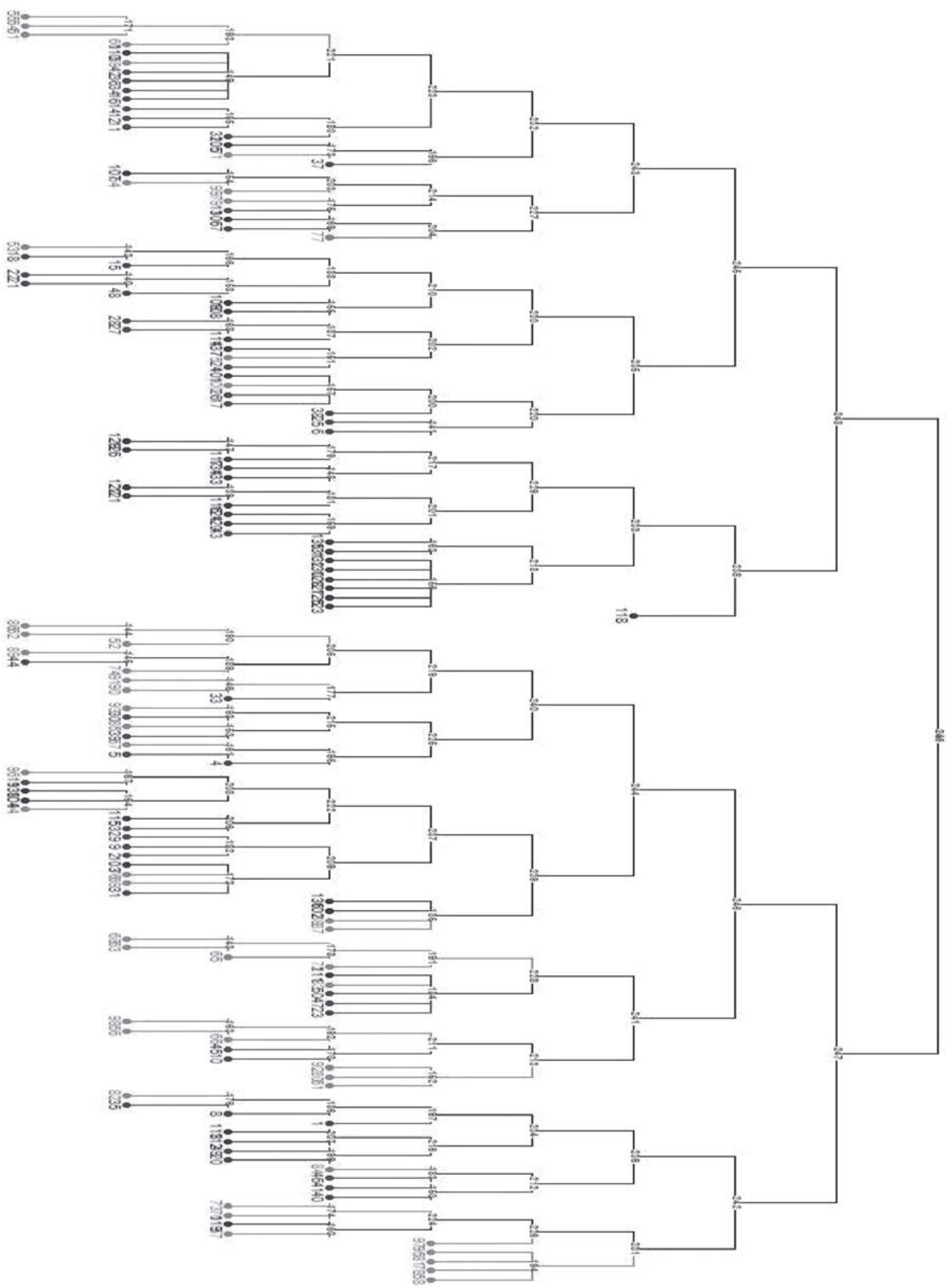

Fig. 2: Dendrogram of burley genotypes constructed based on UPGMA method

from 0.99 to 0.065. The maximum dissimilarity value (0.99) was found between YB-24 and Ky-171 (0.99). Such divergent entries can be used as parents in crossing for obtaining maximum heterosis and desirable segregants in advanced generations (Cruz *et al.*, 2012). The minimum dissimilarity value was between Ky-160 and Ky-171 (0.065). Such pairs are not recommended for use in breeding programs for hybridization as the genetic gain due to selection in resultant cross derivatives will be less due to low degree of variability (Cruz *et al.*, 2004).

The study clearly indicated the presence of morphological diversity in the burley germplasm maintained at ICAR-CTRI. Sarala *et al.*, (2018a & 2018b) also reported the existence of variation in other tobacco germplasm viz., mutant tobacco lines (35 accessions), bidi (205), chewing (175), cheroot (185) and cigar filler (82) germplasm maintained at ICAR-CTRI genebank. Further, Baghyalakshmi *et al.*, (2018) observed genetic diversity in tobacco germplasm that include genotypes evaluated under All India Network Project of tobacco. The diversity available in the burley germplasm can serve as a valuable source for burley tobacco improvement. Breeders can utilize this diversity for improving the leaf yield potential of burley cultivars grown in India.

REFERENCES

Aliyu, B., M.O. Akoroda and S. Padulosi. 2000. Variation within Vigna reticulata Hooke FII. **Nig. J. Gene**. 1-8.

Baghyalakshmi, K., K. Sarala, K. Prabhakara Rao, D. Damodar Reddy, C. Chandrasekhara Rao, P. Soni, P. Vinay, G. Kiran and K. Santinandivelu. 2018. Genetic diversity in tobacco genotypes evaluated under All India Network Project. **Multilogic Sci.** 8 (issue special): 30-34.

Cruz, C. D., A. J. Regazzi and P.C.S. Carneiro. 2012. Modelosbiométricosaplicadosao melhoramentogenético. 4th ed. UFV, **Viçosa**: p.514.

Cruz, C. D., A. J. Regazzi and P.C.S. Carneiro. 2004. Modelosbiometricosaplicadosao melhoramentogenetico UFV, **Vicosa:** p.480.

Fu, Y and D. Somers. 2009. Genome-Wide Reduction of Genetic Diversity in Wheat Breeding. **Crop Sci.** 49: 161–168.

Jeffers, J.N.R. 1967. Two case studies in the application of principal component analysis. **Appl. Stat.** 16: 225-236.

Perrier, X and J.P. Jacquemond-Collet. 2006. **DARwin software**. http://darwin.cirad.fr/darwin/

Sarala, K., K. Baghyalakshmi, K. Prabhakara Rao, D. Damodar Reddy, P. Vinay, G. Kiran and P. Sonia. 2018a. Genetic diversity among mutant germplasm accessions of *Nicotiana* tabacum as determined by morphological parameters. Tob. Res. 44(2): 47-53

Sarala, K., K. Baghyalakshmi, K. Prabhakara Rao, D. Damodar Reddy, P. Vinay, P. Sonia and G. Kiran. 2018b. Characterization of a sub-set of tobacco germplasm and its core collection. In '1st National Genetics Congress' conducted at New Delhi during 14.12.18 to16.12.18 by the Indian Society of Genetics and Plant Breeding, New Delhi and Indian Council of Agricultural Research (ICAR), New Delhi. Abstract No. PS I-8, Page No. 49.

Singh, S. R., N. Ahmed, S. Lal, S. A. Ganie, M. Amin, N. Jan and A. Amin. 2013. Determination of genetic diversity in onion (*Allium cepa*L.) by multivariate analysis under long day conditions. **Af. J. Agri. Res.** 8(45): 5599-5606.