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-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

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

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 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.

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Table 1: Burley lines utilised in the study

S.No.	BY No.	Entry name
1.	BY 1.	Aurelius
2.	BY 2.	Apia
3.	BY 3.	B-103
4.	BY 4.	B-104
5.	BY 5.	B-105
6.	BY 6.	By-49
7.	BY 7.	By- 53
8.	BY 8.	By-64
9.	BY 9.	Burley-1
10.	BY 10.	Burley S-3
11.	BY 11.	Burley-5
12.	BY 12.	Burley-21
13.	BY 13.	Burley-37
14.	BY 14.	Burley-49
15.	BY 15.	Burely-64
16.	BY 16.	Burely-C-22-1
17.	BY 17.	Burely CR-101
18.	BY 18.	Burely CR-179
19.	BY 19.	Burley-181
20.	BY 20.	Burley Sota-49
21.	BY 21.	By-Sota-51
22.	BY 22.	By Sota- 62
23.	BY 23.	Burely Giuseppina
24.	BY 24.	BSRB-1
25.	BY 25.	BSRB-2
26.	BY 26.	Briarvet
27.	BY 27.	Banket A1
28.	BY 28.	Banket A 10
29.	BY 29.	Banket 21
30.	BY 30.	Banket 102
31.	BY 31.	Banket 127
32.	BY 32.	Burely 100 A
33.	BY 33.	Bolsunvowika
34.	BY 34.	Bursanica
35.	BY 35.	Burlina
36.	BY 36.	Burley Spartan
37.	BY 37.	Burley Resistant
38.	BY 38.	Burley MB-2
39.	BY 39.	Burley Maruhatayo
40.	BY 40.	Burley Naga Hatayo
41.	BY 41.	Burely Granreditto
42.	BY 42.	Gold-G
43.	BY 43.	Harwin
44.	BY 44.	Heterosis Burley
45.	BY 45.	Halleys Special
46.	BY 46.	Haronic
47.	BY 47.	Harrow Velvet
48.	BY 48.	Harmony
49.	BY 49.	H-37
50.	BY 50.	H-254-A
51.	BY 51.	Hy-Burely
52.	BY 52.	HDBRG-CTRI
53.	BY 53.	HDBRG-ITC
54.	BY 54.	HDBRG-LP-2
55.	BY 55.	HDBRG-Guntur
56.	BY 56.	IGB
57.	BY 57.	Kanazawa
58.	BY 58.	Kelley
59.	BY 59.	Kentucky
60.	BY 60.	Kentucky-14
61.	BY 61.	Kentucky-15
62.	BY 62.	KY-10
63.	BY 63.	KY-14
64.	BY 64.	KY-15
65.	BY 65.	KY-16
66.	BY 66.	KY-17
67.	BY 67.	KY-19
68.	BY 68.	KY-21
69.	BY 69.	KY-41 A
70.	BY 70.	KY-42
71.	BY 71.	KY-5
72.	BY 72.	K-907
73.	BY 73.	K-8959
74.	BY 74.	Lec-27-2-B
75.	BY 75.	Leca-10
76.	BY 76.	Momi-2
77.	BY 77.	N-501
78.	BY 78.	N-502
79.	BY 79.	N-503
80.	BY 80.	N-506
81.	BY 81.	N-508
82.	BY 82.	NC-55
83.	BY 83.	Red Burley
84.	BY 84.	R-610
85.	BY 85.	Station Stand Up
86.	BY 86.	Sota 6504
87.	BY 88.	Sota 6506
88.	BY 89.	Sota 7303
89.	BY 90.	Spanish Burley
90.	BY 91.	Thesues
91.	BY 92.	T-117
92.	BY 93.	TN-86
93.	BY 94.	TN-90
94.	BY 95.	VA-163
95.	BY 96.	VA-110
96.	BY 97.	VA-510
97.	BY 98.	VA-528
98.	BY 99.	Vam
99.	BY 100.	Virginia Zamoska
100.	BY 101.	WBPR-9

101.	BY 102.	WBPR-10	120.	BY 121.	Banket A1
102.	BY 103.	WBPR-11	121.	BY 122.	Ky 160
103.	BY 104.	Zlatolist	122.	BY 123.	Ky 171
104.	BY 105.	Zlatolist	123.	BY 124.	Li Burley
105.	BY 106.	Banket 102 x BSRB-2	124.	BY 125.	Hi Burley
106.	BY 107.	BSRB-2 x Burley 21	125.	BY 126.	TN-90
107.	BY 108.	TN-86	126.	BY 127.	KTH 2802
108.	BY 109.	TN-90	127.	BY 128.	KTH 2803
109.	BY 110.	Ky-907	128.	BY 129.	NC-7
110.	BY 111.	Ky-8959	129.	BY 130.	KT-200
111.	BY 112.	Kentucky -42	130.	BY 131.	KT-204
112.	BY 113.	Burley -53	131.	BY 132.	KT-206
113.	BY 114.	324c	132.	BY 133.	KT-209
114.	BY 115.	YB21	133.	BY 134.	KT-210
115.	BY 116.	YB22	134.	BY 135.	KT-212
116.	BY 117.	YB23	135.	BY 136.	BRK-1
117.	BY 118.	YB24	136.	BY 137.	BRK-2
118.	BY 119.	YB25	137.	BY 138.	BRK-3
119.	BY 120.	YB4	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

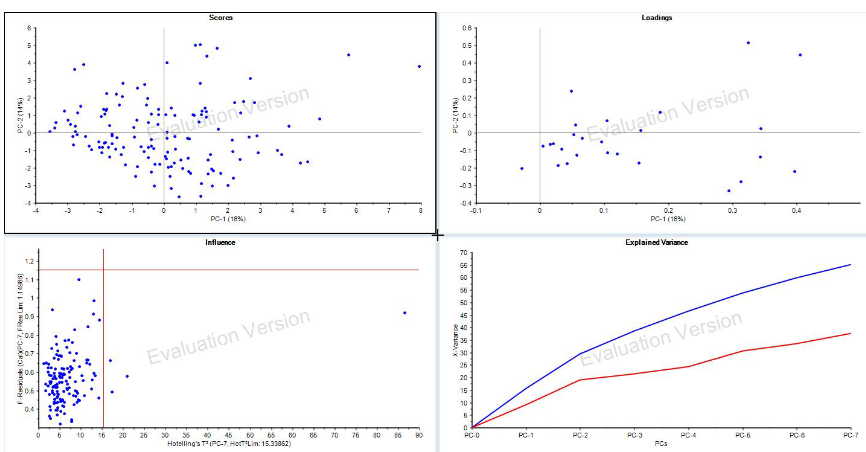
Table 3: Total Variance in burley germplasm explained through principal component analysis Component Initial Eigen values

	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

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



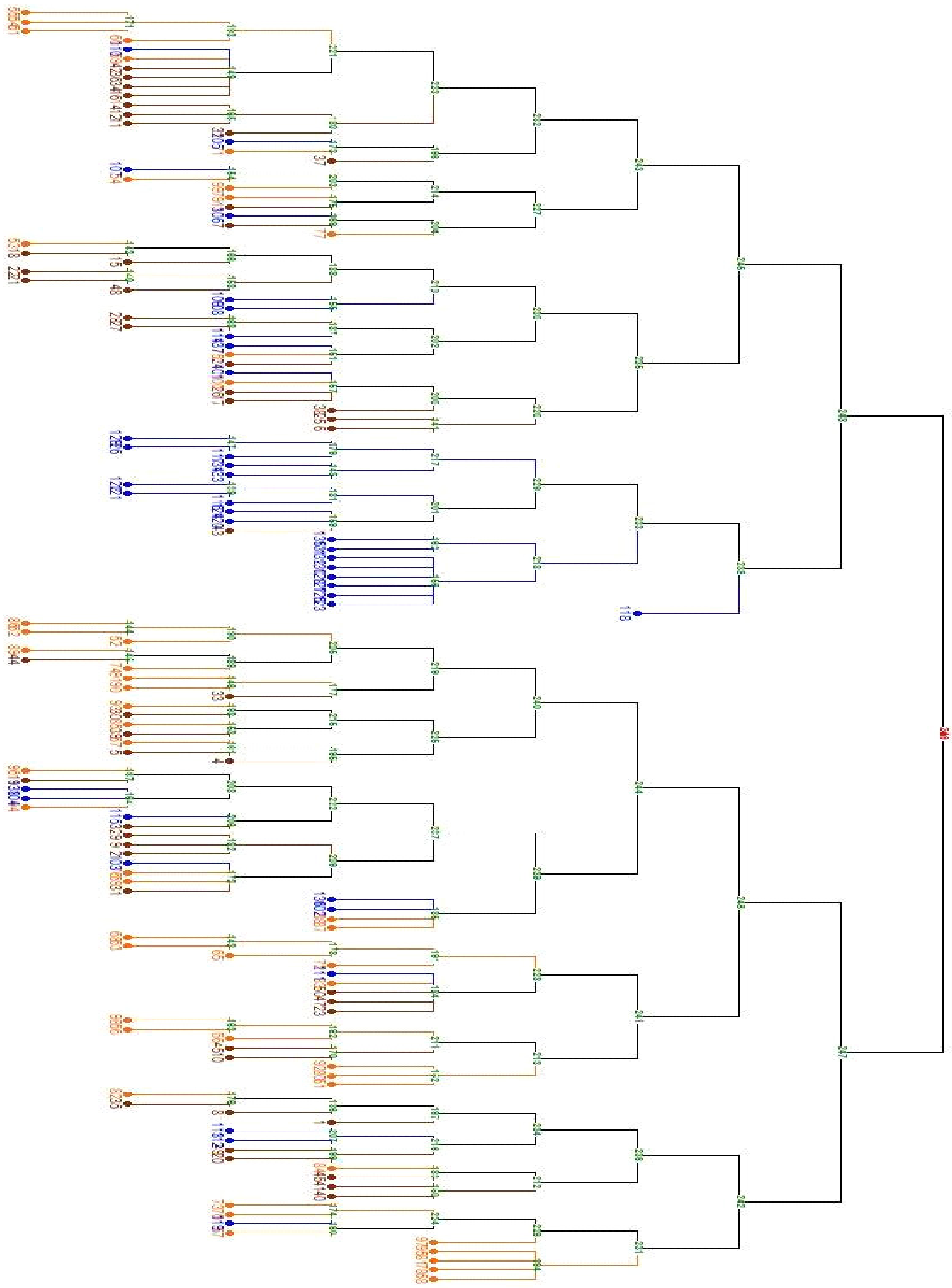


Fig 2. Dendrogram of burley genotypes constructed based on UPGMA method