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THE APPLICATION OF THE CLUSTER ANALYSIS FOR ESTIMATING DIVERGENCE OF TOBACCO *(N.tabacum L.)*

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INTRODUCTION

In the pre-breeding period, breeders attempt to determine phenotypic divergence among genotype germplasm in genetic collections they work with (*Duvick 1984, Prodanović*, 1995, *Dražić,* 1997a, 2007). Breeders' special interest is to determine material diversity over estimates of quantitative traits (*Jovanović et al*., 1993; 1994; 1994a, *Dražić* 2004). Yield and yield components are the most fundamental traits for selection and production.

The method of hierarchical cluster analysis is often applied to determine a form and degree of genotypic similarity for several variables (*Ward 1963)*. Groups (or clusters) obtained by this method, diverge phenotypically different varieties, while similar varieties are linked within groups.

Grouping genotypes of a certain germplasm collection into clusters makes selection of the parental pairs, most favourable for hybridisation, easier to breeders. In relation to a greater number of traits, crosses of the most divergent genotypes, from the most distant groups, can in progenies result into transgressive recombinations.

The aim of the present study was to determine, by the hierarchical cluster analysis, phenotypic divergence of the working germplasm collection of 20 burley tobacco genotypes, originating from various geographic regions and selection periods, under agroecological conditions of eastern Serbia. This study is a continuation of studies on phenotypic divergence within the species *Nicotiana tabacum* L. that were initiated under identical conditions with the collection of flue-cured Virginia tobacco varieties (*Dražić and Prodanović*, 1999).

MATERIAL AND METHODS

Twenty burley tobacco varieties originating from six countries were studied. Varieties Spartak, Poseidon, Bols 3, Tanit 2 and Austria 97 were from Austria (*Bolsunov,* 1961). The local varieties Burley DKH 28 and Burley DKH 33 were derived on the basis of initial material originating from Austria. The variety Burley T has been grown over 25 years in Yugoslavia on the basis of the introduced material (*Dražić 1997., 2003)*. Burley 103 originates from Japan, Harwin from Canada, and Burley HLN from Zimbabve. Varieties Burley 64, Va 590, TN 86, TN 90, SA 130, L8, N88 and N 777 were received from the USA. The six US varieties have been recently developed (*Miller*, 1987, *Palmer et al.*, 1991).

Varieties were grown during several years in the location of Veliko Gradište, in microtrials set up according to the 4-replicate randomised complete block design. For this paper we used results received from two-year experiments. The elementary plot size amounted to 20 m^2. Planting distance was 80 x 50 cm, while planting density amounted to 25,000 plants ha^{-1}. The trial was conducted on yellow sandy soil. The mean air temperature of the growing season (April - September) was about 19^{o}C, while the precipitation sum amounted to over 400 mm.

Ten plants per each replication were sampled for the analysis. The following components of each genotype were esteemed: duration of the growing season (in days), height (cm), leaf number on stalk, length and width of mid plant leaf (cm) and yield (kg ha^{-1}).

Means for all traits (x) for each genotype and each variety in the trial were determined. Variability of traits among genotypes within germplasm collection is presented by variance (S^2) and coefficient of correlation (V%). Significance of differences among genotypes was evaluated by the analysis of variance and F-test.

The Cluster programme of SAS software (1982) was applied to the hierarchical cluster analysis (*Ward*, 1963) in order to esteem the degree of divergence among genotypes. The matrix of Euclidean distances $d_2(e_i, e_j)$ among group means is calculated by this method:

$$d_2(e_i, e_j) = \left[\sum_{k=1}^{p} \frac{(x_{ik} - x_{jk})^2}{\sigma_k^2} \right]^{\frac{1}{2}}$$

The method of single linkage was used, whereby a dendrogram, showing successive fusions of genotypes, culminating at the stage where all groups belonged to the same cluster, was produced to display phenotypic differences among studied varieties.

RESULTS AND DISCUSSION

The estimates of quantitative traits of 20 studied Burley tobacco varieties are presented in Table 1.

Table 1.- Trait means of analysed genotypes
Табела 1.- Средни вредности на својствата на испитуваните генотипови

Genotype Генотип	Growing season Вегетационен период	Height (cm) Висина	Leaf number Број на листови	Leaf length (cm) Должина на листот	Leaf width (cm) ширина на листот	Yield (kg/ha^{-1}) Принос
Berlej T	66	160	20	58	25	1604
Berlej DKH 28	70	155	23	50	31	2160
Berlej DKH 33	74	175	25	56	30	2030
Spartak	83	180	26	55	30	2001
Poseidon	66	165	23	60	33	1990
Bols 3	64	153	20	50	30	1270
Tanit 2	64	170	22	62	38	1990
Austria 97	63	170	22	62	37	1950
Burley 103	74	49	14	26	14	780
Burley Harwin	81	136	19	47	24	1495
Burley HLN	60	130	28	48	25	1261
Burley 64	74	57	13	23	12	560
Va 590	79	48	11	22	10	544
Burley 37	73	36	12	21	10	522
TN 86	94	120	26	50	25	1670
TN 90	108	143	25	48	23	1870
SA 130	105	145	27	47	22	1904
N 777	107	90	15	31	16	784
L 8	109	102	21	41	18	436
N 88	112	95	23	33	15	990

Table 2. Variability parameters of traits of studied genotypes
Табела 2. Варијабилни параметри на својствата на испитуваните генотипови

Trait Својство	Mean Средна вредност	Min Минимум	Max Максимум	Variance Варијанса	Coefficient of variation Коефициент на варијабилноста	F
Growing season Вегетац. период	81	62	110	290,4	21	14,71**
Height Висина	124	36	180	2252,3	38	102,05**
Leaf number Бр. на листови	21	11	28	26,2	24	26,58**
Leaf lenght Должина на листот	45	21	62	175,1	29	30,45**
Leaf width Ширина на листот	23	10	38	69,6	37	25,30 **
Yield Принос	1391	436	2160	501230,0	51	32,43 **

Table 2 presents basic biometric parameters for the entire germplasm collection. Maximum and minimum estimates of traits are especially pointed out, indicating significant differences among varieties. Coefficients of variation regarding traits of observed material ranged from 21% (duration of the growing season) to 51% (yield). F-test expresses statistically very significant differences among genotypes for all traits.

Due to pronounced variability, the hierarchical cluster analysis showed a great divergence of the material. Dendogram of divergency by linkage distances for 20 examined genotypes, marked with ordinal numbers from Table 1 is shown on Figure 1.

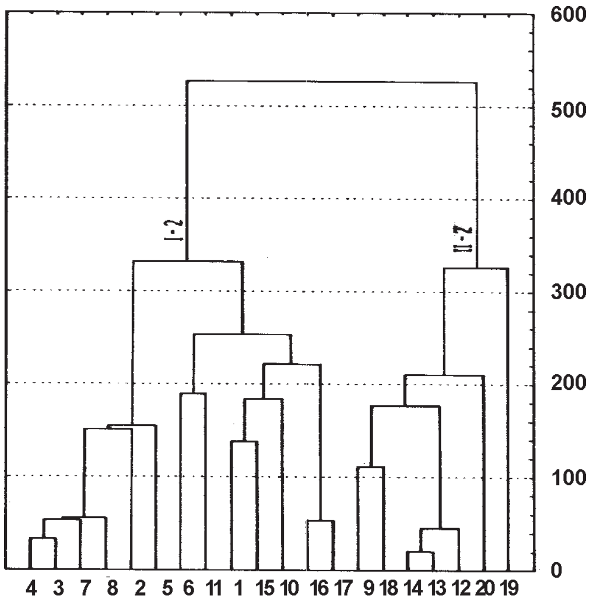

Figure 1. Dendrogram of linkage distances among 20 varieties (yield components and yield)
Графикон 1. Дендрограм на растојанијата на поврзувањата меѓу 20 сорти (компоненти на приносот и принос)

Two genotypic groups are observable in Figure 1. There are 13, i.e. seven varieties in the first (I-2), i.e. the second (II-2), respectively.

The comparison of the varietal distribution across the dendrogram with their estimates in Table 1 shows that linking into groups was done, first of all, according to yield. The group I-2 (Spartak, Burley DKH 33, Tanit 2, Austria 97, Burley DKH 28, Poseidon, Bols 3, Burley HLN, Burley T, TN 86, Burley Harwin, TN 90 and SA 130) encompasses varieties with relatively high yield, ranging from 1261 to 2154 kg ha-1, while the group II-2 (Burley 103, Burley 37, Burley 64, Va 590, L8, N88 and N 777) is characterized by a lower yield (ranging from 435 to 990 kg ha-1). Although the hierarchical cluster analysis was based on a greater number of elementary quantitative traits (yield components) and only one complex trait (yield) it seems that yield effects on grouping was the most important. However, since yield is a result of development of many elementary quantitative traits and depends on their values, elementary and complex traits should not be observed as conflicted sides. It should be noticed that the elementary traits enabling grouping of varieties by their yield were chosen for this study.

With regard to the fact that the stated traits are of the crucial importance for the yield level, the special attention should be paid to them in selection of Burley tobacco.

Linkage of yield components and yield is also confirmed by congruity of grouping of varieties into cluster (Figure 1.). However, there are certain differences especially in the position of the cluster II-2 with varieties L8, N 88 and N 777. The genotypes in the Figure belong to low yielding vareties. This statement points out to the fact that these three recently derived US varieties have a potential to form good yield according to their morphological traits, but they do not achieve such yield under our conditions.

Moreover, it should be mentioned that the form of grouping was not changed when the trait leaf area was omitted if leaf length and width were analysed and vice versa (*Dražić and Prodanović, 1999*). This means that the addition of positively highly interrelated and derived traits in the hierarchical cluster analysis does not affect the form of divergence and therefore the number of observed traits should not be increased. From the practical point of selection it means that greater efficiency can be achieved by monitoring a lower number of more important traits in more abundant material.

The dendrogram show linkage of genotypes in relation to the centre and time of their selection. For instance, all varieties of Austrian origin and local varieties, derived from the initial material of Austrian origin, belong to the same cluster (Bolsunov 1961., 1967., *Dražić* 1997.,1997a). The US varieties were also linked, whereby grouping into sublusters was mainly related to the period of their development (*Heggerstad et al., 1960*). Some authors suggested that varietal linkage into clusters could be, to a great extent, caused by a location of their development, explaining it by the model of a variety development applied in a particular institute and by the use of relatively smaller amount of tested germlasm with regard to combining abilities for parental components in each of the breeding centres (*Jovanović et al., 1994a*).

The produced dendrogram can be used to plan hybridisation. Crosses should be performed among genotypes as divergent as possible from different groups and subgroups, but of satisfactory traits. In such a way, a great number of unnecessary crosses of similar to similar varieties is eliminated, because they cannot result in progenies with the most pronounced gene recombinations for several traits. The mode of grouping of studied varieties point out to potentially good parental combinations.

CONCLUSIONS

Studies on 20 Burley tobacco varieties, among which 17 were introduced, under conditions of eastern Serbia, point to the following:

There are significant differences among genotypes of this germplasm collection based on estimates of variances, coefficients of variations and F-test.

Groups of genotypes primarily linked by estimates of yield were singled out by the hierarchical cluster analysis based on both yield components and yield.

The obtained divergence form for yield points out that selected quantitative traits were exactly those directly affecting yield estimates, and therefore a special attention should be paid to them in Burley tobacco selection.

The location and time of selection of observed varieties also significantly affected their divergence.

Phenotypic divergence established among genotypes can be used in selection of parental pairs in the further breeding programme.

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ПРИМЕНА НА КЛАСТЕР-АНАЛИЗАТА ЗА ОЦЕНА НА ДИВЕРГЕНТНОСТА НА ТУТУНОТ (*N. tabacum* L.)

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Република Србија

Испитувани се шест својства кај дваесет сорти берлејски тутун (должина на вегетациониот период, височина, број на листови, должина и широчина на средниот лист и принос) Утврдени се значајни разлики во нивните вредности, односно голема варијабилност на својствата кај проучуваните генотипови. За процена на степенот на нивната дивергенција, користена е хиерархиската кластер-анализа. Добиен е дендрограм заснован врз дивергенција на сортите спрема поважните својства, вклучувајќи го и приносот. Се издвојуваат два кластера I-2, II-2), односно групи на сорти. Во секој кластер се најдуваат генотипови со слична продуктивност, Што покажува дека избраните елементарни својства основа за оплеменување на приносот на берлејскиот тутун. При планирањето на хибридизациите родителите треба да се одбираат од различни кластери или супкластери за да можат да се рекомбинираат што подивергентни генотипови во поглед на испитуваните квантитативни својства.

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