

## Grouping of cotton varieties by phenotypic stability through cluster analysis

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### Abstract

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A cluster analysis was applied with a view to group 31 cotton varieties by phenotypic stability, determined by four stability measures, for five traits. It was found that the cluster analysis divided the genotypes, distinguishing the groups by phenotypic stability very well, and gave the opportunity to assess the most stable ones of particular importance for selection. Genotypes showed high rating of  $YS_i$  index had different selection value and formed groups that differed in the mean level of trait and stability. Among the genotypes evaluated highly through the  $YS_i$  criterion, there were some ones showed low stability. For all traits under study, groups including genotypes having a high average trait level and high stability, based on all stability measures were distinguished, which are very valuable for selection programs. The varieties possessing complex breeding value (high average trait level and high stability) in terms of productivity were Helius, Viki, Denitsa, Boyana and Philipopolis, in terms of boll weight these were 791–169, Viki, Avangard-264, Eva and Vega and in terms of fiber ginning out turn these were Viki, Boyana and Nelina. The varieties most valuable for fiber length were Natalia, Dorina, Perla-267 and Colorit and for the height of first fruiting branch these were Eva, Natalia, Millennium, Perla-267 and Colorit, combining in the best way mean level of trait and stability. Cluster analysis has emerged as an effective method for grouping genotypes by phenotypic stability measured by different stability methods and can greatly facilitate cotton breeding programs.

*Keywords:* cluster analysis; cotton; economic traits; phenotypic stability; varieties

### Introduction

Grouping of genotypes by phenotypic stability is of great importance for breeding programs. Stability is a very important characteristic of varieties, which requires its study. Stable varieties give more reliable yields in different ecological environments, or when changing environmental conditions.

There are many measures, approaches and concepts for measuring the stability of genotypes. Most widely used methods are the regression methods of Finlay & Wilkinson (1963), Eberhart & Russell (1966), the dispersion method of Shukla (1972), the method of Kang (1993) for simultaneous assessment of yield and stability.

Many researchers have been used these methods to assess the stability and adaptability of different cotton genotypes across different ecological environments to select the superior and adaptable ones (Balakrishna et al., 2016; Güvercin et al., 2017; Patil et al., 2017; Chinchane et al., 2018; Fathi et al., 2018; Iqbal et al., 2018; Pinki et al., 2018; Shashibhushan & Patel, 2020; Deho et al., 2021; Vavdiya et al., 2021).

Recently, PCA (Principal Component Analysis), AMMI (Additive Main Effects and Multiplicative Interactions) and GGE (Genotype and Genotype-Environment interaction) bi-plot analyses have been used to study genotype-environment interaction and genotype stability. Yan & Kang (2003), Fathi et al. (2018) used the bi-plot method to study genotype (G) and genotype-environment interaction (GE). Xu et

al. (2014) used this method to test environments and mega environments. Shahzad et al. (2019), Maleia et al. (2019) appreciated stability and adaptability of local and introduced varieties using the AMMI method. According to Riaz et al. (2013) the AMMI model was highly effective for analyzing experiments in many environments. Some researchers have been used both models simultaneously to analyze and compare the results obtained from different environmental experiments (Moiana et al., 2014; Pretorius et al., 2015; Farias et al., 2016; Maleia et al., 2017; Orawu et al., 2017; Riaz et al., 2019). These analyzes are based on bi-plot graphics and allow to illustrate the information and group the genotypes.

In addition to these methods, there are other multivariate methods that allow grouping of genotypes and environments into homogeneous groups and cluster analysis has been widely used (Westcott, 1986).

The aim of this study was, by applying the cluster analysis, to identify the groups of phenotypic stability in cotton varieties, which will facilitate and make their use in breeding programs more effective.

## Materials and Methods

The study included the varieties: Bulgarian – Chirpan-539, Beli Iskar, IPTP Veno, Boyana, Viki, Plovdiv, Kris (obtained from intraspecific hybridization); Trakia, Helius, Philipopolis, Sirius (obtained by experimental mutagenesis); Avangard-264 (from interspecific hybridization); Perla-267; Vega, Colorit, Rumi, Darmi, Nelina, Natalia and Dorina (obtained from the crosses of lines *G. hirsutum* L. × *G. barbadense* L. with varieties *G. hirsutum* L.; foreign – Deltapine 30, Stoneville 112 – American, Millennium, Eva, 791-169 – Greek, C-9070 – Uzbek, Nazily-84 – Turkish, Tabladila-16 – Spanish, T-08 – Romanian and Siokra-1-4 – Australian.

Four competition cotton variety trials were carried out in the experimental field of Field Crops Institute in town of Chirpan, in the period 2016–2019, on Pellic Vertisols, by the block method, in four replications and a plot of 20 m<sup>2</sup>, by applying the conventional technology for cotton cultivation in our country under non-irrigated conditions. The characters studied were seed cotton yield (kg/ha), boll weight (g), fiber ginning out turn (%), height of 1<sup>st</sup> fruiting branch, very important for mechanized harvesting. 10 plants from each replication were observed.

The years, during which the experiment was conducted, were used as different ecological environments. Statistical analysis of the genotype × environment interaction was performed and different stability parameters were used to assess the phenotypic stability of genotypes in different ecological environments: mean values ( $\bar{x}$ ) of studied characters; stabil-

ity variances ( $s^2_i$ ,  $S^2_i$ ) for linear and nonlinear interactions (Shukla, 1972); ecovalence ( $W^2_i$ ) (Wricke, 1962) and ( $YS_i$ ) criterion (Kang, 1993) for simultaneous assessment of mean level and stability. ANOVA were carried out for each year and over years. The program STABLE (Kang & Magari, 1995) was used to estimate the genotype × environment interaction and calculate the stability parameters ( $s^2_i$ ,  $S^2_i$ ,  $W_i$  and  $YS_i$ ).

Hierarchical cluster analysis was applied and the Euclidean distance was used as a measure for distinction between groups. For clustering the varieties the method proposed by Ward (1963) was used, which minimizes the variation within the groups. Data standardization has been performed in advance.

The years of the study were characterized as follows: in terms of temperature sum all years of the study were warm ( $P = 14.3\text{--}19.4\%$ ); in terms of rainfall, 2017 and 2019 were moderately wet ( $P = 22.6\text{--}33.3\%$ ), 2018 was wet ( $P = 20.9\%$ ) and 2016 was dry ( $P = 93.1\%$ ).

$P$  – security coefficient based on the descending order of years, respectively by temperature sum for May–September and rainfall sum for May–August ( $P\% = n/(m + 1) \times 100$ , where  $n$  was the order number of testing year;  $m$  – the total number of years in descending order of years – climatic norm.

The period 1989–2018 (last 30 years) was considered as climatic norm (Alexandrov et al., 2010).

## Results and Discussion

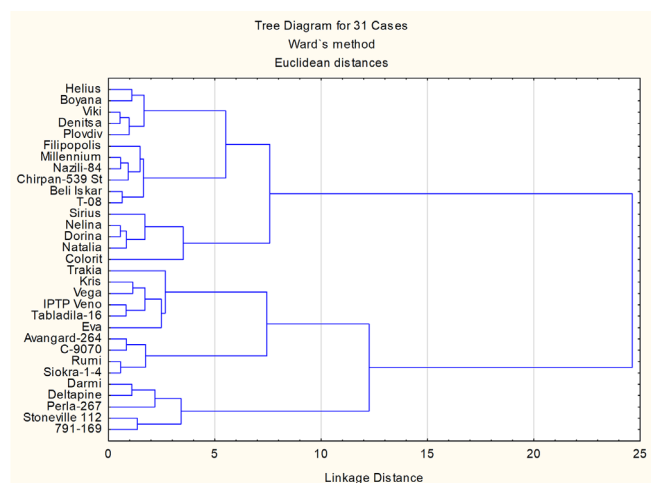
Results obtained from the analysis of phenotypic variance of the studied 5 traits of 31 cotton genotypes tested in 4 environments (2016–2019) are presented in a previous study (Valkova et al., 2022).

The variation by years was significant for all traits and shows the great importance of year conditions. The genotype × environment interaction was significant for all traits, due to the unequal response of genotypes to changes in environmental conditions.

Dendrograms of the performed hierarchical cluster analysis for the examined five traits are presented in Figure 1 – 5.

**Seed cotton yield.** From the dendrogram of conducted hierarchical cluster analysis (Figure 1) it can be seen that for seed cotton yield the genotypes divided into two main clusters. Two subgroups were formed in each main cluster.

The first main cluster includes genotypes had different stability according to the variances  $s^2_i$  and  $S^2_i$  and ecovalence  $W^2_i$  (Table 1). The first subgroup of this main cluster includes varieties produced mean yield from 1435 kg/ha to 1652 kg/ha, above the overall mean of trial (1398 kg/ha) and had high rating of  $YS_i$  (18–33). For some genotypes, the variances  $s^2_i$



**Fig. 1. Grouping of cotton genotypes by phenotypic stability for seed cotton yield**

and  $S^2_i$  were statistically weakly significant, which characterized them as unstable. The Helius and Viki varieties were very similar, high yielding (1648–1652 kg/ha) and had the highest rating of  $YS_i$  (32–33).

Helius variety was stable on the basis of the three stability parameters, the variances ( $s^2_p$ ,  $S^2_i$ ) and ecovalence ( $W^2_i$ ), while Viki variety had lower stability measured with the variance  $S^2_i$ . Denitsa variety also high yielding (1621 kg/ha) had lower stability according to the three stability measures ( $s^2_p$ ,  $S^2_i$  and  $W^2_i$ ) and a lower rating of  $YS_i$  (27). The other varieties of the same subgroup had lower productivity (1435 – 149.3 kg/ha), some had lower stability based on the three parameters ( $s^2_p$ ,  $S^2_i$  and  $W^2_i$ ) and lower  $YS_i$  rating (18–22). The varieties Boyana and Philipopolis, also very similar, were stable according to both stability variances ( $s^2_p$ ,  $S^2_i$ ) and the ecovalence ( $W^2_i$ ) and relatively high rated based on  $YS_i$  (21–25).

Second subgroup of the same main cluster includes varieties produced lower yields (1123–1365 kg/ha), below the overall mean of trial, and had lower rating of  $YS_i$ . Most of them had stable performance based on both stability variances ( $s^2_p$ ,  $S^2_i$ ) and ecovalence ( $W^2_i$ ), but as a result of lower yields they were rated lower based on  $YS_i$  criterion.

The second main cluster includes varieties had different productivity and very unstable performance given the significant stability variances  $s^2_p$ ,  $S^2_i$  and ecovalence  $W^2_i$ . The first subgroup of this main cluster includes the varieties Trakia, high yielding (1659 kg/ha), Tabladilla-16 and Eva, produced

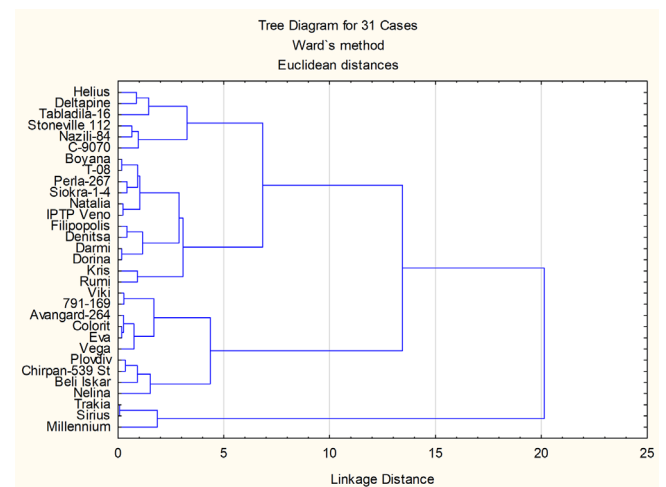
**Table 1. Mean seed cotton yields (2016–2019) and stability parameters  $\sigma^2_i$  and  $S^2_i$  of Shukla (1972), Wricke’s  $W^2_i$  and Kang’s  $YS_i$  (1993) for 31 cotton genotypes**

Genotypes	Mean values	$b_i$	$S^2_{di}$	$\sigma^2_i$	$S^2_i$	$W^2_i$	$YS_i$
<b>First main cluster, 1<sup>st</sup> subgroup</b>							
Helius	1652	1.23	2374.6	6801.6ns	10117ns	20480.3	<b>33*</b>
Viki	1648	1.62	1935.9	9013.3ns	13342.0*	26687.3	32*
Boyana	149.1	1.54	724.4	4656.8ns	6933.0ns	14461.0	25*
Philipopolis	143.5	0.62	2756.3	4874.5ns	7315.1ns	15072.1	21*
Plovdiv	1493	1.46	3276.1	11005.9*	16507*	32279.6	22*
Beli Iskar	1457	0.66	3315.5	10159.6*	15242.4*	29904.4	19*
Denitsa	1621	1.68	2236.7	10600.2*	15818.3*	31141.1	<b>27*</b>
Veno	1471	0.25	2868.1	13400.1*	20053.5**	38998.8	20*
T-08	1453	0.70	4334.9	12732.0*	19100.8**	37123.7	18*
<b>First main cluster, 2<sup>nd</sup> subgroup</b>							
Sirius	1356	1.82	269.5	7279.5ns	10770.6ns	218215	12*
Millennium	1335	0.75	22740	6663.1ns	9972.5ns	20091.7	10
Nazili-84	1357	0.77	3062.7	8812.8ns	13121.6*	26124.7	13*
Chirpan-539 St	1365	1.09	3729.7	10199.3*	15231.0*	30015.7	10
Nelina	1322	1.45	681.7	3549.1ns	5276.8ns	11352.4	8
Dorina	1283	1.24	526.2	1624.3ns	2439.7ns	5950.4	5
Natalia	1123	1.43	547.8	2992.6ns	4474.7ns	9790.5	-2
<b>Second main cluster, 1<sup>st</sup> subgroup</b>							
Trakia	<b>1695</b>	0.94	6085.7	16851.9**	25217.1**	48685.9	26*
Tabladilla-16	1520	-0.07	1627.7	15915.5**	23540.5**	46058.2	20*
Eva	<b>1562</b>	-0.37	1620.5	23459.0**	35058.8**	67228.5	21*

relatively high yields (1520 kg/ha and 1562 kg/ha) and had high  $YS_i$  rating (21–26), but they were very unstable on the basis of both variances ( $s^2_p, S^2_i$ ) and ecovalence ( $W^2_i$ ). The second subgroup includes varieties produced very low mean yields, below the overall mean of trial, with even lower stability based on the stability variances ( $s^2_p, S^2_i$ ) and ecovalence ( $W^2_i$ ) and low rating of  $YS_i$  (-6 to 10).

From the analysis of results it can be concluded that the cluster analysis groups well the varieties by stability for seed cotton yield. The most valuable genotypes high yielding and stable in different environments referred to first subgroup of the 1<sup>st</sup> main cluster. Helius variety was found to be superior combining high productivity and high stability based on all stability measures and had the highest rating of  $YS_i$ . The Viki and Denitsa varieties showed relatively lower stability, rated as one of the most valuable after the Helius variety. Boyana and Philipopolis varieties, had slightly lower productivity, but with high stability on the basis of all stability measures ( $s^2_p, S^2_i$  and  $W^2_i$ ) appeared to be valuable, too. Results obtained confirms previous research, simultaneous assessment of yield and stability based on the  $YS_i$  parameter has identified as the most valuable lines ML-244, ML-288 (Helius variety) and ML 191 (Valkova & Dechev, 2005) and Viki variety (Valkova & Dechev, 2006).

In the first subgroup of second main cluster some varieties (Trakia, Tabladilla 16 and Eva) had high productivity and



**Fig. 2. Grouping of cotton genotypes by phenotypic stability for boll weight**

low stability, which could be included in crosses with stable varieties from the first subgroup of first main cluster in order to improve their stability. Within the subgroups in both main clusters there was also a division of genotypes and their differentiation into smaller groups, there were also very similar in phenotypic stability genotypes.

The two main clusters included varieties similar in  $YS_i$  rating, Denitsa variety in the first main cluster and Trakia

**Table 2. Boll weight average for 2016-2019 and stability parameters  $\sigma^2_i$  and  $S^2_i$  of Shukla (1972), Wricke's  $W^2_i$  and Kang's  $YS_i$  (1993) for 31 cotton genotypes**

Genotypes	Mean values	$\sigma^2_i$	$S^2_i$	$W_i$	$YS_i$
<b>First main cluster, 1<sup>st</sup> subgroup</b>					
Helius	5.3	0.503**	0.752**	1.445	12*
Deltapine	5.5	0.534**	0.801**	1.532	22*
Tabladilla-16	5.3	0.707**	1.060**	2.019	11
Stoneville 112	5.7	0.330*	0.495**	0.962	30*
Nazili-84	5.6	0.398**	0.597**	1.152	24*
C-9070	5.4	0.286*	0.430**	0.838	19*
<b>Second main cluster, 1<sup>st</sup> subgroup</b>					
Viki	5.5	0.032ns	0.048ns	0.124	28*
791-169	5.5	0.012ns	0.018ns	0.067	31*
Avangard-264	5.4	0.142ns	0.213ns	0.433	26*
Colorit	5.4	0.187ns	0.281*	0.560	25*
Eva	5.4	0.176ns	0.263ns	0.527	27*
Vega	5.4	0.082ns	0.123ns	0.265	23*
<b>Second main cluster, 2<sup>nd</sup> subgroup</b>					
Plovdiv	5.3	0.128ns	0.192ns	0.394	12*
Chirpan-539 St	5.3	0.147**	0.219**	0.446	16*
Beli Iskar	5.3	0.012ns	0.017ns	0.069	12*
Nelina	5.0	0.038ns	0.056ns	0.140	0

variety in the second main cluster. Both varieties had high productivity, but were with different stability performance.

**Boll weight.** Three main clusters were formed for the boll weight (Figure 2). The first one was the largest and includes three subgroups. The varieties in this main cluster had boll weight from 5.0 g to 5.7 g and, with the exception of Philipopolis variety, were unstable based on the stability variances ( $s_p^2$ ,  $S_i^2$ ) and ecovalence ( $W_i^2$ ) (Table 2). The first subgroup in this main cluster includes varieties formed boll weight of 5.3–5.7 g, divided into two smaller groups. The second group includes the varieties Stoneville 112 and Nazili-84, which were very similar and had the largest boll weight (5.7 g and 5.6 g). High and significant variances ( $s_p^2$ ,  $S_i^2$ ) defined them as unstable, but as a result of their high genetic effects, they were highly rated based on the criterion  $YS_i$  (30 and 24).

The first subgroup of second main cluster includes the varieties with high stability on the basis of the three stability measures (variances  $s_p^2$ ,  $S_i^2$  and ecovalence  $W_i^2$ ) and high  $YS_i$  rating (23–31). Their boll weight was 5.4–5.5 g. Second subgroup of this main cluster includes varieties also with high stability based on the three stability measures ( $s_p^2$ ,  $S_i^2$  and  $W_i^2$ ), with the exception of Chirpan-539, but had lower boll weight and lower  $YS_i$  rating.

The third main cluster, which separated from the two main clusters, includes only three varieties, which were very unstable in terms of stability variances ( $s_p^2$ ,  $S_i^2$ ) and ecovalence ( $W_i^2$ ) and had very low  $YS_i$  scores.

Clustering regarding the boll weight reveals two subgroups genotypes of importance for cotton breeding programs related to the first and second main clusters: Stoneville 112 and Nazili 84 varieties (1<sup>st</sup> main cluster, 1<sup>st</sup> subgroup), formed the largest bolls, unstable on the basis of the three stability measures ( $s_p^2$ ,  $S_i^2$  and  $W_i^2$ ), had high rating of  $YS_i$  due to their high genetic effects; Viki, 791–169, Avangard-264, Colorit, Eva and Vega (second main cluster, first subgroup), with boll weight of 5.4–5.5 g and high stability according to all stability measures. Highest breeding value was found for the Greek variety 791–169, combining a high mean level of this trait and high stability, had the highest rating of  $YS_i$ .

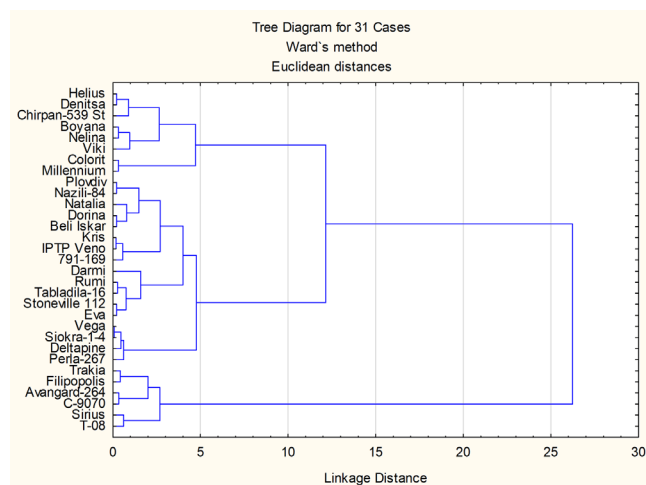
**Fiber ginning out turn.** For the fiber ginning out turn the varieties belong to two main clusters. The first main cluster includes the main part of genotypes, divided into two subgroups. The first subgroup includes the varieties showed the highest fiber ginning out turn (35.7–36.9%). All of them had high rating of  $YS_i$  (21–30), but on the basis of the two stability variances ( $s_p^2$ ,  $S_i^2$ ) and ecovalence ( $W_i^2$ ), some varieties were very stable, others were unstable, which has led to their differentiation into three small groups within the subgroup (Figure 3).

Boyana, Nelina (very similar) and Viki varieties, which are in one group, had high stability based on the stability

variances  $s_p^2$  and  $S_i^2$  and ecovalence  $W_i^2$ , these three varieties combined high ginning out turn and stability, which makes them very valuable for cotton breeding programs. The second subgroup of this main cluster is much larger and at a lower level of division in its formation of smaller groups is observed. The varieties from this subgroup had a lower fiber ginning out turn (33.8–35.5%) and lower scores based on the  $YS_i$  criterion. The varieties Kris, Veno and 791–169, separated in one group, had high stability on the three stability measures ( $s_p^2$ ,  $S_i^2$  and  $W_i^2$ ) and comparatively high rating of  $YS_i$ . The varieties Siokra, Deltapine 30 and Perla-267, also very stable on the basis of the three measures ( $s_p^2$ ,  $S_i^2$  and  $W_i^2$ ), formed another group and they had a low ginning out turn and very low  $YS_i$  evaluations (Table 3).

The second main cluster includes varieties having high fiber ginning out turn (35.5–36.3%), some with high  $YS_i$  scores, but all of them were very unstable on the basis of the three stability measures ( $s_p^2$ ,  $S_i^2$  and  $W_i^2$ ).

From the analysis of results for this trait, it can be summarized that both main clusters include varieties having high ginning out turn and high rating of  $YS_i$ , but with different stability measured by the stability variances  $s_p^2$ ,  $S_i^2$  and ecovalence  $W_i^2$ . All varieties, stable under the three measures, are located in the first main cluster, divided into two subgroups, depending on the trait mean level and rating of  $YS_i$ , divided into smaller groups. All varieties, stable under the three measures, are located in the first main cluster, in two subgroups, depending on the trait mean level and the  $YS_i$  rating, divided into even smaller groups. Viki variety appears to be the most valuable, combining high ginning out turn and high stability, followed by Boyana and Nelina varieties showed slightly



**Fig. 3. Grouping of cotton genotypes by phenotypic stability for ginning out turn**



**Table 3. Ginning out turn average for 2016–2019 and stability parameters  $\sigma^2_i$  and  $S^2_i$  of Shukla (1972), Wricke's  $W^2_i$  and Kang's  $YS_i$  (1993) for 31 cotton genotypes**

Ginning out turn, %					
Genotypes	Mean values	$\sigma^2_i$	$S^2_i$	$W_i$	$YS_i$
<b>First main cluster, 1<sup>st</sup> subgroup</b>					
Heliuss	35.7	0.978*	1.466**	2.865	21*
Denitsa	35.7	0.827*	1.240*	2.442	21*
Chirpan-539 St	36.3	0.908*	1.362*	2.670	28*
Boyana	35.7	0.060ns	0.090ns	0.290	24*
Nelina	35.6	-0.004ns	-0.006ns	0.110	21*
Viki	36.1	0.200ns	0.301ns	0.684	30*
Colorit	36.1	1.887**	2.830**	5.415	23*
Millennium	36.9	1.961**	2.941**	5.624	26*
<b>First main cluster, 2<sup>nd</sup> subgroup</b>					
Natalia	35.0	0.524ns	0.787ns	1.593	6
Kris	35.1	0.287ns	0.431ns	0.927	10
Veno	35.1	0.187ns	0.281ns	0.647	9
791-169	35.3	0.201ns	0.302ns	0.687	14*
Eva	34.4	0.751ns	1.127*	2.230	-1
Vega	34.4	-0.007ns	-0.011ns	0.10	-1
Siokra-1-4	34.4	0.050ns	0.075ns	0.261	-1
Deltapine	34.6	0.164ns	0.246ns	0.581	2
Perla-267	33.8	0.367ns	0.551ns	1.152	-2
<b>Second main cluster</b>					
Trakia	36.3	3.097**	4.644**	8.812	24*
Philippopolis	35.9	3.003**	4.505**	8.550	20*
Sirius	35.8	4.178**	6.267**	11.846	19*

lower trait level and high stability according to all stability measures.

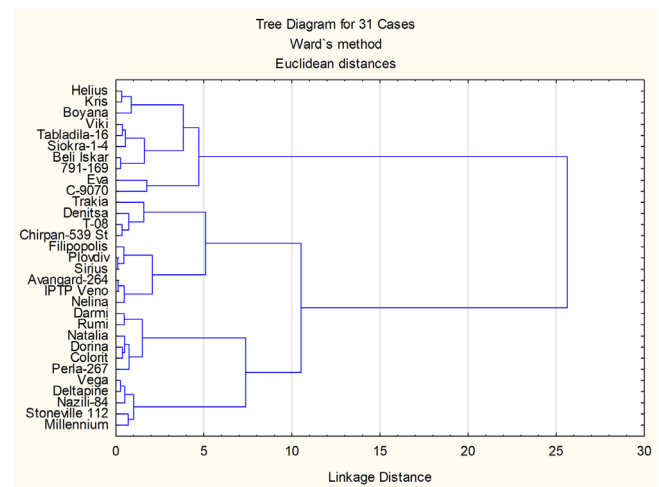
**Fiber length.** For the fiber length, at a lower level of division, the genotypes divided into three main clusters (Figure 4). The first main cluster includes varieties having fiber length of 25.9–26.7 mm, unstable on the basis of the stability variances  $s^2_p$ ,  $S^2_i$  and ecovalence  $W^2_i$ . The varieties from this main cluster divided into three subgroups, the first two having low  $YS_i$  estimates. Eva and C-9070 varieties, possessing the longest fibers for this cluster, and a relatively high  $YS_i$  rating, formed a separate group and were very similar (Table 4).

The second main cluster divided into two subgroups, the first includes varieties unstable on the basis of the stability variances  $s^2_p$ ,  $S^2_i$  and ecovalence  $W^2_p$ , while the second one includes varieties that were highly stable, but due to their shorter fibers had low  $YS_i$  scores.

The third main cluster also divided into two subgroups. The first subgroup includes varieties having fiber length of 26.5–26.8 mm and high stability according to the three stability parameters and the highest  $YS_i$  rating (27–34). The second subgroup includes varieties similar in fiber length

(26.4–26.7 mm), unstable on the basis of the three stability measures, due to which they had lower  $YS_i$  scores.

The analysis of results shows that the varieties stable for



**Fig. 4. Grouping of cotton genotypes by phenotypic stability for fiber length**

**Table 4. Fiber length average for 2016–2019 and stability parameters  $\sigma^2_i$  and  $S^2_i$  of Shukla (1972), Wricke's  $W^2_i$  and Kang's  $YS_i$  (1993) for 31 cotton genotypes**

Fiber length, mm					
Genotypes	Mean values	$\sigma^2_i$	$S^2_i$	$W^2_i$	$YS_i$
<b>First main cluster, 2<sup>nd</sup> subgroup</b>					
Eva	26.7	0.932**	1.399**	2.662	25*
C-9070	26.6	1.254**	1.881**	3.566	20*
<b>Second main cluster, 2<sup>nd</sup> subgroup</b>					
Philippopolis	25.9	0.066ns	0.096ns	0.232	2
Plovdiv	25.8	0.131ns	0.196ns	0.412	0
Sirius	25.8	0.116ns	0.174ns	0.371	-1
Avangard-264	26.1	0.203ns	0.305ns	0.617	9
Veno	26.2	0.219ns	0.330ns	0.663	10
Nelina	26.1	0.152ns	0.228ns	0.473	7
<b>Third main cluster, 1<sup>st</sup> subgroup</b>					
Natalia	26.8	0.172ns	0.258ns	0.529	34*
Dorina	26.6	0.143ns	0.214ns	0.446	30*
Colorit	26.6	0.203ns	0.305ns	0.617	28*
Perla-267	26.5	0.075ns	0.112ns	0.255	27*
<b>Third main cluster, 2<sup>nd</sup> subgroup</b>					
Stoneville 112	26.7	0.465**	0.698**	1.352	24*
Millennium	26.4	0.377*	0.567*	1.104	18*

the fiber length belong to two main clusters, depending on the mean level of trait and their evaluation according to the  $YS_i$  criterion and in each cluster they formed a separate subgroup.

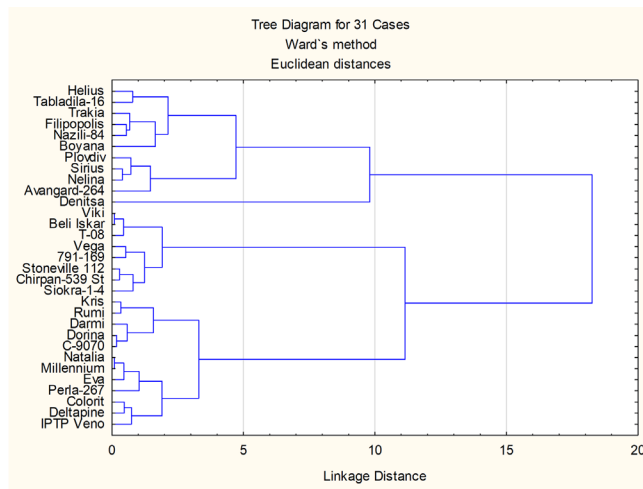
The most valuable genotypes for selection by this trait were found to be the varieties Natalia, Dorina, Colorit and Perla-267, located in the third main cluster, first subgroup, having the longest fibers, stable according to the three stability measures ( $s^2_i$ ,  $S^2_i$  and  $W^2_i$ ) and the most high  $YS_i$  scores. These results are in accordance with those reported by Stoilova & Dechev (2002) and Stoilova (2010) that the varieties Natalia, Darmi, Colorit Avangard-264, etc., containing gene plasma of the *G. barbadense* L. species, combined best fiber length and stability. The varieties in the second subgroup of second main cluster, showed high stability and low  $YS_i$  scores, due to the shorter fibers, could be used in crosses to create highly stable genotypes having longer fibers.

**Height of first fruiting branch.** For this trait, the varieties were divided into two main clusters, each including two subgroups (Figure 5). The first main cluster includes varieties having height of 1st fruiting branch 17.5–18.2 cm, unstable on the basis of the variances  $s^2_i$  and  $S^2_i$  and ecovalence  $W^2_i$  and low (for the first subgroup) to very low (for the second subgroup)  $YS_i$  scores. Only the Helius variety had the highest average value of trait for this cluster, and a relatively high (19)  $YS_i$  rating (Table 5).

The first subgroup of second main cluster includes varieties with lower mean trait values (17.1–17.8 cm) and low  $YS_i$  scores. The varieties of this subgroup were divided into two smaller groups, one of which includes varieties unstable under the measures  $s^2_i$ ,  $S^2_i$  and  $W^2_i$ , and the other – stable under the same stability measures.

The second subgroup of the same main cluster includes varieties having high 1st fruiting branch (17.9–18.3 cm). The varieties of this subgroup formed four smaller groups: the first one includes the varieties Kris and Rumi, unstable on the basis of stability measures  $s^2_i$ ,  $S^2_i$  and  $W^2_i$ , but having high  $YS_i$  rating (28–29); the second one also includes unstable genotypes, with lower  $YS_i$  scores (19–20); the third group includes genotypes possessing the highest selection value, high stability and relatively high  $YS_i$  rating (25–31); the last group includes stable genotypes based on the three stability measures, but having a lower  $YS_i$  score (15–23).

The analysis of results shows that the genotypes stable for the height of first fruiting branch belong to the second main cluster, but to different subgroups. The first subgroup includes genotypes stable according to the stability variances  $s^2_i$ ,  $S^2_i$  and ecovalence  $W^2_i$ , but had low  $YS_i$  scores (from -2 to 6) due to lower trait mean values (17.1–17.7 cm). The second subgroup includes the varieties Eva, Natalia, Millennium and Perla-267, combining in the best way average trait level (18.1–18.3 cm) and stability under the three measures ( $s^2_i$ ,  $S^2_i$  and  $W^2_i$ ) and



**Fig. 5. Grouping of cotton genotypes by phenotypic stability for height of the 1<sup>st</sup> fruiting branch**

high  $YS_i$  scores (25–31). These varieties, for this trait, are of the greatest importance for cotton breeding programs. This subgroup also includes genotypes, distinguished into another small group, that were unstable according to the three stability measures, but had relatively high  $YS_i$  scores (19–29) due to the higher trait mean level (18.1–18.4 cm).

**Table 5. Height of the 1<sup>st</sup> fruiting branch average for 2016–2019 and stability parameters  $\sigma_i^2$  and  $S_i^2$  of Shukla (1972), Wricke’s  $W_i^2$  and Kang’s  $YS_i$  (1993) for 31 cotton genotypes**

Height of the 1 <sup>st</sup> fruiting branch, cm					
Genotypes	Mean values	$\sigma_i^2$	$S_i^2$	$W_i$	$YS_i$
<b>First main cluster, 1<sup>st</sup> subgroup</b>					
Helius	18.2	1.424**	2.137**	4.100	19*
Tabladila-16	18.0	1.705**	2.557**	4.887	12*
<b>Second main cluster, 1<sup>st</sup> subgroup</b>					
Stoneville 112	17.5	0.44ns	0.661ns	1.339	1
Chirpan-539 St	17.1	0.365ns	0.548ns	1.128	-2
Siokra-1-4	17.7	0.207ns	0.311ns	0.684	6
<b>Second main cluster, 2<sup>nd</sup> subgroup</b>					
Kris	18.3	0.995*	1.492**	2.896	28*
Rumi	18.4	0.809*	1.213*	2.374	29*
Darmi	18.2	1.044**	1.565**	3.032	20*
Dorina	18.1	0.830*	1.244**	2.433	19*
C-9070	18.1	0.739*	1.108*	2.176	18*
Natalia	18.2	0.285ns	0.427ns	0.903	28*
Millennium	18.2	0.343ns	0.514ns	1.065	28*
Eva	18.3	0.172ns	0.258ns	0.585	31*
Perla-267	18.1	0.865ns	0.130ns	0.345	25*
Colorit	18.1	0.446ns	0.668ns	1.354	23*
Deltapine	18.0	0.538ns	0.807*	1.612	18*
Veno	17.9	0.262ns	0.393ns	0.838	15*

Summarized results from the analysis of studied traits show that the cluster analysis groups well the genotypes by phenotypic stability. The results obtained are in accordance with the findings of previous studies. Dechev (1998) in durum wheat, Stoilova & Dechev (2003) in cotton, also used the cluster analysis to group the genotypes by phenotypic stability and concluded that it divided the genotypes into groups having different breeding values.

Valkova & Dechev (2012) used PCA (Principal Component Analysis) to assess the phenotypic stability of promising mutant cotton lines. This analysis allows to group genotypes by phenotypic stability. PC1 is associated with the linear and PC2 with the nonlinear effects of genotypes. The positive values of PC1 are for static (biological) stability while the negative values are for dynamic stability, which must be taken into account. Shukla’s (1972) stability variances ( $s^2_p, S^2_i$ ) similarly reflect linear and nonlinear interactions. A higher value of  $s^2_i$  indicates less phenotypic stability, while positive values of PC1 are associated with higher stability. The same authors performed a correlation analysis that showed a significant negative correlation ( $r = -0.63$ ) between PC1 and  $s^2_p$ , and non-significant medium high ( $r = 0.50$ ) between PC2 and  $S^2_i$ . This shows that cluster analysis similarly to PCA can be used for grouping genotypes by phenotypic stability.



For the individual traits, two to three main clusters were formed, each divided into two or more subgroups. Valuable information about the phenotypic stability of traits gave the division of genotypes at a lower level, within the subgroups, and their division into smaller groups.

Genotypes, showed high scores based on the  $YS_i$  criterion, fall into different main clusters or into different subgroups of one main cluster. The cluster analysis divides the genotypes had high rating of  $YS_i$  and differentiates them into smaller groups: a group of genotypes having a high mean level of trait and high stability based on the three stability measures, very valuable for selection; a group of genotypes unstable on the basis of the three stability measures, but highly rated based on the  $YS_i$  index, due to high genetic effects (high mean level of trait); a group of genotypes, with a low mean level of trait, rated highly on the basis of the  $YS_i$  index, due to high stability based on the three stability parameters (the variances  $s_p^2$ ,  $S_i^2$  and ecovalence  $W^2$ ).

The genotypes of these three groups can be included in a selection program to improve the average level of trait and its stability. Many genotypes showed similarity in phenotypic stability for all studied traits, which can be taken into account, in order to reduce the number of crosses and volume of selection in general.

## Conclusions

The cluster analysis divided the genotypes, distinguishing the groups by phenotypic stability, and made possible to single out the most stable ones of special interest for the selection.

Genotypes highly evaluated through the  $YS_i$  criterion had different selection value and formed groups that differed in the trait mean level and stability. Among the genotypes with high rating of the  $YS_i$  criterion some had low stability.

For all studied traits, groups of genotypes having a high average trait level and high stability based on all stability measures were distinguished, which are very valuable for the cotton breeding programs.

Given the trait mean level and stable performance of genotypes, the varieties Heliuss, Viki, Denitsa, Boyana and Philipopolis possessed complex breeding value in terms of productivity, 791-169, Viki, Avangard-264, Eva and Vega – in terms of boll weight, Viki, Boyana and Nelina – in terms of fiber ginning out turn.

The varieties most valuable for fiber length were Natalia, Dorina, Perla-267 and Colorit, and for the height of first fruiting branch these were Eva, Natalia, Millennium, Perla-267 and Colorit, combining in the best way mean trait level and stability.

Cluster analysis has emerged an effective method for grouping genotypes by phenotypic stability measured through different stability methods and can greatly facilitate cotton breeding programs.

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