AGRICULTURAL SCIENCE AND TECHNOLOGY, VOL. 16, No 2, pp 17-27, 2024
Published by Faculty of Agriculture, Trakia University, Bulgaria
ISSN 1313-8820 (print)
ISSN 1314-412X (online)
http://www.agriscitech.eu
DOI: 10.15547/ast.2024.02.013

Genetics and Breeding

Grouping of Bulgarian and foreign cotton varieties by cluster analysis

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(Manuscript received 2 June 2023; accepted for publication 25 March 2024)

Abstract. *During the period 2016-2019, 21 Bulgarian and 10 foreign cotton varieties were included in a trial set by the block method design in four replicates and a harvest plot of 20 m^2. A cluster analysis was applied to the most important economic traits. It was found that based on the clustering by individual years and on average for four years, the varieties were divided into two or three main clusters, mainly on the traits of seed cotton yield, fiber length and lint percentage. The year conditions have influenced the phenotypic manifestation of the studied traits and have had a significant impact on the genotypes clustering by the individual years. The clustering of the varieties based on the average data for four years showed different degrees of genetic similarity and distance between them and with the standard variety - Chirpan-539. The varieties Helius, Trakia, Philipopolis and Plovdiv, which were highly productive, were genetically very similar and it is appropriate to include them in one selection program together with the varieties Natalia, Dorina, Vega, Perla-267, Kolorit, (Bulgarian selection), Stoneville 112, Deltapine 30 (American) and 791-169 (Greek), which were genetically more distant from them and have shown other valuable traits.*

Keywords: cotton, *G. hirsutum* L., genetic diversity, economic traits

Introduction

Cotton production in Bulgaria is mainly based on Bulgarian cotton varieties belonging to the species *G. hirsutum* L. Development of early-maturing varieties possessing high genetic potential for yield and varieties with improved fiber quality are the priorities in the selection programs. Basic selection methods are classical intra- and inter-specific hybridization and experimental mutagenesis.

Many new high yielding varieties and varieties with improved fiber quality have been created. Especially valuable for the cotton production and selection programs are the varieties Trakia, Helius, Denitsa, Philipopolis, Sirius and the newest Tsvetelina and Perun, combining earliness and high productivity, and a number of other valuable qualities (Valkova, 2009; 2014a; 2014b; Valkova and Bozhinov, 2010; Koleva and Valkova, 2019), Kolorit, Darmi, Natalia, Dorina, Rumi and IPK Nelina, achievements in the selection of fiber quality (Stoilova and Saldzhiev, 2008a; 2008b; 2010; Stoilova and Nistor, 2012; Stoilova and Meluca, 2013).

Many researchers have studied genetic and environmental variation using methods that allowed grouping of genotypes and environments by similarity, such as cluster analysis, PCA (Principal Component Analysis), AMMI (Additive Main Effects

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and Multiplicative Interaction) analysis, and others. In cotton, these analyses have also been used (Ashokkumar and Ravikesavan, 2011; Cao Wenmei et al., 2011; Daohua et al., 2011; Zhang and Abdelraheem, 2017; Zhang et al., 2017). Some authors assessed genetic diversity using different multivariate methods simultaneously (Kamali et al., 2011; Kamalha et al., 2018; Rathinavel, 2018; Sarwar et al., 2021).

Cluster analysis was widely used in cotton to assess the genetic distance, based on various traits, between a certain set of genotypes, respectively the genetic diversity (RathinaveL, 2018; Jarwar et al., 2019; Sarwar et al., 2021).

In our country this method has been used to study the genetic similarity and genetic distance in promising cotton lines and varieties obtained from the application of different selection methods (Stoilova and Dechev, 2003a; 2003b; Dimitrova et al., 2004a; 2004b; Stoilova et al., 2005; Stoilova and Bozhinov, 2006; Stoilova and Valkova, 2010; Valkova and Dechev, 2012).

The aim of this study was to group Bulgarian and foreign cotton varieties by applying cluster analysis based on the most valuable economic traits, with a view to their more effective use in breeding programs.

Material and methods

The study included 21 Bulgarian and 10 foreign cotton varieties: Bulgarian - Chirpan-539, Beli Iskar, IPTP Veno, Boyana, Viki, Plovdiv, Kris (created by intraspecific hybridization), Trakia, Helius, Philipopolis, Sirius (by experimental mutagenesis), Avangard-264, Perla-267, Vega, Kolorit, Rumi, Darmi, Nelina, Natalia and Dorina (by interspecific *G. hirsutum* L. × *G. barbadense* L. hybridization); foreign - Deltapine 30, Stoneville 112 - American, Mellennium, Eva, 791-169 - Greek, C-9070 - Uzbek, Nazili-84 - Turkish, Tabladila-16 - Spanish, T-08 - Romanian and Siokra-1-4 - Australian.

All studied varieties were included in a trial, carried out during the period 2016 - 2019, in the experimental field of the Field Crops Institute in the town of Chirpan, on soil type Pellic Vertisols, by the block method design, in four replicates and a plot of 20 m^2. The traditional technology for cotton growing in our country under non-irrigated conditions has been applied. The following indicators have been taken into account: height of the first fruiting branch setting (cm) - an important indicator for cotton mechanized harvesting; seed cotton yield (kg/ha); boll weight; fiber length by the “butterfly” method (mm) and lint percentage (%). A total of 10 plants were observed from each replication. Mathematical and statistical processing was performed on the data from all recorded indicators (Lidanski, 1988). The ANOVA 123 program was used.

Hierarchical cluster analysis was applied and the Euclidean distance between the different groups of genotypes was used as a measure of difference between them. For clustering the varieties, the Ward’s method (1963) was used, which minimizes the variation within the groups. Data standardization has been performed in advance.

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

P – Security coefficient determined on the basis of the years in descending order, respectively by the temperature sum for May-September and the rainfall sum for May-August. $P\%=n/(m+1)\times100$, where n was the order number of the year of testing; m – the total number of years included in the descending order of years - climatic norm.

The period 1989-2018 (last 30 years) was considered as the climatic norm.

Results and discussion

The data for the seed cotton yield by years and on average for four years (2016-2019) are presented in Table 1. Variation of yields was observed both by varieties and by years. In the individual years, the yields varied from 830 kg/ha (for the Kolorit variety in 2016) to 1953 kg/ha (for the Trakia variety in 2018). The highest average seed cotton yields were realized in 2017 - from 1394 kg/ha for Natalia variety to 1942 kg/ha for Viki variety. The yields were the lowest in 2016, but for some varieties, they were higher than those obtained in 2017 or 2018, or in both years.

On average for the four years, the yields obtained from the Bulgarian varieties varied from 1123 kg/ha to 1695 kg/ha, and from the foreign ones - from 1290 kg/ha to 1562 kg/ha. The Helius, Trakia and Viki varieties had significantly higher seed cotton yields - 1648–1695 kg/ha than the standard variety Chirpan-539 and exceeded it by an average of 20.7 - 24.2%. The lowest yields were obtained from the Natalia and Kolorit varieties - 82.3% and 83.6% of the standard.

Table 1. Productivity of the varieties (seed cotton yield) in 2016-2019 and average for 4 years

Varieties	Seed cotton yields kg/ha				Average kg/ha	In % to Chirpan-539
Years	2016	2017	2018	2019		
Helius	1396*	1877***	1526	1526	1652	121.0*
Trakia	1360*	1781**	1688**	1953*	1695	124.2*
Boyana	1162	1770**	1535	1498	1491	109.2
Viki	1305	1942***	1562	1782	1648	120.7*
Philipopolis	1263	1523	1389	1563	1435	105.1
Plovdiv	1150	1735*	1683**	1406	1493	109.4
Denitsa	1218	1894***	1726**	1647	1621	118.7
Kris	1433**	1568	1146	1502	1412	103.4
Sirius	1046	1734*	1272	1373	1356	99.3
Avangard-264	1013	1659	1224	1597	1373	100.5
Darmi	1564***	1488	1043	1313	1352	99.0
Rumi	1179	1698	954^{0}	1250	1270	93.0
Nelina	1009	1581	1346	1351	1322	96.8
Natalia	839	1394	1182	1077^{00}	1123	82.3
Dorina	1111	1565	1287	1167^{0}	1283	94.0
Vega	1520**	1462	1264	1240	1372	100.5
Perla-267	1431**	1508	1057	1079^{000}	1269	93.0
Kolorit	830	1610	1019	1104^{0}	1141	83.6
Beli Iskar	1445**	1658	1222	1504	1457	106.7
Veno	1539***	1595	1266	1482	1471	107.7
Stoneville 112	980	1476	1551	923^{000}	1233	90.3
Deltapine 30	1355*	1405	1338	847^{000}	1236	90.5
Millennium	1339*	1573	1201	1228	1335	97.8
791-169	867	1595	1621	1213	1324	97.0
Eva	1566***	1449	1508	1725	1562	114.4
T-08	1159	1489	1560	1606	1453	106.4
C-9070	1106	1633	1697**	1265	1425	104.4
Nazili-84	1326*	1578	1397	1128^{0}	1357	99.4
Tabladila-16	1605***	1552	1555	1367	1520	111.3
Siokra-1-4	991	1510	1557	1101^{0}	1290	94.5
Chirpan-539 St	1055	1513	1324	1568	1365	100.0
GD 5.0%	276	179	317	363	266	19.5
GD 1.0%	367	239	422	483	353	25.9
GD 0.1%	477	311	548	628	456	33.4

P>0.05 – non-significant; P<0.05 - *; P<0.01 - **; P<0.001 - ***

Table 2 presents the average data for four years for the boll weight as an important structural element of productivity, fiber length and lint percentage, and the height of 1st fruiting branch setting, an important indicator for the mechanized harvesting of cotton. The boll weight varied from 5.0 g to 5.7 g for the American variety Stoneville 112. Compared to the standard variety, some varieties had insignificantly larger boll weight, others had insignificantly smaller one. Bulgarian varieties had boll weight of 5.0 g to 5.5 g, foreign ones - from 5.3 g to 5.7 g. The American Stoneville 112 and the Turkish Nazili-84 varieties had the largest bolls - 5.6-5.7 g and in this respect they were superior to the Bulgarian varieties.

Table 2. Agronomic traits and fiber technological properties for the period 2016-2019 (4 years' average)

Variety	Boll weight g	Height of 1st fruiting branch cm	Lint percentage %	Fiber length mm
Helius	5.3	18.2**	35.8	25.9
Trakia	5.2	17.8*	36.3	25.7
Boyana	5.3	17.5	35.7	26.2
Viki	5.5	17.6	36.1	26.2
Philipopolis	5.2	18.0**	35.9	25.9
Plovdiv	5.3	17.9*	35.5^{0}	25.8
Denitsa	5.2	17.7	35.7	26.0
Kris	5.0	18.3***	35.1^{00}	26.0
Sirius	5.2	17.7	35.8	25.8
Avangard-264	5.4	18.0**	35.5^{0}	26.1
Darmi	5.1	18.2**	35.2^{00}	26.3
Rumi	5.2	18.4***	34.9^{000}	26.5*
Nelina	5.0	17.7	35.6	26.1
Natalia	5.4	18.2**	35.0^{000}	26.8***
Dorina	5.1	18.1**	35.1^{00}	26.6**
Vega	5.4	17.7	34.4^{000}	26.5*
Perla-267	5.3	18.1**	33.8^{000}	26.5*
Kolorit	5.4	18.1**	36.1	26.6**
Beli Iskar	5.3	17.6	35.2^{00}	26.3
Veno	5.3	17.9*	35.1^{00}	26.2
Stoneville 112	5.7*	17.5	34.7^{000}	26.7**
Deltapine 30	5.5	18.0**	34.6^{000}	26.5*
Millennium	5.2	18.2**	36.9	26.4
791-169	5.5	17.8*	35.3^{00}	26.4
Eva	5.4	18.3***	34.4^{000}	26.7**
T-08	5.3	17.7	35.7	26.2
C-9070	5.4	18.0**	35.5^{0}	26.6**
Nazili-84	5.6	17.9*	35.5^{0}	26.4
Tabladila-16	5.3	18.0**	35.0^{000}	26.2
Siokra 1-4	5.3	17.7	34.4^{000}	26.3
Chirpan-539 St	5.3	17.1	36.3	26.0
GD 5.0 %	0.4	0.7	0.8	0.5
GD 1.0 %	0.5	0.9	1.0	0.6
GD 0.1 %	0.7	1.2	1.3	0.8

P>0.05 – non-significant; P<0.05 - *; P<0.01 - **; P<0.001 - ***

The lint percentage varied by varieties from 33.8% for the Perla-267 variety to 36.9% for the Millennium variety. Bulgarian varieties had lint percentage of 33.8 - 36.3%, foreign ones - 34.4 - 36.9%.

The fiber length by varieties varied within narrow limits, from 25.7 mm to 26.8 mm for the Bulgarian varieties and from 26.2 mm to 26.7 mm for the foreign ones. The standard variety Chirpan-539 had fiber length of 26.0 mm. Of the Bulgarian varieties, Rumi, Natalia, Dorina, Vega, Perla-267 and Kolorit had 0.4-0.8 mm longer fiber than that of the standard variety. Of the foreign varieties, longer fiber by 0.5-0.7 mm than that of the standard variety was found for Stoneville 112, Deltapine 30, Eva and C-9070.

The height of setting of the first fruiting branch by varieties varied from 17.1 cm to 18.4 cm for Bulgarian varieties and from 17.5 cm to 18.3 cm for foreign ones. The lowest setting of the 1st fruiting branch was reported for the standard variety and all others significantly and insignificantly surpassed it. The highest setting of the 1st fruiting branch was found for the Rumi variety of the Bulgarian varieties and for the Eva variety of the foreign ones.

Based on the analyzed traits, clustering of the genotypes was performed and the dendrograms obtained from the hierarchical cluster analysis by the individual years are presented in Figures 1-4. The dendrograms show that during the individual years of the study the genotypes were clustered into two (2016, 2017 and 2019) or three (2018) main clusters.

The location of genotypes shows that in the different years, many of them moved from one main cluster to another. The Helius variety retained its cluster during the four years of study. Eighteen varieties changed their main cluster in just one year, seven varieties were in the same main cluster for two years, in the other two years they were in another main cluster, and five varieties were in one main cluster for two years, and in the other two years they were in two different main clusters.

From the dendrogram for 2016 (Figure 1), it can be seen that most of the varieties belonged to the second main cluster, divided into two large groups (two subclusters). The varieties Tabladila-16, Darmi, Eva and Veno belonged to the same group, but to different subgroups, which reveals the presence of some genetic differences between them at a lower level of division. These four varieties in 2016 have achieved the highest seed cotton yields of 1539-1605 kg/ha and significantly exceeded the standard variety Chirpan-539 by 45.9-52.1%.

The Spanish Tabladila-16 and the Bulgarian Darmi varieties were very similar, which in addition to the seed cotton yield, both were equal in boll weight, which had very low values - 4.5 g, fiber length and lint percentage, and height of setting of the 1st fruiting branch. The other two varieties Eva (Greek) and Veno (Bulgarian) had larger bolls. Eva variety had longer fiber but lower lint percentage, and Veno variety had lower setting of the 1st fruiting branch.

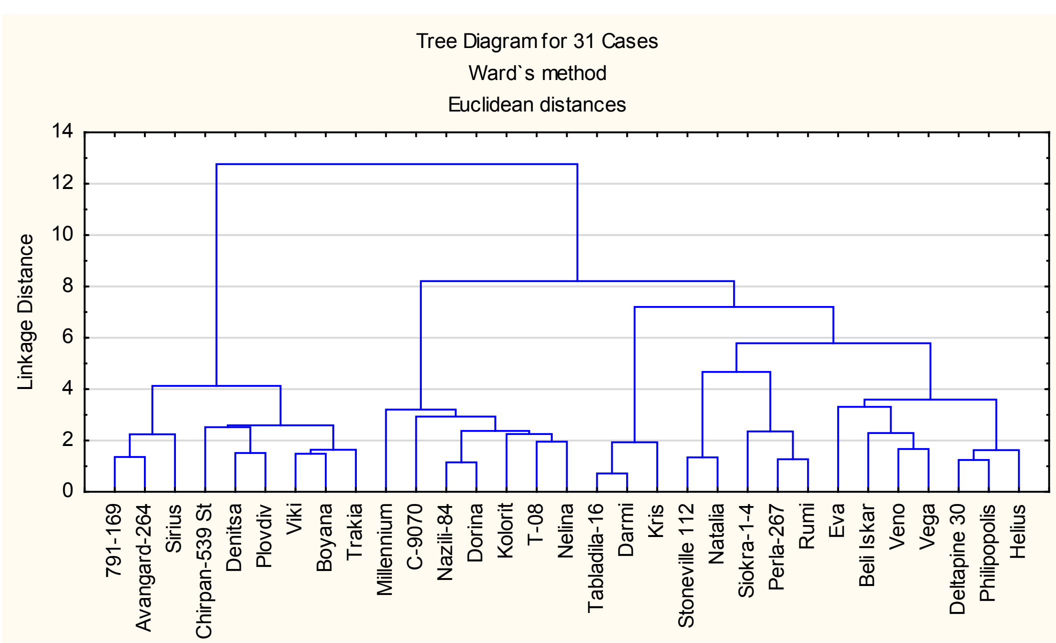

Figure 1. Dendrogram of 31 cotton varieties based on 4 traits for 2016

The varieties Kris, Perla-267, Beli Iskar and Vega belonged to the same group and in seed cotton yields of 1431-1520 kg/ha they also significantly exceeded the standard variety by 35.6-44.1%. Beli Iskar and Vega varieties were in the same subgroup together with Eva and Veno varieties, and Kris variety was in the same subgroup with Darmi and Tabladila-16 varieties, which shows that they were very similar.

Helius and Trakia varieties, with relatively equal seed cotton yields of 1396 kg/ha and 1360 kg/ha, respectively 32.3% and 28.9% above the standard variety, were at a great distance from each other, in different main clusters. The Trakia variety showed much lower setting of the 1st fruiting branch, which distanced it from the Helius variety.

The standard variety Chirpan-539 was in the first main cluster, which distanced it from the highest yielding varieties in the second main cluster. The Denitsa and Plovdiv varieties were very similar to it.

A significant change occurred in the grouping of genotypes in 2017, when 21 varieties out of 31 changed their main cluster (Figure 2). This year, the varieties were also divided into two main clusters, each with two subgroups. The Helius, Denitsa, Viki, Trakia, Plovdiv and Sirius varieties, which realized significantly higher yields than the standard variety, were in the same subgroup of the second main cluster, which shows that they were similar. Denitsa and Helius varieties were very similar, Viki differed from them. These three varieties had the highest seed cotton yields of 1877-1942 kg/ha, which was 24.1-28.3% above the standard variety. Boyana variety which also showed higher seed cotton yield of 1770 kg/ha (17.0% above the standard variety) was located in another major cluster and it was in the same subgroup with the standard variety Chirpan-539. Boyana variety had lower values of lint percentage and height of setting of 1st fruiting branch, which separated it from them.

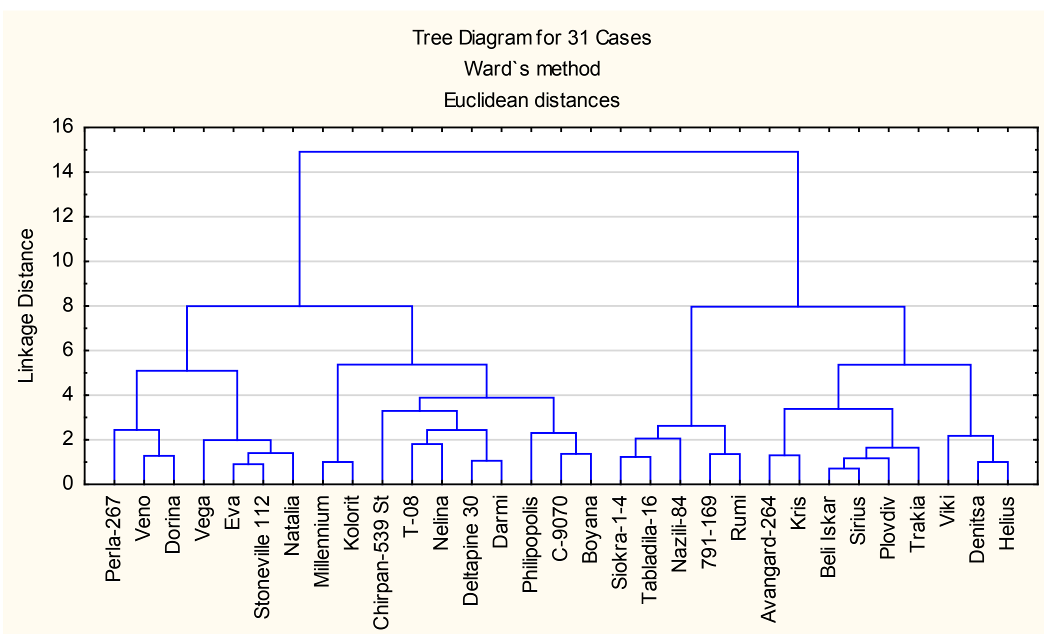

Figure 2. Dendrogram of 31 cotton varieties based on 4 traits for 2017

In 2018, the varieties were divided into three main clusters, 13 retaining their main cluster from 2017 (Figure 3). The Helius, Plovdiv, Trakia and Denitsa varieties kept their main cluster, but were in different subgroups, while Viki and Sirius varieties were in the other two main clusters. In the same cluster, but in different subgroups, were Eva and Veno, which have changed their places again. The C-9070 variety also changed its main cluster and it was in the same subgroup with the Denitsa and Trakia varieties. The standard variety Chirpan-539 and Philipopolis variety also changed their cluster and were in the same subgroup with Plovdiv and Helius varieties. The Trakia, Plovdiv, Denitsa and C-9070 varieties which showed significantly higher seed cotton yields (of 1683-1726 kg/ha) than the standard variety (27.1-30.4% above it) were in the same group, and only Plovdiv variety was in another subgroup. In the three clusters, in all subgroups, very similar genotypes were observed.

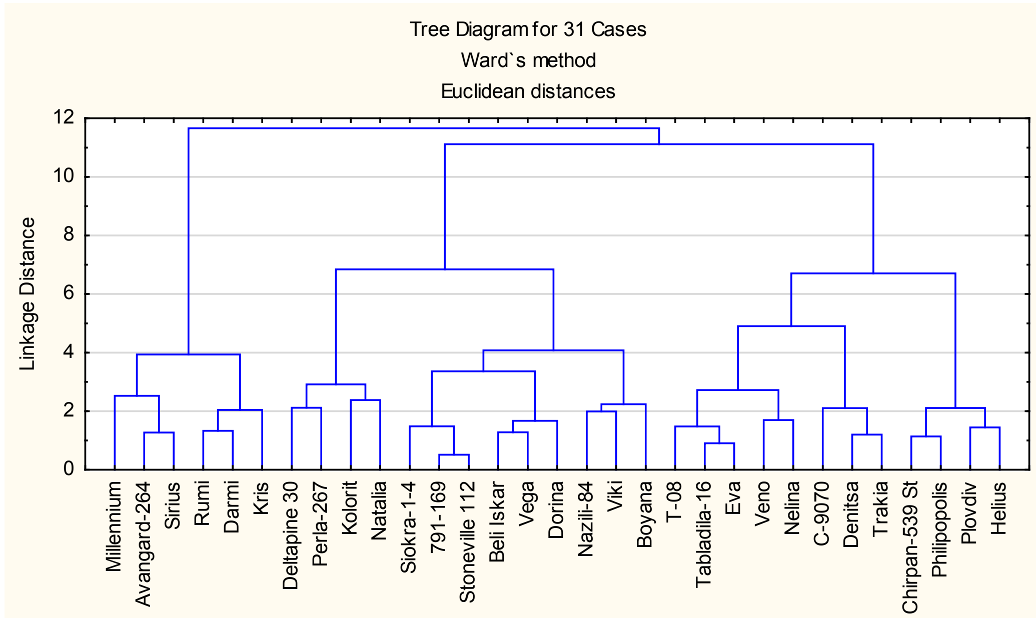

Figure 3. Dendrogram of 31 cotton varieties based on 4 traits for 2018

In 2019, the varieties were grouped into two main clusters, in four subgroups (Figure 4). The Trakia variety, which retained its cluster, had the highest productivity in that year. The Kris, Plovdiv, Boyana, Sirius and Philipopolis varieties were very similar to Trakia variety and they were located in the same subgroup. The Beli Iskar, Viki, Helius, Veno and Chirpan-539 varieties were in the same main cluster, but in another subgroup, they also kept their cluster. The C-9070 and Tabladila-16 varieties changed their main cluster again. There were also many similar varieties in the two main clusters and subgroups.

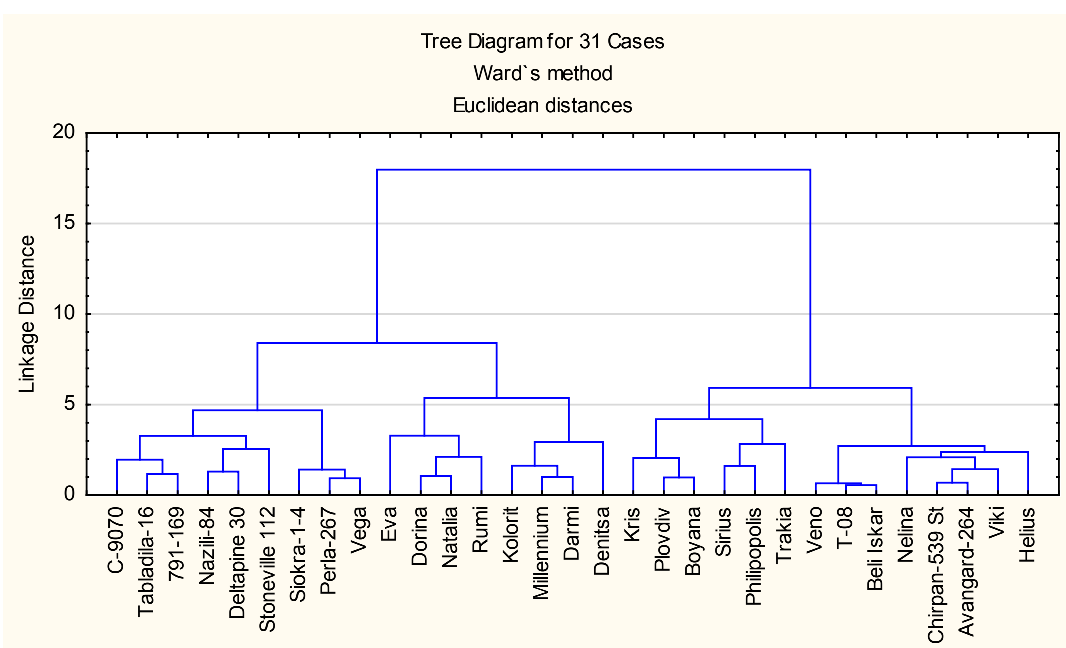

Figure 4. Dendrogram of 31 cotton varieties based on 4 traits for 2019

Based on the averaged results, the varieties were grouped into two main clusters, each one with two subgroups (Figure 5). The first main cluster included varieties with longer fiber, in some cases identical to the standard. The C-9070 and Eva varieties had 0.6-0.7 mm significantly longer fiber than the standard variety and by 4.4-11.3% insignificantly higher seed cotton yield. The two varieties were very similar to each other and were in the same subgroup. The Tabladila-16 variety belonged to the same subgroup, by 11.3% higher yield and 0.2 mm longer fiber. These three varieties had lower lint percentage than the standard variety, but had larger bolls and set higher their fruiting branches. The varieties Stoneville 112 and Natalia, distinguished by significantly the longest fiber, 0.8 mm longer than that of the standard, were in the same main cluster, but in different subgroups. Both varieties had lower productivity and lower lint percentage than the standard variety, but compared to it set higher the 1st fruiting branch, the Stoneville 112 variety had the largest boll weight.

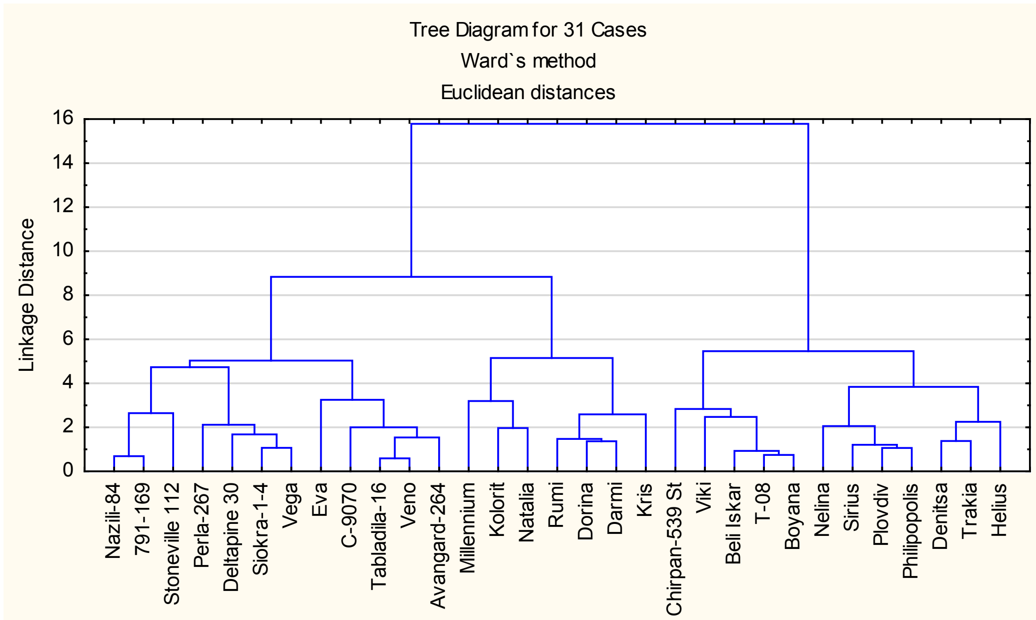

Figure 5. Dendrogram of 31 cotton varieties based on 4 traits on average for the period 2016 – 2019

The varieties Helius, Trakia, Viki, with significantly higher seed cotton yields of 1648-1695 kg/ha (by 20.7-24.2%) than the standard, Boyana, Philipopolis, Plovdiv, Denitsa, Beli Iskar and T-08, with insignificantly higher yields of 1435-1621 kg/ha (by 5.1-18.7%) than it, were located in the second main cluster, but in different subgroups. Denitsa, Trakia and Helius varieties, also Plovdiv and Philipopolis varieties proved to be very similar to each other and were located in the same subgroup. The standard variety Chirpan-539 was in the same cluster, but in the other subgroup. Beli Iskar, T-08 and Boyana varieties were very similar to it. These three varieties surpassed insignificantly by 6.4-9.2% the standard variety in seed cotton yield, they had slightly longer fiber and lower lint percentage in comparison to it, as for the boll weight and the height of first fruiting branch setting they were equal to it.

Clustering based on the averaged data shows that the first main cluster included 19 genotypes (61.3%). Natalia, Dorina, Vega, Perla-267 and Kolorit varieties changed their main cluster in only one year (2016). These varieties were Bulgarian selection for fiber quality. The American Stoneville 112 and Deltapine 30 varieties, and the Greek 791-169 variety also showed stability over the years and changed their cluster in just one year, the two American ones in 2016, the Greek one in 2017.

The second main cluster included 12 varieties (38.7%). The Helius variety showed high stability and retained its cluster throughout the years. The Trakia, Philipopolis and Plovdiv varieties changed their cluster in only one year (2016). These varieties were Bulgarian selection for productivity. Helius and Trakia were obtained by experimental mutagenesis. Philipopolis and Plovdiv were created by intraspecific hybridization. Of the foreign varieties, the most stable of this cluster was T-08, which changed its cluster only in 2017. The standard variety Chirpan-539 was in different main clusters, in one in the first two years, and in another - in the last two years.

Singh et al. (2009) reported that in clustering 68 different genotypes in 10 clusters, there was no relationship between geographical origin and genetic diversity of genotypes. The clustering was random and independent. Rathinavel (2018) also concluded that the clustering of 101 varieties was based on morphological traits and not on their geographical origin. The results obtained in this study confirm that the clustering of the studied varieties was based on their economic traits.

Daohua et al. (2011) using the cluster analysis, found that samples of *G. hirsutum* L., *G. barbadense* L. and *G. arboreum* L. clustered their pedigree. Gopinath et al. (2009) reported 8 clusters in the grouping of 60 different genotypes. The boll weight and number of bolls per plant, and 2.5% staple length were of the greatest importance in their clustering.

The Bulgarian varieties were obtained from two differently purposeful breeding programs - for productivity and for fiber quality, by applying different selection methods, and based on four-year average data, they were referred to different main clusters, depending on the selection direction, selection methods and preliminary selection by traits. Foreign varieties were genetically more distant from high-yielding Bulgarian varieties, these were later in maturing and did not give reliable yields under our conditions. The seed cotton yield, fiber length and lint percentage were of the greatest importance for the clustering of varieties.

The summarized results of the analysis of dendrograms showed that the grouping of varieties by cluster analysis, in the four experimental settings, corresponding to the years of research, was highly dependent on the year conditions, which refer to the so-called uncontrollable factors, and had a substantial influence on the variability of traits. The transition of one genotype from one cluster to another, when the environmental conditions changed, was probably due to the genotype-environment interaction, reported by other authors (Dechev, 1997). The clustering of genotypes according to average data partially included their stability by traits and years, as well as their drought resistance, considering that 2016 was dry, unfavorable for cotton in terms of rainfall, 2017 and 2019 were moderately wet, 2018 - wet.

The duration of the research made it possible to assess the yield parameters and other economic qualities, as well as the stability of the genotypes, sufficiently precisely.

The dendrogram based on cluster analysis provides visual information about the remoteness of genotypes. It is assumed that genotypes phenotypically differing in more traits are genetically more distant. Genetic distance is measured as phenotypic distance (Kabir et al., 2009).

The clustering of genotypes on the basis of average data for over four years revealed different degrees of genetic similarity and genetic distance between them and with the standard variety - Chirpan-539.

It is justified to include genetically distant genotypes in the same crossing scheme in order to create greater genetic diversity.

The inclusion of similar varieties, from one main cluster, but in different subgroups, can lead to faster selection progress.

Conclusion

The conditions of years affected the phenotypic manifestation of the studied traits and had a substantial influence on the clustering of genotypes by individual years. The clustering of the studied varieties based on the average data revealed different degrees of genetic similarity and genetic distance between them and with the standard variety Chirpan-539. The genotypes, in three of the four experimental settings, and on average for four years, were divided into two main clusters, the seed cotton yield, fibre length and lint percentage were of the greatest importance for their clustering. The genetically distant varieties the clustering of which was not highly influenced by the year conditions are of interest for inclusion as parental components in the same crossing scheme in order to create greater genetic diversity. The Helius, Trakia, Philipopolis and Plovdiv varieties, which proved to be high-yielding, were genetically very similar to each other and it is appropriate to include them in one selection program together with the varieties Natalia, Dorina, Vega, Perla-267, Kolorit, (Bulgarian selection), Stoneville 112, Deltapine 30 (American) and 791-169 (Greek), which were genetically more distant from them and have shown other valuable traits as longer fiber, higher lint percentage, bigger bolls. Many varieties were genetically similar to each other for the studied economic traits and their inclusion in crosses between them can lead to faster selection progress.

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