

Identification and characterization of drought tolerant local populations of *Cucurbitaceae*

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Abstract

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For a three-year period (2019-2021), local populations of the *Cucurbitaceae* family were studied in a field experiment to establish drought resistance. The experiment is two-factor, set by non-standard method, with direct sowing, in two replications, with a systematic distribution of variants, with a natural deficit of soil moisture, intensifying with the progress of vegetation and control under irrigated conditions. Evaluation information has been collected according to the current ECPGR and UPOV descriptors. Physiological analyzes of leaf morphometry parameters, water exchange and relative leaf chlorophyll content were performed twice during the fruit growth phase. Two local populations of *Cucurbita pepo* var. *saccharata* with good yield potential in arid conditions and stable response to environmental conditions in terms of growth intensity and water exchange have been identified: BGR6545 and BGR6547. The populations BGR6545, BGR6547, BGR3329, B9E0020 and B9E0043 have the highest values of morphometric indicators for drought tolerance. The highest yields in ambient conditions without irrigation were reported for accessions BGR6545, BGR6547, B9E0057 and B9E0092.

Keywords: *Cucurbitaceae*; drought tolerance; chlorophyll

Introduction

Climate change-associated drought stress in plants is one of the major environmental factors that cause a reduction in plant growth, development, and productivity. Therefore, an improvement to obtain superior genotypes pumpkin that are highly adaptable to arid and semi-arid conditions remains the main objective of the future breeding efforts (Seymen et al., 2019). There is a review which outlines some of the basic growth-analysis techniques for describing different aspects of productivity and attempts to summarize investigations on physiological, morphological, and ecological aspects of productivity and the relationship of these factors to eating quality in the most important domesticated species (Loy, 2004). Water relation directly or indirectly provides the information about the water status of plants under water deficit conditions that may be in the form of relative water content, leaf water

potential, leaf osmotic potential and turgor potential (Akram, 2011). Water is necessary to maintain the optimum growth and physiological activities involved in different processes necessary for plant growth, development and ultimately yield (Hussain et al., 2008). In the antenna complex of the chloroplasts, chlorophylls are the critical pigments that capture the light to be transformed into carbohydrates during photosynthesis. The use of chlorophyll content as a trait may contribute to increase light interception and efficiency of conversion, and therefore to maintain/increase crop yield under stress (Anjum et al., 2011; Kapoor et al., 2020). In another study on the effect of drought stress and various levels of nitrogen on pumpkin, it was found that increasing drought stress could reduce the yield and yield components, leaf area, chlorophyll a, b content, and net photosynthesis as well as stomata conductance but could increase proline content and sub-stomatal CO₂ (Aghai & Ehsanzade, 2011).

C. argyrosperma, *C. ficifolia*, *C. maxima*, *C. moschata* and *C. pepo* are differentiated by the morphological characteristics: habitat of growing; stems; leaves in distal nodes; indument petioles and primary veins in the lower surface of blades; receptacle in staminate flowers; corolla; filaments; peduncle, size, shape, surface and color of fruit; pulp; shape, size and color of seeds. That squashes, pumpkins, zucchinis and gourds are very diverse locally, regionally and worldwide, having a wealth of innumerable strains, landraces and varieties. In cultivated plants, fruits are produced in a wide variety of shapes, sizes, colors and types of surfaces (OECD, 2016). For use in different directions in the selection are interesting features: shrubby habit; red/ orange color of the fruit, increased content of dry matter and carotene – suitability for use in the processing industry; yield; long-term storage – for feed purposes; marketability – taste, appearance, preservation of quality, transportability; suitability for intensive technologies – responsiveness to fertilizers, watering, thickening of crops; physiological properties – precocity, resistance to diseases, pests and adverse climatic conditions, including drought resistance (Burenin et al., 2014; Koleboshina et al., 2017).

In modern conditions, specialized selection is needed and focusing the priorities in creating the final selection products not only on the main useful economic characteristics, but also introducing an adaptive direction in developing models of future varieties (Koleboshina et al., 2017). Therefore, the main tasks in the study of plant genetic resources are the distribution of genetic sources by selectively important traits and methodological developments to identify the biological potential of resources in order to make fuller use of them (Burenin et al., 2014).

The aim of the present study is to identify drought-tolerant local populations of *Cucurbitaceae* and to evaluate the most promising accessions by descriptive characteristics so that they can be used as sources for selection.

Material and Methods

For a three-year period (2019-2021), a total of 43 local populations and varieties of the *Cucurbitaceae* family were studied in a field experiment to establish drought resistance. The origin of the accessions is from 21 different locations in Bulgaria. Their botanical diversity initially includes 4 species, 6 varieties and 7 groups by variety. The experiment is two-factor, set by a non-standard method, with direct sowing, in two replications, with a systematic distribution of variants. The plants are grown in non-irrigated conditions on *Pellic Vertisol*, at pH7, sufficient light and natural deficit of soil moisture, intensifying with the progression

of vegetation. The agro-technological and plant protection measures were carried out according to the standard technology adopted for the region of central southern Bulgaria. The control is placed under irrigated conditions. Soil moisture was monitored with a Rapitest Moisture Meter.

In the average total amount of precipitation during the study period no significant deviations from the climatic norm for the region were found. Differences are observed in the distribution of precipitation during the growing season and in the appearance of spontaneity in their nature. At the beginning of the vegetation the soil moisture is optimal for the development of the crops. As a result of the heavy rains, even physiological deformations were observed in the plants – cracking of the stems and change in the structure of the flowers. Drought begins in July with rising temperatures and reaches critical levels in September, when rainfall is 80% below the multiannual norm (Figure 1). Upon reaching the coefficient of wilting of the crops during the period of fruit growth, irrigation norms are applied by drip irrigation in an amount until the turgor of the leaves is restored and reaches 40% FC (Field capacity).

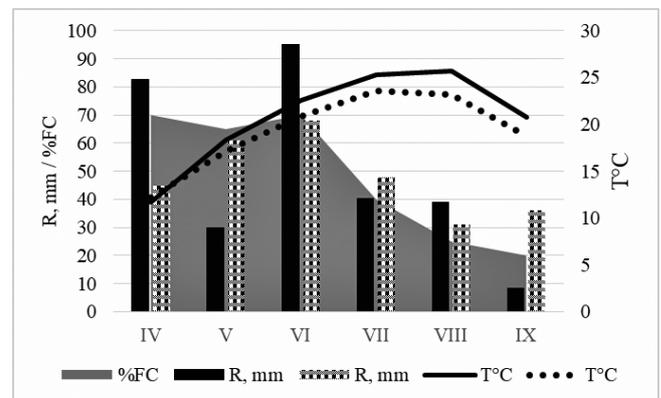


Fig. 1. Agrometeorological conditions

Accessions with irreversible damage and unsatisfactory results were gradually dropped from the sowing scheme. For the others (Table 1) evaluation information was collected according to the current descriptors: Minimum list of descriptors for leafy vegetables, Minimum descriptors for *Cucurbita* spp., Cucumber, melon and watermelon of ECPGR and Useful grouping characteristics of UPOV. A cluster analysis of the accessions according to the evaluation indicators with IBM SPSS Statistics 19 and variation analysis of the yields were performed.

During the phase of fruit growth twice physiological analyzes of leaves were performed as follows:

Table 1. List of research objects in the field experiment (only the harvested accessions are indicated, the first column gives the numbers in the experimental setup used in laboratory and statistical analyzes)

№	BGR	TEMNO	TAXON	ACCENAME / COLLSITE
1	3324	806353	<i>Cucurbita pepo</i> var. <i>saccharata</i> , type <i>other</i>	local population, v. Belomorets, Turgovishte
2	6517	78E6359	<i>Cucurbita pepo</i> var. <i>saccharata</i> , type <i>other</i>	local population, v. Venetz, Razgrad
3	6543	80E7437	<i>Cucurbita pepo</i> var. <i>saccharata</i> , type <i>pumpkin</i>	local population, v. Tzvetkova Bora, Mihailovgrad
4	6545	81E7548	<i>Cucurbita pepo</i> var. <i>saccharata</i> , type <i>other</i>	local population, v. Beslen, Blagoevgrad
5	6547	81E7550	<i>Cucurbita pepo</i> var. <i>saccharata</i> , type <i>pumpkin</i>	local population, v. Skurt, Blagoevgrad
6	3329	806373	<i>Cucurbita maxima</i>	local population, v. Antonovo, Turgovishte
7		78E6383	<i>Cucurbita pepo</i> var. <i>saccharata</i> , type <i>pumpkin</i>	local population, unknown origin
8		B4E0046	<i>Cucurbita pepo</i> convar. <i>giromontia</i> , type <i>zucchini</i>	local population, v. Karadjalovo, Parvomai
9		B4E0049	<i>Cucurbita moschata</i>	local population, v. Karadjalovo, Parvomai
10		B4E0085	<i>Cucurbita pepo</i> convar. <i>giromontia</i> , type <i>zucchini</i>	local population v. Malak chardak, Plovdiv
11		B4E0087	<i>Cucurbita pepo</i> var. <i>saccharata</i> , type <i>olkurbis</i>	local population v. Malak chardak, Plovdiv
12		B4E0102	<i>Cucurbita pepo</i> var. <i>saccharata</i> , type <i>olkurbis</i>	local population v. Malak chardak, Plovdiv
13		A9E1089	<i>Cucurbita maxima</i>	local population, v. Biser, Harmanli
14		B4E0145	<i>Cucurbita moschata</i>	local population, v. Gorski Izvor, Dimitrovgrad
15		B4E0048	<i>Cucurbita moschata</i>	local population, v. Karadjalovo, Parvomai
16		A9BM111	<i>Cucurbita moschata</i>	variety Moschatna
17		A9BM112	<i>Cucurbita maxima</i>	variety Plovdivska 814
18		A9BM113	<i>Cucurbita pepo</i> convar. <i>giromontia</i> , type <i>zucchini</i>	variety Izobilna
19	3326	806360	<i>Cucurbita pepo</i> var. <i>saccharata</i> , type <i>pumpkin</i>	local population, v. Venetz, Razgrad
20		B9E0020	<i>Cucurbita moschata</i>	local population, v. Ognyanovo, Pazardzhik
21		B9E0030	<i>Cucurbita moschata</i>	local population, v. Sinitovo, Pazardzhik
22		B9E0043	<i>Cucurbita moschata</i>	local population, v. Sinitovo, Pazardzhik
23		B9E0054	<i>Cucurbita maxima</i>	local population, v. Inje voivoda, Sozopol
24		B9E0057	<i>Cucurbita maxima</i>	local population, v. Inje voivoda, Sozopol
25		B9E0067	<i>Cucurbita moschata</i>	local population, v. Inje voivoda, Sozopol
26		B9E0092	<i>Cucurbita moschata</i>	local population, v. Bolyartsi, Asenovgrad
27		B9E0093	<i>Cucurbita maxima</i>	local population, d. Proslav, Plovdiv

Leaf morphometry parameters and water exchange – fresh weight (FW), dry weight (DW) and relative water content (RWC) (Beadle, 1993; Turner, 1981). The last parameter was determined in percent by the formula: $RWC (\%) = (FW - DW) / (TW - DM) \times 100$, where FW – initial leaf weight (g); TW – leaf weight in full turgor after 24 hours immersed in water (g); DW – dry weight of leaf after dried in dry chamber for 8 h at 105°C (g).

Relative leaf chlorophyll content, expressed as Chlorophyll content index (CCI) was measured with chlorophyll content meter-CCM 200 plus, manufactured by Opti-Sciences Inc, NH, USA. The physiological assessment was carried out *in vivo* on the field. The measurements were taken on two dates per vegetation period from average samples of leaves. The dates of the measurements were 16/06/2020 and 17/08/2020 for first year and 21/07/2021 and 01.09/2021 for second year. From each genotype measurements of 15 leaves (n = 15) are made.

Results and Discussion

The calculation of the potential yield of the three botanical pumpkin species shows that in most accessions the drought has a negative effect on productivity, with a decrease of up to 90% in some populations (Table 2). Similar results for reducing yields to 80-82% under drought stress have been reported by Moradi (2019) and Seymen et al. (2019). The effect of increasing watering stress in experiments conducted by Zeynali et al. (2018) and Moradi (2019) is a significant reduction in all studied parameters, seed and fruit yield and their components. One of the reasons for this is that the absorption of nutrients is reduced in conditions of water shortage. Hamzei et al. (2015) found significant consequences of irrigation on all traits ($p < 0.01$), with the lowest yields obtained with the consumption of 320 mm of water, comparable to the amount of precipitation in our experience. Restriction of water during the period of plant growth causes

a reduction in the number of fruits per plant and the number of seeds in the fruit by interrupting feeding and shortening the period of plant growth. Another reason is that environ-

Table 2. Variation analysis of average yields (where there were normally developed fruits), t/da

№	M	VC	Sm%	Proof
St.	3.5	2	1	*
1	1.5	5	2	P= -0.1%
2	0.5	14	3	P= -0.1%
3	1.2	6	2	P= -0.1%
4	5.5	1	1	P= 0.1%
5	6	1	1	P= 0.1%
6	1.91	4	2	P= -0.1%
9	0.31	3	2	P= -0.1%
11	1.5	5	2	P= -0.1%
12	1.35	3	2	P= -0.1%
14	0.53	18	4	P= -0.1%
16	1.2	6	2	P= -0.1%
18	2.1	3	2	P= -0.1%
19	1.5	5	2	P= -0.1%
21	0.6	1	1	P= -0.1%
22	3.45	1	1	P= -1%
23	2.62	3	2	P= -0.1%
24	3.75	1	1	P= 0.1%
25	0.37	5	2	P= -0.1%
26	4.75	1	1	P= 0.1%
27	1.07	5	2	P= -0.1%

mental conditions at the time of anthesis are important. The formation of female flowers decreases in the conditions of a long day and high temperatures. And when these conditions are combined with drought, pollen can lose viability rapidly (OECD, 2016). Also, drought stress occurrence during reproductive stages caused reduction in chlorophyll content, reduction in photosynthesis period and material translocation by current photosynthesis and reduction in proportion of supplied remobilization of materials. This reflects significant on plant length, nod and branch number/ main stem, leaf and fruit number/ plant, seed number/ fruit, 100-seed weight, grain yield and chlorophyll index of pumpkin (Hamzei & Babaei (2017).

Table 3 shows the results of physiological evaluations of accessions in the field experience reported in 2020. The meteorological conditions before and during the evaluation of the accessions are typical for this period of the year. The fallen precipitation 20 days before the first reading are 40.0 l/m², and 30 days before the second reading – about 30.0 l/m² at higher average daily temperatures by 3.0°C. From these values we can conclude that there is a drought, especially at the second assessment date, which is confirmed by the measured 30.0% FC of the soil. The accessions B9E0020, B9E0043, 806360, B4E0102 and 806373 have the highest values of morphometric indicators of fresh and dry weight of the leaves.

Most waterlogged leaves, with the exception of 806373, have a lower fresh and dry mass, which is characteristic

Table 3. Average results of leaf morphometry parameters and relative leaf chlorophyll content measured during vegetation

№	2020				2021			
	FW, g	DW, g	RWC, %	CCI	FW, g	DW, g	RWC, %	CCI
1	5.01±0.39	1.00±0.32	79.6±3.8	19.90±0.93	5.23±0.40	1.17±0.19	72.50±5.2	19.20±1.41
2	7.35±1.11	1.39±0.48	82.2±4.2	24.06±1.84	3.98±0.32	0.93±0.12	68.70±3.2	24.55±2.10
3	6.04±1.03	1.14±0.42	85.3±4.9	26.88±2.37	7.91±0.62	1.85±0.24	67.30±4.9	28.05±3.15
4	6.24±1.01	1.28±0.27	83.1±4.3	21.33±0.66	7.43±0.82	1.56±0.21	80.10±3.1	26.55±3.06
5	6.66±0.90	1.48±0.39	77.8±3.9	28.41±2.90	7.29±0.39	1.74±0.22	69.13±4.2	24.20±2.14
6	7.58±2.03	1.46±0.41	83.2±3.3	25.92±1.93	6.90±0.58	1.36±0.18	85.20±3.9	22.30±1.79
9	4.60±0.82	1.19±0.44	69.2±4.8	40.92±2.42	8.04±1.06	1.69±0.37	74.12±4.1	32.25±4.94
11	5.72±1.75	1.24±0.32	82.3±3.1	25.74±1.36	7.58±0.57	1.79±0.21	66.20±5.1	28.30±2.67
12	7.01±1.56	1.57±0.41	76.2±4.2	30.71±1.37	4.06±0.24	0.95±0.10	67.40±3.7	26.45±1.74
14	4.40±0.58	1.06±0.35	74.3±4.7	33.85±2.14	5.41±0.40	1.25±0.24	71.20±4.6	25.60±1.91
16	6.20±0.97	1.36±0.51	73.9±3.5	25.77±1.47	5.99±0.69	1.39±0.12	68.70±3.5	27.60±3.01
18	5.31±0.99	1.05±0.31	77.2±4.1	28.42±1.35	5.15±0.95	1.30±0.35	62.30±5.5	23.40±1.74
19	7.76±1.50	1.70±0.57	79.2±3.5	26.50±1.73	5.91±0.93	1.18±0.18	82.50±4.1	20.95±0.89
20	6.11±1.38	1.54±0.48	73.5±3.8	28.72±1.30	6.84±0.35	1.76±0.18	60.20±4.5	28.00±3.23
22	8.45±2.12	2.52±0.74	71.2±4.1	34.05±2.17	7.17±1.03	1.57±0.15	84.10±4.1	28.90±2.56
24	8.56±2.00	1.85±0.58	71.2±3.8	27.3±2.52	10.71±1.00	2.52±0.57	69.1±3.50	24.5±2.01
26	6.52±1.23	1.51±0.41	73.1±3.4	26.1±2.85	7.3±0.81	1.64±0.21	73.1±3.10	26.3±2.57

of more drought-tolerant species. However, 806373 and 806360 combine high fresh and dry leaf weights with a higher than average RWC for the accessions tested. The reported CCI values vary widely, which is related to the variation of morphological parameters, more pronounced in the fresh leaf mass. The highest CCI values were reported for the accessions with numbers B4E0049, B9E0043, B4E0145, B4E0102 and B9E0020. The accessions B9E0043, B4E0102 and B9E0020 have higher fresh and dry weight values than the average of the sample. The presence of a positive correlation in these indicators is characteristic of this type of research (Chipilski & Uhr, 2021; Ahmad et al., 2022). Interestingly in this case, all the mentioned accessions with low RWC, i.e. in which there is a stronger water deficit, reacted by increasing the concentration of cell juice, but this did not lead to the degradation of chlorophyll pigments and reduce the intensity of growth.

In the second year the meteorological conditions before the evaluation of the accessions are more unfavorable compared to 2020, as three weeks before the first reading fell only 19.9 l/m², while for the same period before the second reading fell about 32.0 l/m². The measured soil moisture for both dates is below 40.0% FC. For accessions B4E0049, 80E7437, B4E0087, 81E7548, 81E7550, B9E0043 and B9E0020 statistically significant highest values of fresh and dry leaf mass were reported, the last two samples having high values of fresh and dry leaf weight in 2020. The highest values of RWC have accessions 81E7548, B9E0043, 806360, 806373, B4E0049. In 2021 the average hydration of the leaves of the studied accessions is lower than the values for the previous year. To some extent, the rule that lower hydrated leaves have lower morphometric parameters is reaffirmed. For the majority of accessions, the chlorophyll content index ranges from 23.0 to 28.0 units, with only sample B4E0049 reporting a result of 32.2 units. After that, B9E0043, B4E0087, B9E0020 and 80E7437 are arranged as a result. As in the previous year, the positive dependence between dry weight and chlorophyll content index was confirmed for the leading samples on these indicators. However, in contrast to last year, there was a relatively similar response to a decrease in the CCI in the accessions. Similar results have been reported in drought samples of squash (*Cucurbita pepo* L.) (Selda et al., 2016; Abd El-Mageed et al., 2016). This result in combination with the lower RWC indicates stronger drought stress and stronger growth inhibition of all accessions.

Of the populations studied in the field experiment, four accessions emerged as promising with good yield potential, with a statistically significant positive difference in ambient conditions compared to the average standard of conventional production under irrigated conditions (Table 2). Two of them



Fig. 2. Accession №4 (BGR6545 / 81E7548) *Cucurbita pepo* var. *saccharata*, local population from v. Beslen, Blagoevgrad municipality



Fig. 3. Accession №5 (BGR6547 / 81E7550) *Cucurbita pepo* var. *saccharata*, type pumpkin, local population from v. Skurt, Blagoevgrad municipality

were collected during expeditions within the current project – №B9E0057 and №B9E0092, and the other two are part of the basic national collection of long-term storage in the genebank in IPGR – Sadovo – BGR6545 and BGR6547. From the analysis of the results obtained in the two-year laboratory study we found the most stable reaction of the accessions with catalog numbers B9E0020 and B9E0043, and with BGR3329, BGR6545 and BGR6547 to environmental conditions in terms of growth intensity and water exchange. Therefore, as a result of the overall study, the following local populations with the best results in terms of both botanical and economic drought resistance were identified: BGR6545 (Figure 2) and BGR6547 (Figure 3). Both accessions belong to the botanical species *Cucurbita pepo* var. *saccharata*, to different types according to the morphological features of the fruit. Both accessions represent local populations collected in 1981 from different villages in Southwestern Bulgaria (Table 1). They reported higher values of fresh mass, dry leaf mass, RWC, CCI and at the same time higher yields in field conditions.

In the cluster analysis performed on the basis of the morphological features of the vegetative and reproductive organs of plants (by descriptor), both identified accessions are in one cluster with the widespread in the country variety Izobilna (*Cucurbita pepo* convar. *giromontia*, type Zucchini), appearing in The National Variety List of Bulgaria, which is included for comparison in the experimental setup (Figure 4).

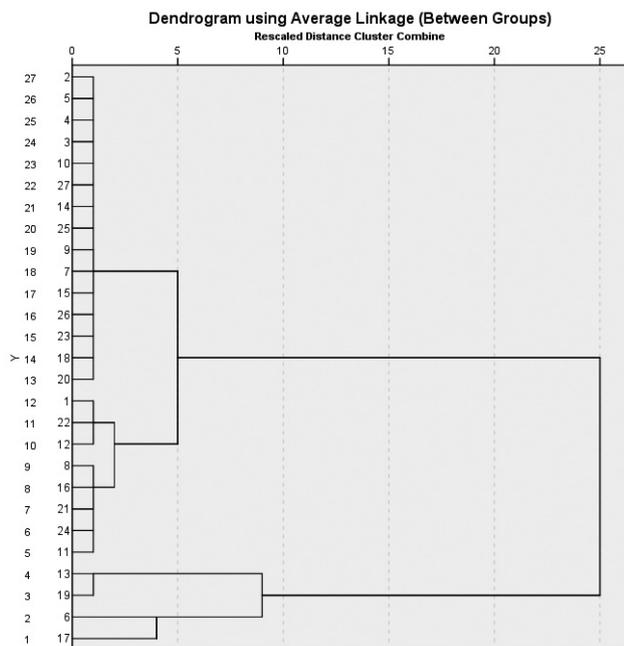


Fig. 4. Clustering of accessions based on morphological features (according to ECPGR and UPOV)

The main difference is in the creeping habit and the shape of the fruit. In the analysis based on economic characteristics, including dry matter, sugar content, number of days from germination to flowering, node number with first set and fruit yield, the two accessions were grouped in a separate cluster, mainly due to accelerated flowering and high yields (Figure 5).

The shortened period until the beginning of flowering does not affect the early maturity and the period of fruiting. Both accessions are middle-maturing. BGR6547 is characterized by compact ripening of the fruit, and BGR6545 – with the longest period of fruiting, as at the time of harvest in mid-October 50% of the fruit is not yet ripe. In the whole sample of genotypes, neither positive nor negative correlation was found between the morphometric parameters and the size of the yields, i.e. laboratory analyzes are a guide to drought tolerance, but are not indicative of the yield of test specimens. It can be concluded that thanks to the current project, in addition to identifying drought tolerant local populations, the national collection of *Cucurbitaceae*, located in the Bulgarian Gene Bank, added valuable specimens during the current expeditions, showed good economic drought resistance – high yields in arid conditions (B9E0057, B9E0092) and correspondingly good botanical drought resistance – high values of the indicators characteristic of drought-resistant genotypes (B9E0020, B9E0043).

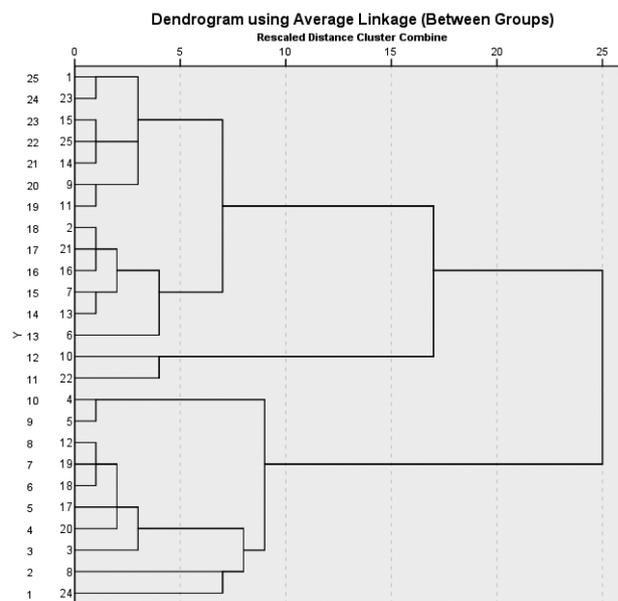


Fig. 5. Clustering of accessions on the basis of economic characteristics (dry matter, sugar content, onset of flowering, knot with first fruit and yield)

Conclusions

The Bulgarian National Gene Bank stores accessions of local populations suitable for use as a genetic basis for creating drought-tolerant varieties of the genus *Cucurbita*: BGR3329 (*C. maxima*), BGR6545 and BGR6547 (*C. pepo*).

New accessions with good botanical drought resistance – B9E0020 and B9E0043 (*C. moschata*) and economic drought resistance – B9E0057 (*C. maxima*) and B9E0092 (*C. moschata*) have been added to the collection of *Cucurbitaceae* through the present project.

Two local populations of *Cucurbita pepo* var. *saccharata* have been identified with good yield potential in arid conditions and stable response to environmental conditions in terms of growth intensity and water exchange: BGR6545 and BGR6547.

The values of the morphometric indicators can be used in the selection as a guide for the botanical drought resistance of plants and do not correlate with fruit yields.

The populations BGR6545, BGR6547, BGR3329, B9E0020 and B9E0043 have the highest values of morphometric indicators for drought tolerance.

The highest yields in ambient conditions without irrigation were reported for accessions BGR6545, BGR6547, B9E0057 and B9E0092.

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