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Research Article

Divergence analysis of *Cucurbita pepo* L. population for seed oil production

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Abstract

The work aim was to analyze the divergence of the genotypes of common pumpkin (*Cucurbita pepo* L.) and to select suitable parents for the breeding program to increase the production of cold-pressed oil. For the analysis of the yield components of the genotypes, the fruits of the common pumpkin were collected in autumn and winter 2021-2022 in central and Western Serbia. There were 19 genotypes (G1-G19) collected randomly in a larger geographical area. The following analyses were carried out: fruit weight, seed weight per fruit, seed number per fruit, thousand seeds weight, percentage of seeds in the total weight of the fruit, percentage of kernel in the total weight of the seed and seed oil content. The results revealed highly significant differences between the common pumpkin genotypes for analyzed traits at a significance of $P \leq 0.05$, indicating the presence of sufficient, considerable genetic variability between the different genotypes. The most significant seed yield components in the pumpkin fruit itself were in significant positive correlations with the oil content in the seeds. The genotypes were divided into 5 clusters. The highest seed weight per fruit and seed oil content were represented in G16, G9 and G12. The results indicate that crossing parents G13, G7, G14, G6, G8, G4, G3, G11 on one side and G16, G9, G12 on the other side is expected to result in high heterosis and a high probability of new combinations to obtain higher yielding cultivars of common pumpkin with higher oil content.

Keywords: cold pressed oil; common pumpkin; divergence analysis; genotypes; seed yield components

Introduction

The Balkan region is characterized by small farms with a mixture of arable farming, livestock breeding and fruit growing production. Most farms have gardens where you can find different genotypes of autochthonous species and varieties of different crops. They have their value, which is not yet recognized on the market.

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Many farms grow common pumpkin (*Cucurbita pepo* L.) which is used for human consumption and for feeding pigs. A secondary product of this production is pumpkin seeds, which are thrown away, due to their poor digestibility in the animals' digestive tract and remain unused. Pumpkins are mainly grown in semi-intensive or extensive systems without the use of pesticides and with no or very low levels of mineral fertilizers, which gives their oil additional value. Pumpkin seeds contain 21-40% protein and are high in potassium, sodium, iron, calcium, zinc, phosphorus, copper and manganese (Amin *et al.*, 2019; Rezing *et al*., 2019). It contains 19 amino acids (Amin *et al*., 2019; Rezing *et al*., 2013). It is a good source of fatty acids (Meru *et al*., 2018, Montesano *et al*., 2018, Geranpour *et al*., 2019), phenolic compounds (Andjelkovic *et al*., 2010; Rezing *et al*., 2019), tocopherols (Naziri *et al*., 2016) and phytosterols (Rezing *et al*., 2019, Montesano *et al*., 2018).

Pumpkin seeds are an important food that positively affects human health (Dotto and Chacha, 2020). They are baked due to their high protein content or used as a food additive in various products such as bread, salami, sausage and mayonnaise (Villamil *et al*., 2023). Pumpkin seeds contain up to 50% oil (Ayyildiz *et al*., 2019). By cold pressing pumpkin seeds, you can obtain very high-quality salad oil, which has a high market price (Dhatt *et al*., 2020). The importance of pumpkin seeds is also recognised by pharmaceutical companies as they contain high levels of antioxidants (Hosen *et al*., 2021). Pumpkin seeds have a pronounced anthelmintic effect (Chari *et al*., 2018; Li *et al*., 2020), antidepressant effect (LaChance *et al*., 2018; Kosari-Nasab *et al*., 2019), antidiabetic and hypoglycemic effect (Marbun *et al*., 2018; Chinsembu, 2019), antihyperplastic effect on the prostate (Leibbrand *et al*., 2019), cytoprotective effect (Konturek *et al*., 2017) and numerous other positive effects on human health (Heim *et al*., 2018; Morakul *et al*., 2019).

In the past, not much work was done on hybridising the common pumpkin, as it was considered a less valuable food for the poorer sections of the population. Today, however, in the age of increasing demand for healthy and organically produced food, the pumpkin is becoming more and more important. Modern research is focused on hybridization, and efforts are aimed at increasing the yield and quality of the fruit, resistance to diseases and pests, the possibility of growing in certain habitats, and extending the shelf life of the fruit (Hosen *et al*., 2021). Identifying the diversity of pumpkin genotypes is necessary for the conservation, appropriate utilization, and development of current cultivars (Shafin *et al*., 2020; Rai *et al*., 2023). Genetic diversity of natural pumpkin populations enables breeders to create new, higher quality varieties with desired traits (Govindaraj *et al*., 2015, Sajid *et al*., 2022).

The present study was conducted to identify the genetic divergence and the best performing genotypes of common pumpkin based on quantitative traits and subsequently select suitable parents for the pumpkin breeding program for the production of cold pressed oil from seeds.

Materials and Methods

Biological material

For the analysis of the yield components of the genotypes, the fruits of the common pumpkin were collected in autumn and winter 2021-2022 in central and Western Serbia. A total of 19 genotypes (G1-G19) were collected randomly from the 19 of village locations (Table 1).

Based on interviews with producers, samples of pumpkin fruits grown without the use of pesticides and mineral fertilizers were selected. Five fruits of each genotype were randomly sampled.

Table 1. Locations from where pumpkin genotypes were collected

Genotypes	Locations	Coordinates	Altitude
G1	Dugojnica	42°40′34″ N, 22°07′19″ E	465 m
G2	Mikulja	44°22′08″ N, 20°58′57″ E	176 m
G3	Luka	43°34′12″ N, 19°31′22″ E	467 m
G4	Kosovica	43°30′47″ N, 20°10′38″ E	855 m
G5	Opaljenik	43°31′12″ N, 20°09′00″ E	903 m
G6	Maleševo	43°48′33″ N, 21°03′22″ E	377 m
G7	Bečanj	43°52′55″ N, 20°34′28″ E	296 m
G8	Stanjevo	43°28′00″ N, 21°02′36″ E	479 m
G9	Radaljevo	43°38′31″ N, 20°10′34″ E	657 m
G10	Stapari	43°52′15″ N, 19°43′57″ E	884 m
G11	Balosave	43°52′21″ N, 20°47′12″ E	275 m
G12	Požegrmac	43°32′19″ N, 19°28′32″ E	957 m
G13	Donji Dubac	43°40′06″ N, 20°20′20″ E	806 m
G14	Hrtkovci	44°52′32″ N, 19°45′59″ E	76 m
G15	Lužnice	44°06′17″ N, 20°49′03″ E	280 m
G16	Pretoke	43°56′17″ N, 20°37′51″ E	331 m
G17	Kasidoli	43°33′02″ N, 19°27′11″ E	567 m
G18	Kumanica	43°28′14″ N, 20°13′40″ E	819 m
G19	Veliki Kupci	43°27′55″ N, 21°14′39″ E	237 m

Experimental procedures

The collected fruits were stored in the Plant Production Laboratory at the Faculty of Agronomy in Čačak. The analyses were carried out on each fruit individually. The weight of the fruit was measured, then the fruit was cut open and the seeds were separated. The seeds were dried at room temperature. The mass of the air-dried seeds was then measured. The number of seeds per fruit was determined by counting. Based on the data obtained, the weight of the thousand seeds and the percentage of seeds in the total weight of the fruit were calculated for each genotype. Fifty seeds were taken from each fruit, weighed and shelled by separating the seed coat from the kernel. The mass of the kernel was measured, after which the percentage of kernel in the total weight of the seed was calculated.

The remaining seeds of each genotype were measured and used to analyses the oil content. Crude oil content (%) was determined by extraction with petroleum ether from ground whole pumpkin seeds. The extractions were carried out in a solvent extractor (Velp. Scientifica-SER148, Italy) under the following conditions: Temperature 110 °C, immersion 30 min, washing 60 min and recovery 30 min. The residual solvent was removed in a drying oven at 105 °C.

The average annual temperature for the sampled sites is 11-13 °C, and the annual precipitation sum is 500-700 mm (MYS, 2022). The weather conditions during the growing season in 2021 were extremely unfavorable, with a cold spring and a dry summer, which did not favor the growth and development of the common pumpkin.

Statistical procedures

The results obtained were subjected to an analysis of variance (genotypes as a random effect) with a significance level of $P \leq 0.05$. The differences between the mean values were tested using the LSD test. The interdependence of the analyzed quantitative characteristics was assessed by calculating the simple correlation coefficient based on 57 measurements. Mahalanobi's D2 statistical analysis was used to assess genetic diversity. Statistical analyses were performed using the Statistica 10 software (StatSoft, 2010).

Results and Discussion

The average fruit weight of all genotypes of common pumpkin was 4.22 kg and ranged from 1.33 to 12.52 kg. The highest fruit weight was recorded in G12 (Figure 1). G17 had significantly higher fruit weight compared to all other genotypes except G12. G16 had significantly higher fruit weight compared to all other genotypes except G12 and G17. G5, G7, G9, G10 and G19 had significantly higher fruit weight compared to G1, G2, G3, G4, G8, G11, G14, G15 and G18. G1, G2, and G3 had the lowest fruit weight. Sedláčková and Avagyan (2022) state that the fruit weight of *Cucurbita pepo* L. genotypes in Slovakia varieties in an interval of 0.52-12.74 kg. The average fruit weight of *Cucurbita pepo* L. genotypes in Kenya was 3.50 kg (Jepkemboi *et al*., 2023), 2.21 kg for genotypes in Italy (Darrudi *et al*., 2018), and 4.44 kg for genotypes in Bulgaria (Haytova *et al*., 2020).

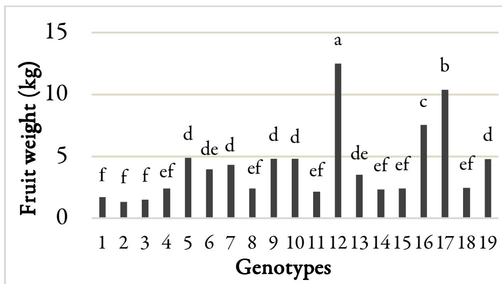

Figure 1. Fruit weight of pumpkin

Values characterised by different lower-case letters differ significantly (P≤0.05) according to the LSD test

The average dry seed weight per pumpkin fruit for all genotypes in this trial was 63.7 g and ranged from 16.7 to 111.0 g (Figure 2). G12 had the highest seed weight per fruit with more than 100 g. G9 and G16 had a seed weight per fruit of more than 90 g, which was significantly higher than G1, G2, G3, G4, and G15, which had the lowest values. G2, G3, and G4 had a seed weight per fruit of less than 50 g and G15 less than 20 g. According to Sedláčková and Avagyan (2022), the dry weight of seeds per fruit of *Cucurbita pepo* L. in Slovakia was between 4.53 and 187.4 g. The average dry weight of seeds per fruit for genotypes in Kenya was 47 g (Jepkemboi *et al*., 2023), and in Italy 57 g (Darrudi *et al*., 2018).

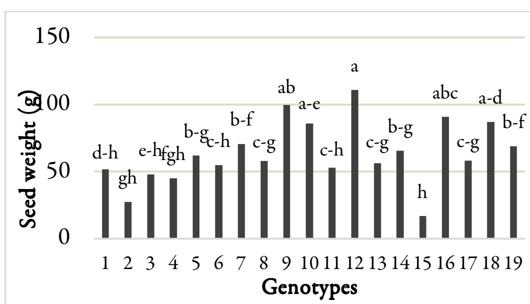

Figure 2. Seed weight per fruit of pumpkin

Values characterised by different lower-case letters differ significantly (P≤0.05) according to the LSD test

In this trial, the seed number per pumpkin fruit varied between the genotypes in an interval from 229 g in G15 to 445 in G9. The average seed number per fruit for all genotypes was 364. G9, G16, and G17 had a significantly higher seed number per fruit compared to G2 and G15 (Figure 3). The other genotypes did not differ significantly from each other. Khalili and Nejatzadeh (2021) reported that the average seed number per fruit of *Cucurbita pepo* L. in Iran ranged from 241 to 463. The average seed number per fruit for genotypes in Kenya was 32.6 (Jepkemboi *et al*., 2023), and in Italy 422 (Darrudi *et al*., 2018).

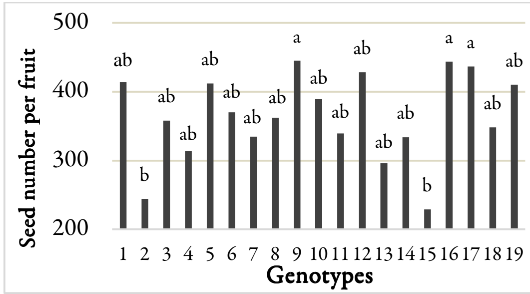

Figure 3. Seed number per pumpkin fruit

Values characterised by different lower-case letters differ significantly (P≤0.05) according to the LSD test

The average thousand seeds weight of all genotypes in this trial was 185.2 g and ranged from 100.2 to 254.3 g. G7, G9, G10, G12, G13, G16 and G18 had a thousand seeds weight of more than 200 g (Figure 4). G2, G3, G6, G8, G11, and G19 had a thousand seeds weight between 150 and 200 g. G1, G5, G15 and G17 had a thousand seeds weight between 100 and 150 g. G18 had a higher thousand seeds weight than all other genotypes except G7, G9, G10, G12, G13, and G16. According to Khalili and Nejatzadeh (2021), the thousand seeds weight of *Cucurbita pepo* L. in Iran was between 197.8 and 300.0 g, and according to Sedláčková and Avagyan (2022), it was 15.4-475.7 g for the Slovakian genotypes. The average thousand seeds weight in genotypes in Italy was 14.8 g (Darrudi *et al*., 2018). In the Republic of Serbia, Rabrenović *et al*. (2012) indicate that the thousand seed weight of three cultivars of oil pumpkin (*Cucurbita pepo* L.) with seed coat varied in the interval 236-254 g.

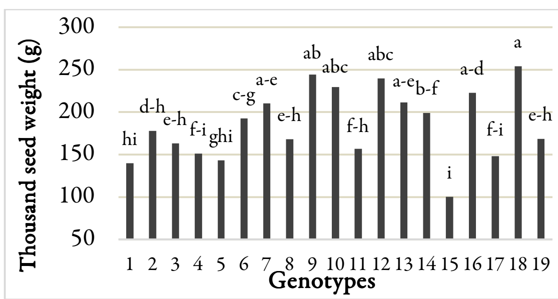

Figure 4. Thousand seeds weight of pumpkin

Values characterised by different lower-case letters differ significantly (P≤0.05) according to the LSD test

Percentage of seeds in the total weight of the pumpkin fruit varied between the genotypes in an interval of 0.56% to 3.55%. The average percentage of seeds in the total weight of the fruit was 1.9% for all genotypes. G18 had a significantly higher percentage of seeds in the total weight of the fruit compared to all other genotypes except G1, G3, and G14 (Figure 5). G1 and G3 had a significantly higher percentage of seeds in the total weight of the fruit compared to G5, G6, G7, G12, G15, G16, G17, and G19, which had the lowest percentage of seeds in the total weight of the fruit, less than 1.5%, except G7. The average percentage of seeds in the total weight of the fruit was 3.2-8.0% for Polish genotypes (Fedko *et al*., 2020) and 2.59 for Italian genotypes (Darrudi *et al*., 2018).

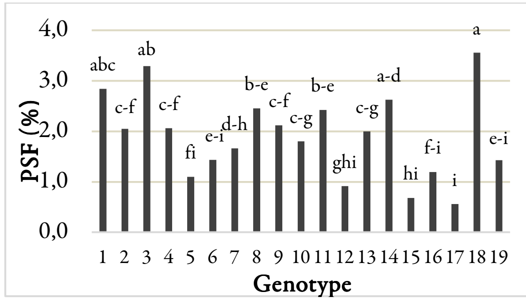

Figure 5. Percentage of seeds in the total weight of the pumpkin fruit (PSF)
Values characterised by different lower-case letters differ significantly (P≤0.05) according to the LSD test

The average percentage of kernel in the total weight of the pumpkin seed was 71.7 for all genotypes in this trial and ranged from 44.5 to 83.2%. G19 had a significantly higher percentage of kernel in the total weight of the seed compared to G2, G5, G13, G15 and G17 (Figure 6). G3 and G11 had significantly higher percentage of kernel in the total weight of the seed compared to G5, G13, G15 and G17. G5 had the lowest seed yield, significantly lower than all others except G17. Rabrenović *et al.* (2012) indicated that the average percentage of kernel in the total weight of the seed in three cultivars of oil pumpkin (*Cucurbita pepo* L.) with seed coat in the Serbia was in the interval of 73.0-77.5%.

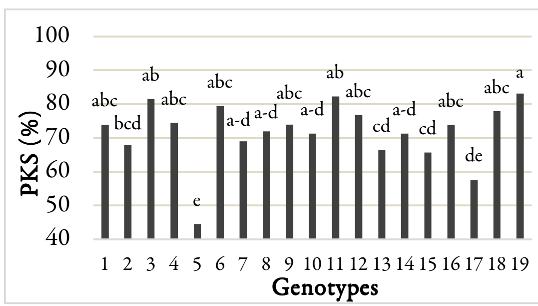

Figure 6. Percentage of kernel in the total weight of the seed (PKS)
Values characterised by different lower-case letters differ significantly (P≤0.05) according to the LSD test

The oil content in the pumpkin seeds varied between the genotypes in an interval of 3% to 42%. The average oil content in the fruits was 25.7% for all genotypes. G6, G8, G12 and G14 had significantly higher seed oil content compared to G2, G3, G4, G5, G11, G13, G15 and G17 (Figure 7). G15 had the lowest seed oil content, which was significantly lower than that of all other genotypes except G5 and G13. In addition to the low percentage of kernel in the total weight of the seed, G15 had visibly weak and shriveled seeds, which together with the small seeds number per fruit is a characteristic of the genotype. The oil content of *Cucurbita pepo* L. seeds in genotypes from Poland was 12-33% (Fedko *et al*., 2020), and Serbia was 34.53% (Cvetković *et al.*, 2021), and the maximum oil content of pumpkin seeds from Iran was up to 46% (Khalili and Nejatzadeh, 2021).

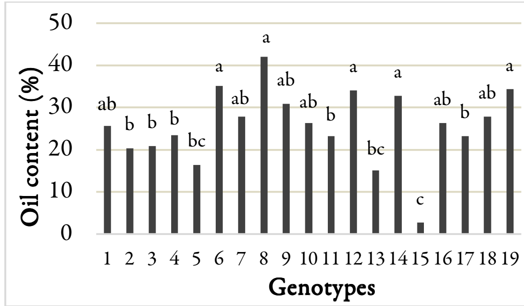

Figure 7. Oil content in pumpkin seed
Values characterised by different lower-case letters differ significantly (P≤0.05) according to the LSD test

The analysis of variance revealed highly significant differences between the 19 common pumpkin genotypes for analyzed traits at a significance of P≤0.05, indicating the presence of sufficient, considerable genetic variability between the different genotypes. These results are consistent with the results of other studies. According to Hosen *et al*. (2021), the genetic diversity of the germplasm, i.e. the population of common pumpkin, includes traits such as fruit shape, seed size and pulp thickness in addition to those mentioned above. The results of these studies are confirmed by the results of Kumar *et al*. (2017), Kiramana and Isutsa (2018), Thirumdasu and Chatterjee (2018), Brdar-Jokanović *et al*. (2019), Gomes *et al*. (2020), Rai *et al.* (2023). The large divergence of natural populations of common pumpkin means that they can be used with great success in a breeding program programmed for the development of high-yielding and high-quality varieties, as shown by Dalda-Şekerci *et al*. (2020). In recent times, the demand for different variations of pumpkin fruits, both in appearance and quality, has been very pronounced, which is why researchers have been encouraged to improve cultivars (Dhatt *et al*., 2020; Rai *et al*., 2023).

The weight of the fruit in this experiment was in a significant positive correlation with the mass of seeds per fruit and the number of seeds per fruit and in a significant negative correlation with the percentage of seeds in the total weight of the fruit (Table 2). The mass of seeds per fruit had a significant positive correlation with the number of seeds per fruit and the thousand seeds weight. The oil content in the seeds had a significant positive correlation with the weight of seeds per fruit, the number of seeds per fruit and the thousand seeds weight. A significant positive correlation was also recorded between the percentage of seeds in the total weight of the fruit and the percentage of kernel in the total weight of the seed. The results indicate that the most significant yield components and the seed yield itself were found in significant positive correlations with the oil content in the seeds. According to the results of Sharaf *et al*. (2021) fruit mass of common pumpkin was significantly positively correlated with fruit yield per plant, while according to Rai *et al*. (2023), fruit mass and total yield of common pumpkin were significantly positively correlated with seed diameter. Similar results are indicated by Darrudi *et al*. (2018). This confirms the fact that selection for a higher fruit yield also affects the increase in seed yield.

Table 2. Coefficients of correlation among fruit weight (FW), seed weight per fruit (SWF), seed number per fruit (SNF), thousand seeds weight (TSW), percentage of seeds in the total weight of the fruit (PSF), percentage of kernel in the total weight of the seed (PKS) and oil content in the seed of common pumpkin; (n = 95)

	SWF	SNF	TSW	PSF	PKS	OC
FW	0.61 *	0.62 *	0.31	-0.67 *	-0.19	0.2
SWF		0.73 *	0.82 *	-0.01	0.19	0.56 *
SNP			0.32	-0.16	0.02	0.52 *
TSW				0.22	0.29	0.48 *
SR					0.47*	0.22
KR						0.45

* Significant at the P ≤ 0.05

The composition of D2 clusters showed that the genotypes were classified into five clusters, of which two clusters (A and E) contained two representatives each (Table 3). Cluster A included genotypes 2 and 15, and cluster E included genotypes 10 and 18, which indicates their divergence in relation to the other genotypes. Cluster D included two subclusters, one with G16 and the other with G9 and G12. Cluster C was similar and included G1, G5, G17 and G19 classified into several subclusters. Cluster B included the largest number of genotypes, it was divided into 2 subclasses, which were divided into groups. One group consists of G13, G7 and G14 and the other G6, G8, G4, G3, G11. For further selection work, genotypes from divergent clusters/subclusters will be selected in order to achieve the greatest recombination of traits that determine the fruit weight and quality of common pumpkin seeds.

Table 3. Composition of D2 clusters for 19 common pumpkin genotypes

Clusters	List of genotypes of *Cucurbita pepo* L. under each cluster	Total number of genotypes
A	G2, G15	2
B	G13, G7, G14, G6, G8, G4, G3, G11	8
C	G19, G1, G5, G17	4
D	G16, G9, G12	3
E	G10, G18	2

The pattern of clustering proved the existence of a significant amount of variability. Genotypes are grouped into different clusters irrespective of their geographical origins. This means that the genetic constitution of the varieties was more important than their origin and genetic divergence analysis was widely used to determine the genetic relationship among the genotypes and find out the suitable genotypes for future breeding programs. Genetic diversity analysis also helps in tagging and eliminating duplicate accessions from genetic stock.

The divergence among the genotypes in the same cluster is indicated by the divergence within the cluster. Inter cluster divergence, one on either hand, denotes the divergence between the genotypes of various two groups. Based on main character relations, a critical analysis of clusters revealed that they were heterogeneous within and between one another (Table 4). The lower D2 value between their characters indicated a close genetic relationship between these genotypes in one cluster and those in the other cluster. These findings are supported by the results obtained by Swain *et al*. (2022).

Table 4. Average inter and intra cluster D2 values for 19 common pumpkin genotypes

Clusters	A	B	C	D	E
A	50.0	218.7	155.1	119.5	143.3
B		81.9	176.3	274.2	218.3
C			83.5	170.0	116.6
D				32.3	101.4
E					48.4

Intra-cluster distances were lower than distances between different clusters. The largest inter-cluster distance was recorded between clusters B and D (274.2), then between clusters A and B (218.7), and clusters B and E (218.3). Genotypes classified in the mentioned clusters can be used as parents in breeding work to obtain a wide range of variability and transgressive segregations. The small distance between clusters D and E indicates that the selection of parents from such clusters may result in a narrow genetic base. Similar results are indicated by Swain *et al*. (2022). The intra-cluster distance was the largest for cluster C (83.5) and the smallest for cluster D (32.3). This shows that the chances for the development of good segregations by crossing parents within a cluster are the highest in cluster C, and the least in cluster D. Similar results in terms of genetic divergence of genotypes and the results of Mahalanobi's D2 statistical analysis were described in moschata pumpkin

(*Cucurbita moschata* Duch. Ex Poir), for numerous morphological characteristics of the plant and fruit (Rajput *et al*., 2021; Verma *et al*., 2020; Panging *et al*., 2023, Kumar *et al*., 2023; Da Silva *et al*., 2024).

Mean values by clusters in this experiment for all seven traits are shown in Table 5. Cluster D genotypes had the highest values for fruit weight, number of seeds per fruit, percentage of kernel in the total weight of the seed and oil content. This is followed by cluster E, whose genotypes had high values for seed weight per fruit, thousand seeds weight, percentage of seeds in the total weight of the fruit, percentage of kernel in the total weight of the seed, and oil content. Genotypes from cluster A had the lowest values for most of the analyzed traits. According to Darruda *et al*. (2018) for further selection in common pumpkin, genotypes from the cluster with the highest fruit yield, seed weight per fruit and thousand seeds weight should be used. Similar results were indicated by Gomes *et al*. (2024) in moschata pumpkin.

Table 5. Cluster mean for 19 pumpkin genotypes for observed traits

Cluster	FW (kg)	SWF (g)	SNF	TSW (g)	PSF (%)	PKS (%)	OC (%)
A	1.87	21.95	236.50	139.10	1.36	66.75	11.50
B	2.82	56.39	338.50	181.43	2.24	74.55	27.56
C	5.44	60.23	418.25	149.75	1.48	64.80	24.95
D	8.29	100.53	439.00	235.50	1.41	74.83	30.47
E	3.63	86.35	368.50	241.90	2.68	74.60	27.08

FW – Fruit weight, SWF – seed weight per fruit, SNF – seed number per fruit, TSW – thousand seeds weight, PSF - percentage of seeds in the total weight of the fruit, PKS - percentage of kernel in the total weight of the seed, OC – Oil content in seed

Conclusions

The analyzed genotypes showed significant variability for the analyzed traits. They were classified into 5 clusters that differed significantly from each other. The largest number of genotypes belonged to cluster B. The highest seed yield and seed oil content were obtained by genotypes within cluster D. Parents for the hybridization program should be selected based on genetic distance, the correlation ratio of the desired traits, and the size of the mean value for the traits in the cluster with the highest heterosis. Based on this, the expectation is that crosses between parents belonging to the most divergent clusters will yield maximum heterosis and wide trait variability. The results indicate that by crossing genetically different clusters B and D, high heterosis and a high probability of new combinations can be expected in order to obtain desirable segregations with a higher oil yield. In further research, they will serve for the development of more productive varieties of common pumpkin with a higher oil content.

Authors' Contributions

Conceptualization: Dalibor Tomić, Vladeta Stevović, Vladimir Zornić. Methodology: Dalibor Tomić, Mirjana Radovanović, Vesna Đurović. Writing—review and editing: Dalibor Tomić, Vladeta Stevović, Vladimir Zornić, Milomirka Madić. Resources: Miloš Marjanović, Đorđe Lazarević. Supervision: Nenad Pavlović. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article

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