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Original scientific article

VARIABILITY AND CORRELATION BETWEEN PROPERTIES OF MAIZE HYBRID SEEDS OF DIFFERENT FRACTIONS OBTAINED AFTER SEED PROCESSING AND THE INITIAL SEEDLING GROWTH

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The shape and the size of maize seeds are the most variable traits, which are determined by a genotype and environmental conditions. The aim of this study was to understand the effects of the mechanism of the relationship and significance of seed variability on germination and morphology of seedlings. The seeds of five hybrids ZP388, ZP434, ZP555, ZP606, and ZP6263 were used in this study. The following seed traits were analysed: physical ones: seed length (L), thickness (T) and the width (W); morphological ones: seed weight (SW), seedling length (SLW), root length (RL), shoot length (SL) and seed germination (G) as a phydiological trait.There are statistically significant differences not only among physical traits of the seeds of the five hybrids ($p\leq0.05$), but also among the morphological traits ($p\leq0.05$). Statisticlly significant differences ($p\leq0.05$). in the

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width (W), length(L) and thickness (T) of seeds of all hybrids were determined in the small flat fraction (SP). The large rounded seed fraction (KO) mainly differed in the width and thickness between hybrids ZP434 and ZP 555, while the large flat seed fraction differed the most in the length between these two hybrids. Large-flat (KP) seed fractions are also characterized by the highest germination (99%). As the seed weight increases, the seedling weight decreases (R^2=0.527). Segmentation within hybrids according to the diversity of morphological and physiological properties of seeds was carried out according to the seed size, fraction and seedling weight, while the other parameters were less important. The characteristic of all hybrids is that large seeds of the KP fraction have high germination and well-developed seedlings.

Keywords: Zea mays, seed fractions, seed size, germination, seedling

INTRODUCTION

The aim of contemporary agriculture is to provide sufficient food for a growing population while using minimum potential (ROUPHAEL *et al*., 2020; PUNIA *et al*., 2020). In the previous three decades, several technical solutions have been proposed to improve agricultural production and adapt to climate changes in order to maximize system productivity (CALVO *et al*., 2014). Management improvements in seed maize production were of vital importance for maize grain yield (MANDIC *et al*., 2016; LONG *et al*., 2010). Breeding of maize hybrids and organization of production equally contributed to the increase in yield (DUVICK *et al*., 2005).

Vigour is one of the qualitative traits of seeds due to which the seed is considered to be a key element in succeeding in growing crops (FINCH-SAVAGE and BASSEL, 2015). The main reserve components in seed tissues are proteins, lipids and carbohydrates, accumulated during maturation. (BEWLEY *et al*., 2013; BAREKE, 2018; ZHAO *et al*., 2018). These storege components are involved in germination and formation of seedlings and, accordingly, are directly related to seed vigour (RAJJOU *et al*., 2012; BEWLEI *et al*., 2013; PRAZERES and COELHO, 2016; WU *et al*., 2017). The importance of quality seed material has been increasing over years due to changes with changes in environmental conditions from year to year is growing. Soil temperature and moisture are the main factors that regulate germination processes and seed vigor (GIBSON-ROY *et al*., 2006). Seed germination is largely dependent on the influence of the environment, which stimulates the creation of various phytohormones and other molecules (LATA and PRASAD, 2011; SIGNORA *et al*., 2002). One of the major phytohormones in the regulation of seed germination is abscisic acid (ABA) (DING *et al*., 2015; LIM *et al*., 2013; ZHANG *et al*., 2019).

Previous research in arabidopsis, rice and wheat has revealed loci that controlled seed germination. (LANDJEVA *et al*., 2010; YUAN *et al*., 2016).

Considering maize, previous studies have focused mainly on research genetic control for seed germination ability under stress conditions.

Our goals were to improve the understanding of seed germination ability in maize and to identify phenotypic properties as causative agents that affect ability of seeds to germinate.

MATERIALS AND METHODS

The following five hybrids develoved at the Maize Research Institute, Zemun Polje, were used in the trial: ZP388 (FAO300), ZP434 (FAO400), ZP555 (FAO500), ZP606 (FAO600), and

ZP6263 (FAO600). According to the morphological properties and chemical composition of the grain, all hybrids are classified as yellow dent maize hybrid.

The seeds were obtained from the seed material produced by farmers, with the application of all standard cropping practices for the maize production and measures prescribed provided by the Rulebook Regulation on the production of seed maize (Official Gazette of the Republic of Serbia, 2006; issue 2006/06).

Seed maize harvesters were used to harvest maize. Shelling, drying and processing were done at the Maize Research Institute.

The basic seed obtained by standard processing on the laboratory selector (CEA Carter-Day, International, Minneapolis, Minnesota, USA),was used to separate seeds using the cylinder sieves with rectangular holes slots of 4.7 mm and 5.7 mm. The seeds were then classified into small and large fractions. Seeds of 6.5 mm to 11.0 mm were then passed through the Ø 8.5 mm calibrator and all seeds that fell through the calibrator were considered to be small (both flat and round fractions). The seeds that remained on the calibrator were consider to be large seed (round and flat fraction). A 1000-g sample was drown from each fraction, and analyses of seed germination (G), root length (RL), shoot length (SL), seedling weight (SLW), seed weight (SW), and physical traits of seeds: length (L), width (W) and thickness (T) of seeds were performed in the laboratory.

Seed weight was determined for each fraction, using the standard 100x4 seed method. After which seed physical properties (length, width and thickness) were determined using seeds from each of 3×10-seed replicate.

Seed germination was determined according to the ISTA method (ISTA Rules, 2019): growth chamber, sand as a substrate, 20/30^{o}C, alternating lighting 16/8h. Morphological traits of seedlings were determinate after germination. Shoots were randomly selected from samples of four replicates of 100 seeds to determine the shoot length, root length and seedling weight ((10×3)×4).

The obtained data were processed with mathematical and statistical methods using the IBM SPSS 19.0 statistical package (free of charge version). Mean values and variability for all parameters were determined using descriptive statistics. The significance of the factors was shown by the analysis of variance (ANOVA), and the significance of the differences using the LSD test. The regression analysis and correlation coefficients were used to determine the interdependence.

In all research variants, a cluster analysis was applied to the natural structure between observations based on multiple criteria and maximizing internal homogeneity and external heterogeneity.

RESULTS AND DISCUSSION

There are statistically significant differences among physical traits of the seeds of five hybrids ($p \leq 0.05$), as well as among the morphological traits ($p \leq 0.05$). Significance of differences in width, thickness and length of seeds among hybrids varied over the seed size and shape. The most significant differences in small seeds were recorded in the seed length in small flat seeds (Table 1). On the other hand, this fraction did not show any significant differences among hybrids for seed thickness. The greatest differences in large fractions were established between hybrids ZP434 and ZP 555 for the width and thickness in the large rounded seed fraction and for the length in the large flat seed fraction (Table 1).

Table 1. Variability of physical properties of ZP hybrid seeds in relation to seed fraction

Hybrid	SO			SP			KO			KP		
	W	T	L	W	T	L	W	T	L	W	T	L
ZP 388	7.57 ^{b}	5.85 ^{b}	9.88 ^{ab}	8.03 ^{a}	4.50 ^{a}	10.01 ^{b}	9.05 ^{a}	6.98 ^{ab}	9.61 ^{ab}	8.64 ^{b}	5.19 ^{a}	10.95 ^{ab}
ZP 434	8.28 ^{a}	6.94 ^{a}	9.06 ^{a}	7.96 ^{a}	4.56 ^{a}	9.54 ^{c}	8.92 ^{a}	6.65 ^{b}	9.76 ^{ab}	9.22 ^{a}	4.94 ^{ab}	11.19 ^{a}
ZP 555	7.58 ^{b}	5.72 ^{b}	10.39 ^{ab}	7.68 ^{ab}	4.42 ^{a}	10.16 ^{b}	8.54 ^{b}	7.28 ^{a}	9.27 ^{ab}	8.70 ^{b}	5.32 ^{a}	10.04 ^{b}
ZP 606	7.77 ^{b}	6.25 ^{ab}	10.45 ^{ab}	7.97 ^{a}	4.54 ^{a}	11.01 ^{a}	8.77 ^{ab}	6.70 ^{b}	9.92 ^{a}	8.75 ^{b}	5.34 ^{a}	10.89 ^{ab}
ZP 6263	7.50 ^{b}	5.73 ^{b}	10.92 ^{a}	7.60 ^{b}	4.44 ^{a}	11.17 ^{a}	8.89 ^{ab}	6.94 ^{ab}	8.76 ^{b}	9.10 ^{a}	4.74 ^{b}	11.42 ^{a}

SO-small round fraction, SP-small flat fraction, KO-large round fraction, KP- large flat fraction, W-width, T-thickness, L-length, lowercase letters a, b, c, d-significance of the difference at the level of $p \leq 0.05$.

In the production, the seeds are divided into two fractions of shape (round and flat) and two fractions of sizes (small and large). According to this division classification, significant differences regarding both criteria were determined between hybrids ZP 434 and ZP 606. The greatest seed weight was observed in the hybrid ZP 606 in both small fractions (SO and SP) and in one large fraction (KP). The smallest seed with the lowest weight (215.97 g) was recorded in the SP fraction of hybrid ZP 388 (Table 2). Maize is one of the most variable plants in terms of the seed shape and size. Breeders develop hybrids with a determined structure of small and large, flat and round seeds. Different environmental conditions more or less contribute to the variability of these traits. Heterogeneity that occurs in seed size is defined as the ability of a plant to create different shapes and sizes in order to maintain the viability of the seed (BHATT and SANTO, 2016).

Table 2. Variability of 1000 - seed weight (g) in relation to the seed fractions of ZP hybrid

Hibrid	SO	SP	KO	KP
ZP 388	254.83 b	215.97 b	376.30 ab	343.87 ab
ZP 434	272.63 b	214.37 b	368.57 b	330.47 b
ZP 555	317.40 a	269.57 ab	361.53 b	333.93 b
ZP 606	331.47 a	288.23 a	386.90 a	367.30 a
ZP 6263	298.97 ab	262.30 ab	390.17 a	347.07 ab
Average	295.10	250.10	376.70	344.50

SO-small round fraction, SP-small flat fraction, KO-large round fraction, KP- large flat fraction, lowercase letters a, b, c, d-significance of the difference at the level of $p \leq 0.05$.

Five traits were used in the study to assess the seed quality of five hybrids. Each trait showed great variability. The analysis of the mean values for seed germination revealed a high percentage of germinated seeds 92-99%, with a variability of 2.41 (Figure 1-A). Such high germination is common for seeds after harvest. The most common cause of drop in germination percentage is seed age and stressful production and storage conditions. Seed germination ability, as an important agronomic trait, affects the vegetative growth and crop yield formation, which is controlled by genetic and environmental factors (HAN *et al.*, 2014). Research performed by MUHARREM (2008) has shown that the germination variability of seed of different sizes also depends on the amount of water absorbed by the seeds.

Remaining parameters also varied within standardized environmental conditions and different genotypes. The rank for the shoot length was 8.39 cm with variance 2.79 (Figure 1-B), root length, with variance of 7.54 (Figure 1-C) and seedling weight ranged from 0.76 g to 1.4 g (Figure 1- D). Predicting the emergence of a seedling and its growth are important steps in establishing a crop. There are numerous models that predict the growth of seedlings under non-limiting physical conditions (WEAICH *et al.*, 1996). The length of shoot and seedling depends on air and soil temperature, but also on a soil type (SHALHEVET *et al.*, 1995).

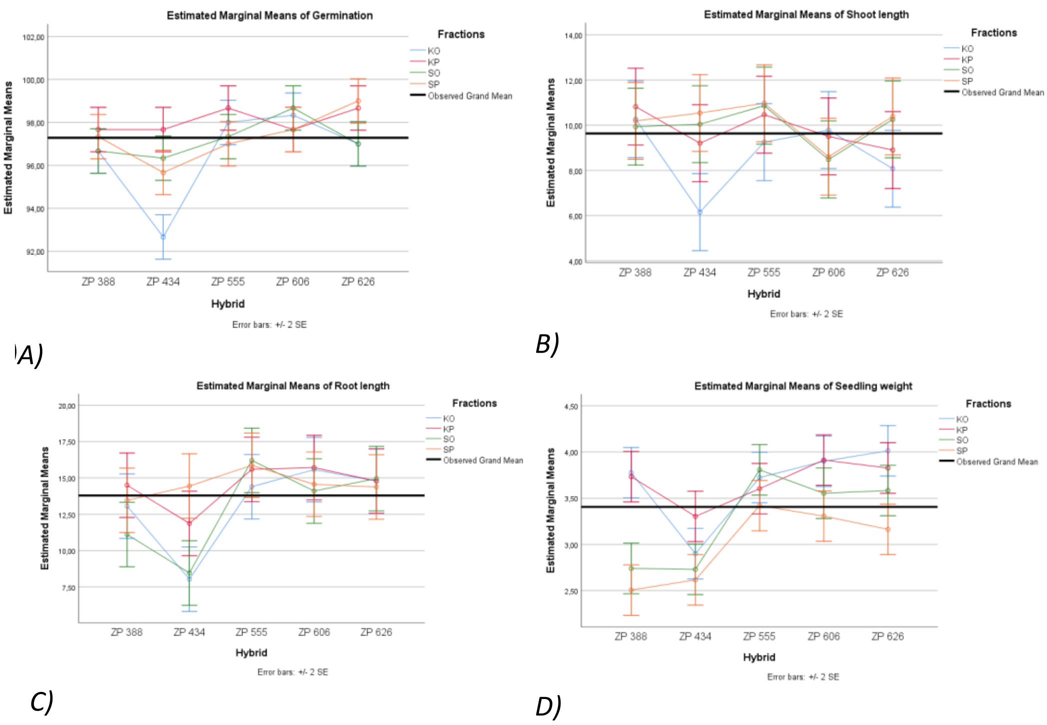


Figure 1. The effect of genotype and seed fraction (KO-large round, KP-large flat, SO-small round, SP-small flat) of maize hybrids on the variability of mean values of seed characteristics. A) -mean values for germination; B) -mean values for shoot length; C) - mean values for root length; D) -mean values for seedling weight

Shoot length, root length and seedling weight varied the most for ZP 434 compared to other hybrids, especially with seeds belong to the large rounded fraction. Germination and the root were above the grand mean for all fractions in hybrids ZP 555, ZP 606 and ZP 6263. The shoot length was above the average values, for both (SO and SP), in hybrids ZP 388, ZP 434 and ZP 555. In contrast, the shoot of seeds of the large rounded fraction was short in all observed hybrids. Based on morphological parameters, it was determined that values of all parameters were above average in seeds of the hybrid ZP.

Based on regression analysis, the interdependence of the seedling weight and the seed size was determined, R^2 0.527 (Table 3). The results are in agreement with the results obtained by KAYA (2008), who concluded that the decrease seed size, led to the decrease in the length of roots and shoots.

Table 3. Regression analysis of seed weight significance for morphological and physiological traits

Depend Variable	R	R^2	Adjusted R^2	Std. Error	Change Statistics		
					R^2 Change	F Change	Sig. F Change
Seedling weight	0.727[a]	0.527	0.520	0.351	0.528	64.861	0.000
Germination	0.055[a]	0.003	-0.014	1.563	0.003	0.176	0.677
Shoot lenghth	0.326[a]	0.106	0.091	1.594	0.106	6.877	0.011
Root length	0.065[a]	0.004	-0.013	2.764	0.004	0.244	0.623

a. Predictors: (Constant), Seedweight

Morphological, chemical, and physical properties of seeds are genetically determined, their degree of expression depends on the conditions under which the seed was developed. According to the results of VANCETOVIC *et al*. (2020), different populations can be used to simultaneously improve the agronomic, biochemical, chemical and physical properties of seeds. These authors found that small seeds were superior for some of these traits. The genetic base of the hybrids used in the experiment is more or less distant, and the interdependence of two or more traits is specific. Seed germination is a feature that is significantly correlated with the shoot length, root length and seedling weight. There are also significant correlations between shoot length and root length. The shoot length showed a small correlation with other properties. This trait varies positively in relation to the root length (r=0.297 and negatively in relation to the seed weight (r=-0,326). Seed weight is correlated with seedling weight ($p \leq 0,01$) and shoot length ($p \leq 0,05$) (Table 4).

Solutions based on the identification of groups within different populations, allows segmentation within the population according to the diversity of morphological and physiological traits of seeds.

Using the cluster analysis and distance coefficients, all results gained in the experiment were divided into three homogeneous groups.

Table 4. Quantitative dependence of morphological, physical and physiological properties of seeds (Pearson coefficient correlation)

	Germination	Shoot length	Root length	Seedling weight	Seed weights
Germination	1	0.294*	0.535**	0.399**	0.055ns
Shoot length	0.294*	1	0.297*	0.022ns	-0.326*
Root length	0.535**	0.297*	1	0.556**	0.065
Seedling weight	0.399**	0.022ns	0.556**	1	0.727**
Seed weights	0.055ns	-0.326*	0.065ns	0.727**	1

*- significance at the level of p≤0.05, ** - significance at the level of p≤0.01, ns- no significance at the level of p≤0.05, p≤0.01.

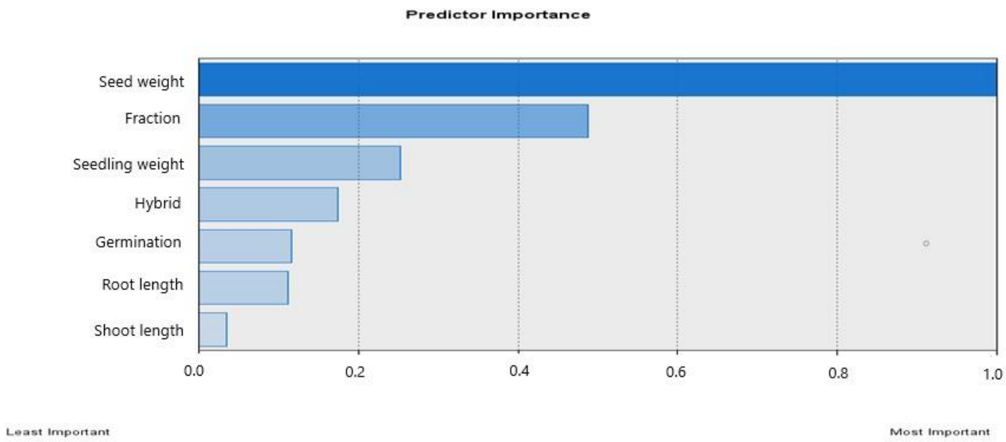
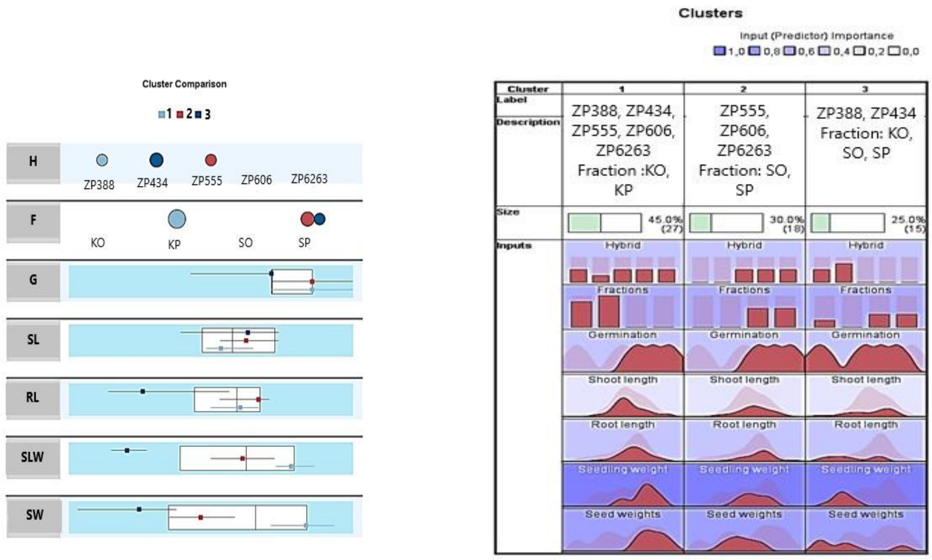


Figure 2. Significance of maize hybrid seed traites in determining the size and the number of clusters

The seed weight, then the seed fraction and the seedling weight had the greatest impact on the formation of homogeneous groups (Figure 2). The first cluster consists of hybrids ZP 388, ZP 434, ZP 555, ZP 606 and ZP 6263. The seed belonged to large flat and large rounded fraction weighing more than 300 g. The second cluster contains seeds whose weight corresponds to the small rounded and small flat fractions of hybrids ZP 555, ZP 606 and ZP 6263, while the third cluster contains seeds of hybrids ZP 388 and ZP 434, belonging to the small flat and large rounded fractions (Figure 3-B).



A) B)

Figure 3. Distance of mean values of seed characteristics of maize hybrids between A) Median seed characteristics by clusters, B) frequency by clusters compared to the total population. H-hybrid, F-fraction, G-germination, SL-shoot length, R-root length, SLW-seedling weight, SW-seed weight, KO-large round, KP- large flat, SO-small round, SP-small flat seed fraction.

Table 5. The effect of seed clustering of maize hybrids according to the diversity of morphological and physiological characteristics of seeds

Claster	Dependent Variable	Mean	95% Confidence Interval for Mean		5% Trimmed Mean	Median	Variance
			Lower Bound	Upper Bound			
1	Germination	97.81	97.42	98.21	97.85	98.00	1.00
	Shoot length	9.59	8.99	10.18	9.55	9.21	2.26
	Root length	14.47	13.68	15.26	14.51	14.62	3.98
	Seedling weight	3.75	3.64	3.87	3.76	3.78	0.08
	Seed weights	359.79	350.93	368.66	359.91	361.53	502.27
2	Germination	97.78	97.22	98.33	97.81	98.00	1.24
	Shoot length	9.93	9.16	10.70	9.96	9.96	2.41
	Root length	15.01	13.99	16.04	15.00	15.45	4.25
	Seedling weight	3.47	3.33	3.62	3.47	3.46	0.09
	Seed weights	294.76	281.93	307.58	295.01	294.10	665.01
3	Germination	95.73	94.72	96.75	95.81	97.00	3.35
	Shoot length	9.37	8.20	10.54	9.50	10.02	4.47
	Root length	11.10	9.47	12.73	11.10	10.31	8.65
	Seedling weight	2.70	2.58	2.82	2.70	2.68	0.05
	Seed weights	265.07	232.55	297.58	262.05	254.83	3447.05

In three clusters (1-3), the highest frequency was established in hybrids ZP 388, ZP 555 and ZP 434, respectively (Figure 3-A). The first cluster encompassed the seed with the highest germination and weight of seedlings and seed, with well-developed root and shoots. The second cluster consisted of the seed of medium germination, with the seedling of well-developed shoots and the longest roots. The third cluster consisted the seed of the lowest germination, the lowest seedling weight, equally developed shoots and roots and the lowest seed weight (Table 5). Seed vigour is an important property of seed quality, which is reflected in germination, seedling growth, seed longevity, tolerance. In recent years, the emphasis has shifted from seed vigour to improving genetic potential (LIU, *et al*., 2017). Genetic analyses, such as the analysis of gene loci in the seeds, has made it possible to investigate the inheritance of seed vigour. This analysis was mainly focused on grain filing, seed size and maturity (ZHANG *et al*., 2020).

Claster groups of hybrids differ according to seed morphology. The distance between the formed groups is significant for germination, root length, seedling weight and seed weight (Table 5).

Table 6. Analysis of variance of morphological and physiological traites of maize hybrid seeds (ANOVA)

ANOVA	Sum of Squares	df	Mean Square	F	Sig.
Germination	25.706	2	12.853	6.290	0.003
Shoot length	9.589	2	4.795	1.760	0.181
Root length	77.310	2	38.655	5.993	0.004
Seedling weight	0.593	2	0.296	15.365	0.000
Seed weight	138201.688	2	69100.844	129.602	0.000

These greatest differences were established for the seed weight and the seedling weight ($p \leq 0.05$). Germination and the root length differed significantly between the first and the third cluster and between the second and the third cluster ($p \leq 0.05$) (Table 7). Remaining traits differed significantly among all three clusters.

Table 7. Determining the significance of differences between clusters (LSD)

Dependent Variable	Cluster (I-J)		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Germination	1	2	0.0370	0.39101	0.925	-0.7459	0.8200
		3	2.0815*	0.41381	0.000	1.2528	2.9101
	2	3	2.0444*	0.44924	0.000	1.1449	2.9440
Root length	1	2	-0.5391	0.69436	0.441	-1.9295	0.8514
		3	3.3746*	0.73484	0.000	1.9031	4.8461
	2	3	3.9137*	0.79775	0.000	2.3162	5.5111
Seedling weight	1	2	0.2826*	0.08367	0.001	0.1151	0.4501
		3	1.0561*	0.08854	0.000	0.8788	1.2335
	2	3	0.7736*	0.09612	0.000	0.5811	0.9660
Seed weights	1	2	65.0339*	10.86144	0.000	43.2842	86.7835
		3	94.7256*	11.49467	0.000	71.7079	117.7432
	2	3	29.6917*	12.47884	0.021	4.7032	54.6801

Based on observed means. The mean difference is significant at the p≤0 ,05 level

CONCLUSION

In these studies, the seed germination ability was observed in relation to five different properties of seeds in laboratory conditions where the effect of the external environment was controlled.

These traits showed high phenotypic variability, suggesting that some traites are rich in genetic variability for germination ability.

By applying the cluster analysis and using multiple criteria, different hybrids were combined according to similarity of morphological and physiological seed traits into the structure of observations. The seed weight was the criterion according to which homogenization was performed.. Other criteria were not significant for the groupings. By segmenting the experimental population, a group of large seeds (either of large rounded or large flat fraction) weight over 300 g stood out. The seeds of all hybrids of this size had the same physiological characteristics of seeds and seedlings. The seeds of a smaller weight were divided into two clusters according to a genotype and traits of both seeds and seedlings. The seeds of lower weight had a greater variability by all criteria. According to the results, the seed size is the primary factor in determining seed germination and the seedling weight.

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VARIJABILNOST I KORELACIJA IZMEĐU OSOBINA HIBRIDNOG SEMENA KURUZA RAZLIČITIH FRAKCIJA DOBIJENIH POSLE DORADE SEMENA I POČETNOG RASTA KLIJANACA

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Izvod

Oblik i veličina semena kukuruza su najpromenljivije osobine koje određuju genotip i uslovi životne sredine. Cilj ovog istraživanja je bio da se sagleda značaj varijabilnosti semena i njen odnos sa klijavošću semena i morfologijom klijanaca. U radu je korišćeno seme pet hibrida ZP388, ZP434, ZP555, ZP606 i ZP6263. Analizirane su sledeće osobine semena: fizičke: dužina (L), debljina (T) i širina (V); morfološke: masa semena (SV), dužina semena (SLV), dužina korena (RL), dužina izdanka (SL) i klijavost semena (G) kao fiziološka osobina.Postoje statistički značajne razlike ne samo među fizičkim osobinama semena pet hibrida $p\leq0,05$) već i među morfološkim osobinama ($p\leq0,05$). Statistički značajne razlike ($p\leq0,05$). širina (V), dužina (L) i debljina (T) semena svih hibrida su određene u sitno pljosnatoj frakciji (SP). Frakcija krupnog okruglog semena (KO) se uglavnom razlikovala po širini i debljini između hibrida ZP434 i ZP 555, dok se frakcija krupnog plosnatog semena najviše razlikovala po dužini između ova dva hibrida. Krupno-plosnata (KP) frakcija semena takođe se odlikuju najvećom klijavošću (99%). Sa povećanjem mase semena, težina semena se smanjuje (R2=0,527). Selekcija unutar hibrida prema raznovrsnosti morfoloških i fizioloških osobina semena vršena je prema veličini semena, frakciji i masi semena, dok su ostali parametri bili manje važni. Karakteristika svih hibrida je da krupno seme KP frakcije ima visoku klijavost i dobro razvijene klijance.

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