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GENETIC DIVERGENCE OF HOT PEPPER LINES (*Capsicum spp.*) IN HYBRID COMBINATIONS

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Abstract

Breeding programs for developing new genotypes emphasize earliness, fruit quality, yield potential, resistance to economically significant diseases, tolerance to abiotic stress, and plant morphology. Hybrids offer numerous advantages over varieties, including higher yields, fruit uniformity, resistance to plant pathogens, and the manifestation of heterosis. These are the primary reasons for initiating research on hybrid combinations of hot peppers. This study aimed to develop a hybrid hot pepper capable of meeting market demands. Seven experimental pepper hybrids were created by crossing the *ms* line HM5 (nuclear male sterility) as the female parent with seven pure lines (LHP 1–7) as the male parents, producing seeds of hybrid combinations. In 2023, a randomized block design experiment with three replications of 40 plants each was conducted in a greenhouse at the Institute for Vegetable Crops Smederevska Palanka. Harvesting was performed at the technological maturity stage. The most important morphological fruit traits and dry matter content (DMC) were analyzed on 10 fruits (kapya type) from each hybrid combination. The results revealed that hybrid combinations had a statistically significant impact on parameters such as fruit weight, length, width, pericarp thickness and DMC. The hybrid HM5xLHP6 showed the highest fruit weight (119.70 g), HM5xLHP4 exhibited the longest fruit (18.26 cm), while HM5xLHP1 and HM5xLHP3 demonstrated the largest fruit width (6.02 cm) and pericarp thickness (5.2 mm) respectively. High DMC values were recorded for HM5xLHP7 and HM5xLHP5 (10.5% and 9.40%). The number of fruits per plant (11.40-16.80) and the number of chambers (2) were consistent across hybrids, with no statistically significant differences observed. By comparing market-available varieties with hybrids, which are still limited in number, the results of this study highlight the superiority of hybrids for cultivation in greenhouse. Hybrids demonstrated justified economic effects for both fresh consumption and industrial processing.

***Key words*:** crossing method, sterile lines, morphological traits

Introduction

Hot peppers (*Capsicum spp.*) hold a significant position among vegetable crops due to their diverse applications in fresh consumption, food processing, medicine, and agronomy. They belong to the family *Solanaceae*, which contains compounds beneficial to human health,

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including vitamins, flavonoids, carotenoids, capsaicinoids (responsible for pungency), volatile oils, fatty acids, proteins, fibers, and minerals (Mendes et al., 2019; Nejati Sini et al., 2024). Hot peppers are cultivated year-round in greenhouse. The global annual production of peppers is estimated at 42.3 million tons, grown on 3.7 million hectares in 2020., emphasizing their economic and agronomic importance (Mori et al., 2022, Yan et al., 2024). Among approximately 30 species of hot peppers, five have been domesticated: *Capsicum annuum, C. frutescens, C. baccatum, C. chinense,* and *C. pubescens* (Martinez et al., 2021). Enhancing these species through the development of high-quality hybrids has proven to be a key step toward increasing yield and quality (Andrés Lobato-Ureche et al., 2023).

Hybridization enables breeders to combine desirable parental traits, such as resistance to abiotic stresses, high yields, and fruit uniformity (Wei et al., 2020). Combining ability studies are useful for predicting hybrid performance (Rohini et al., 2017). Numerous studies have demonstrated the critical role of heterosis and combining ability in hybrid pepper development. Heterosis significantly enhances productivity, which is vital in addressing global climate change and food security challenges (Singh et al., 2023). For instance, Devi and Sood (2018) reported positive heterosis for fruit length, fruit number per plant, and total yield. Various selection methods have allowed breeders to develop hybrids with specific traits, including improved disease resistance, enhanced nutritional composition, and optimized yields (Tudor et al., 2019; Vazquez-Espinosa et al., 2020). However, the development of hybrid hot peppers in Serbia remains limited, despite the availability of numerous varieties developed through different selection methods (Cvikić et al., 2024), with very few hybrids.

The selection process requires prior determination of the inheritance mechanisms of desired traits, which serves as a foundation for defining breeding strategies. Genetic divergence among populations is fundamental for identifying parents that provide high heterotic effects in progeny (Rego et al., 2011). The diversity in fruit size, shape, color, and dry matter content offers significant potential for breeders to create novel and distinct pepper hybrids. Nascimenta et al. (2014) observed significant reciprocal effects on most morpho-agronomic traits and fruit quality. For traits governed by non-additive gene effects, such as pericarp thickness, fresh matter, fruit length, seed yield per fruit, fruit number per plant, days to fruiting, and total soluble solids, hybrid production is identified as the optimal strategy.

The objective of this study was to develop a hot pepper hybrid by integrating genetic and breeding perspectives, with a focus on phenotypic and genotypic variations, heterosis, and combining ability as a foundation for improving production and quality traits.

Materials and methods

In this study, seven experimental pepper hybrids were used as plant material. These hybrids were developed by crossing the *ms* line HM5 (nuclear male sterility) as the female parent with seven pure lines, designated as LHP1, LHP2, LHP3, LHP4, LHP5, LHP6, and LHP7, which are homozygous lines developed at the Institute for Vegetable Crops, Smederevska Palanka. Hybrid seeds of seven experimental hybrids were produced in 2022 through the hybridization of the following combinations: H1 - HM5xLHP1, H2 - HM5xLHP2, H3 - HM5xLHP3, H4 - HM5xLHP4, H5 - HM5xLHP5, H6 - HM5xLHP6, and H7 - HM5xLHP7. In the process of creating pepper hybrids based on sterile lines (*ms* and *cms*), *ms* lines are predominantly used due to their stability and easier maintenance compared to *cms* lines, which are often unstable and challenging to sustain. In *ms* lines (nuclear sterility), segregation occurs, resulting in 50% sterile plants (used as female parents for hybridization) and 50% fertile plants (used for maintaining sterility). The *ms* line HM5, used as the female parent in these crosses, was obtained by segregating a foreign hybrid through pedigree selection.

In 2023, seeds of the hybrid combinations were sown in mid-March in pots measuring 6x6 cm, producing seedlings for further research. After 50-55 days, the plants formed 6-8 permanently developed leaves with a stem height of about 20 cm. At the end of May, the seedlings were transplanted in the greenhouse, with a distance of 30 cm between the plants in

a row. The greenhouse is 10 x 12 m, low tunnel type. The soil is covered with a black-colored mulch film, standard thickness for vegetable crops, 80 cm wide. The experimental trial was conducted in a randomized block design with three replications, each consisting of 40 plants. Standard agronomic practices (drip irrigation system, fertilization with additional feeding, and pest and disease control) were applied during the growing season. Harvesting was done at technological maturity, when the pepper fruits were light yellow in color. The selected pepper fruits were taken from the first harvest (August 9). The parameters analyzed included fruit weight, length, width, pericarp thickness, fruit number per plant, and the number of locules, determined as average values from samples of 10 fruits. The experimental hybrids studied belong to the medium-early maturity group and are characterized by indeterminate growth, robust and compact plants, and pendant fruits of the kapya type. Measurements were taken using a digital caliper. Dry matter content was determined by drying the fruits at 105°C to a constant mass. Sensory analysis was performed to evaluate fruit taste through organoleptic testing.

The data obtained were statistically analyzed using analysis of variance (ANOVA) and tested using the LSD test (Least Significant Difference) at significance levels of 5% and 1%. Correlation analysis (*Pearson's correlation coefficient*) was used to determine the relative dependence of the parameters, with coefficients tested at 5% and 1% significance levels. The statistical analysis was conducted using IBM SPSS Statistics, version 26.0, and the results were presented in tables and graphs.

Resuts and discussion

The results of the study on key morphological characteristics of hot pepper fruits are presented in Table 1, showing the average values of the analyzed parameters. Based on the statistical analysis of data from all hybrid combinations, fruit weight demonstrated a statistically highly significant effect. The highest average value was recorded in the experimental hybrid H6 (HM5xLHP6), with a weight of 119.70 g (Figure 1). This hybrid exhibited a heterotic effect for this trait, which provides an advantage over other combinations. In contrast, hybrid H7 (HM5xLHP7) showed a significantly lower value (85.56 g). The crossing of two pure pepper lines successfully produced F_1 offspring, demonstrating significant heterosis effects, especially in traits essential for stable production.

Table 1. Average values of the tested parameters of the experimental hybrids
Tabela 1. Prosečne vrednosti ispitivanih parametara eksperimentalnih hibrida

Experimental hybrid	FWe (g)	FLe (cm)	FWi (cm)	PT (mm)	NCh	NFp	DMc (%)
HM5xLHP1	114.92	17.16	6.02	5.20	2.00	12.00	7.20
HM5xLHP2	116.88	16.94	5.66	4.20	2.20	13.00	7.80
HM5xLHP3	107.26	16.24	5.50	5.40	2.00	16.60	7.90
HM5xLHP4	112.02	18.26	4.98	4.40	2.40	13.20	7.10
HM5xLHP5	104.44	17.96	4.72	3.80	2.20	16.80	9.40
HM5xLHP6	119.70	18.00	5.40	4.00	2.20	11.40	8.80
HM5xLHP7	85.56	15.40	5.06	4.00	2.00	16.00	10.50
Average	108.68	17.14	5.33	4.40	2.14	14.14	8.39
F-test	4.132	4.279	11.765	2.901	0.578	2.215	26.097
Sig.	**	**	**	*	ns	ns	**
LSD (p<0.05)	16.509	1.474	0.379	0.107	0.580	4.447	0.718
LSD (p<0.01)	22.374	1.997	0.515	0.145	0.786	6.026	0.974

Legend: Few - Fruit Weight; FLe - Fruit Length; FWi - Fruit Width; PT - Pericarp Thickness; NCh - Number of chambers; NFp - Number of fruits per plant; DMc - Dry matter content; $P < 0.01$very significant (**); $P < 0.05$ significant (*); $P > 0.05$ no significant (ns).

According to Bharadwej et al. (2007), fruit weight is one of the most critical components in pepper breeding, significantly influencing yield. Hot pepper hybrids exhibit greater tolerance to abiotic stress, leading to higher yields (Wei et al., 2020). The uniformity of hybrid fruits simplifies cultivation techniques and can reduce production costs (Rani et al. 2021). The experimental hybrids in this study produced fruits of the kapya type, suitable for fresh consumption and processing.

Picture 1. Experimental hot pepper hybrid (HM5xLHP6)
Slika 1. Eksperimentalni hibrid ljute paprike (HM5xLHP6)

The availability of hot pepper hybrids (*Capsicum spp.*) is limited compared to chili or sweet pepper hybrids (Kirana et al., 2024). The same author highlighted heterosis and reciprocity as critical indicators in F_1 hybrids, valuable for hybrid trait development. Hybrid development through heterosis can result in a 30–50% yield increase (Nalla et al., 2021). Traits such as fruit length, width, and pericarp thickness (Figure 2) are particularly important, especially when peppers are grown for industrial purposes. The longest fruit, measuring 18.26 cm, was recorded in hybrid H4 (HM5xLHP4), followed by H6 (HM5xLHP6) with 18.00 cm. Fruit length demonstrated a statistically highly significant effect across all hybrid combinations. A significant value was also observed in H1 (HM5xLHP1), with a fruit width of 6.02 cm. Kirana et al. (2024) reported heterosis in fruit weight per plant but not in fruit length or number of fruits, indicating variability in genetic effects across different traits.

Further studies noted significant improvements in key characteristics such as plant height, fruit maturity, fruit weight per plant, yield per hectare, and vitamin C content. Genotypic evaluation revealed distinct genetic differences between F_1 hybrids and their parents, including reciprocal F_1s. These phenotypic and genotypic changes suggest that hot peppers could become more productive and of higher quality when developed as hybrids, indicating a promising direction for future breeding programs. Amorim et al. (2021) also observed heterosis in productive traits of hot peppers but not in fruit length or the number of fruits per plant. Pericarp thickness showed a statistically significant effect in the hybrid combinations,

with average values ranging from 3.80 to 5.40 mm. Danojević and Medić-Pap (2018) reported a pericarp thickness of 5.80 mm in a newly selected sweet pepper variety. Similarly, Cvikić et al. (2007) documented a pericarp thickness of 4.40 mm in F_1 hybrids.

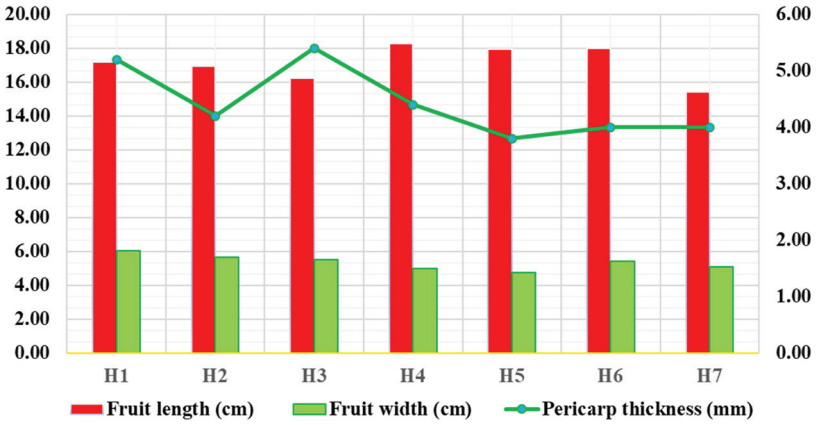

Figure 1. Average values of fruit length and width (cm) and pericarp thickness (mm)
Grafikon 1. Prosečne vrednosti dužine i širine ploda (cm) i debljine perikarpa (mm)
Legend/legenda: H1- HM5xLHP1, H2- HM5xLHP2, H3- HM5xLHP3, H4- HM5xLHP4, H5- HM5xLHP5, H6- HM5xLHP6, H7- HM5xLHP7

In industrial processing for pepper products, the dry matter content (%) of fruits plays a crucial role. Lama et al. (2020) emphasized that fruits with higher dry matter content have an advantage in storage, reducing mass loss during storage. Agronomic traits like fruit weight, pericarp thickness, and dry matter content are critical for industrial production, as noted by Nascimento et al. (2014). Significant values for dry matter content (Figure 2) were observed in the hybrid combinations HM5xLHP5 and HM5xLHP7 (9.40% and 10.50%, respectively). Experimental hybrid HM5xLHP6 also performed well, with a dry matter content of 8.80%.

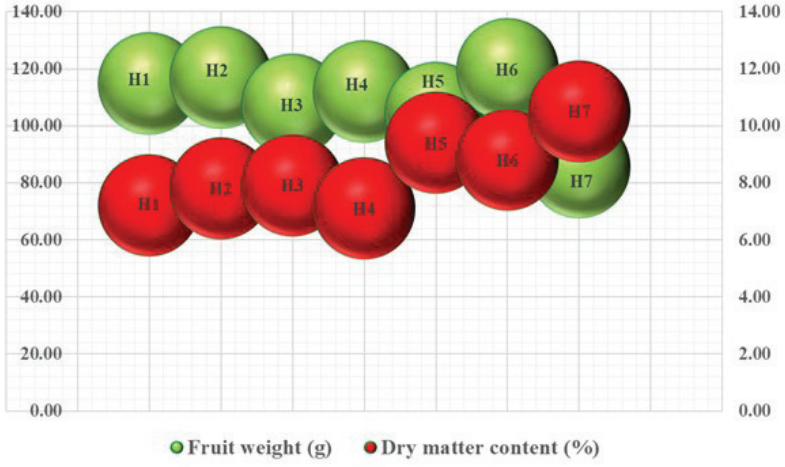

Figure 2. Average values of fruit weight (g) and dry matter content (%)
Grafikon 2. Prosečne vrednosti težine ploda (g) i sadržaj suve materije (%)
Legend/legenda: H1- HM5xLHP1, H2- HM5xLHP2, H3- HM5xLHP3, H4- HM5xLHP4, H5- HM5xLHP5, H6- HM5xLHP6, H7- HM5xLHP7

Fruits with greater weight exhibited higher pericarp thickness values, as reported by Danojević and Medić-Pap (2018). All hybrids combinations had a uniform number of fruit chambers (2), with no statistically significant effect observed for this trait. The number of fruits per plant ranged from 11.40 to 16.80 (Figure 3), with the highest value recorded in HM5xLHP5 (16.80). The approximate values for this trait suggest no statistically significant differences.

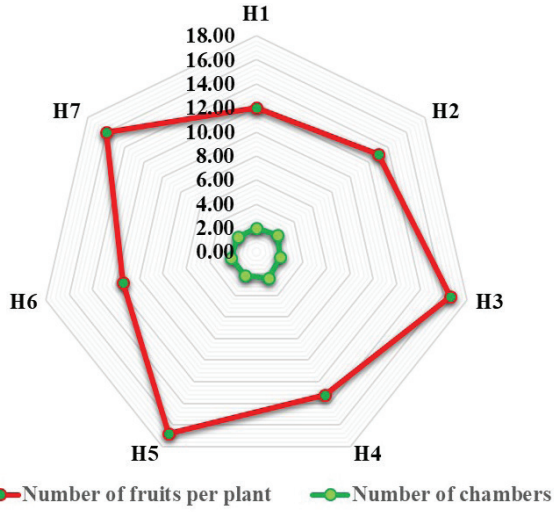

Figure 3. Average values of the number of fruits per plant and the number of chambers in experimental hybrids
Grafikon 3. Prosečne vrednosti broja plodova po biljci i broja komora kod eksperimentalnih hibrida.
Legend/legenda: H1- HM5xLHP1, H2- HM5xLHP2, H3- HM5xLHP3, H4- HM5xLHP4, H5- HM5xLHP5, H6- HM5xLHP6, H7- HM5xLHP7

The yield per plant in the experimental hybrids ranged from 2 to 4 kg, highlighting the high productivity of these hybrids. Following sensory organoleptic analysis, the experimental hybrids were characterized by spiciness. Hybrid seeds are a vital component of cultivation technology for this vegetable species, supporting rural development and economic growth (Sayekti et al., 2020). These hybrids are suitable for greenhouse production, where plants form a large number of fruits that ripen successively for harvest.

Table 2. Correlation coefficients analyzed for pepper fruit parameters
Tabela 2. Analizirani koeficijenti korelacije za parametre ploda paprike

Parameters of fruit pepper	FWe	FLe	FWi	PT	NCh	NFp	DMc
FWe (g)	1	0.481**	0.541**	0.348*	0.450**	-0.315	-0.503**
FLe (cm)	0.481**	1	-0.100	-0.041	0.009	-0.169	-0.306
FWi (cm)	0.541**	-0.100	1	0.561**	0.042	-0.303	-0.416*
PT (mm)	0.348*	-0.041	0.561**	1	0.134	0.007	-0.432**
NCh	0.450**	0.009	0.042	0.134	1	-0.237	-0.159
NFp	-0.315	-0.169	-0.303	0.007	-0.237	1	0.180
DMc (%)	-0.503**	-0.306	-0.416*	-0.432**	-0.159	0.180	1

Legend: Few - Fruit Weight; FLe - Fruit Length; FWi - Fruit Width; PT - Pericarp Thickness; NCh - Number of chambers; NFp - Number of fruits per plant; DMc - Dry matter content; ** Correlation is significant at the 0.01 level; * Correlation is significant at the 0.05 level.

Correlation analysis revealed positive correlations between nearly all fruit traits (Table 2). Significant and strong positive correlations were established between fruit weight and fruit length ($r = 0.481^{**}$), fruit weight and fruit width ($r = 0.541^{**}$), fruit weight and pericarp thickness ($r = 0.348^{**}$), and fruit weight and the number of chambers ($r = 0.450^{**}$). Danojević et al. (2016) also reported a positive correlation between fruit weight and pericarp thickness, aligning with the results of this study. A negative correlation was observed between fruit weight and dry matter content ($r = -0.503^{**}$) and between fruit weight and the number of fruits ($r = -0.315^{**}$).

Conclusion

The evaluation of genetic divergence among experimental hot pepper hybrids revealed statistically significant differences, providing valuable insights for improving breeding programs. Fruit weight, a key trait for yield potential, varied significantly among hybrids, with the highest value recorded in the experimental hybrid HM5xLHP6. Fruit length and dry matter content also exhibited statistically significant effects. The fruits are uniform, hot-tasting, and belong to the medium-early maturity group with a kapya type shape. Due to its high potential, the hybrid HM5xLHP6 was selected and submitted for official registration to the Commission for the Recognition of New Varieties at the beginning of 2024, with the first year of testing completed. The observed genetic diversity and significant phenotypic expressions of traits highlight the potential of these hybrids for enhancing the assortment and production of high-value hot peppers.

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Author contributions

Conceptualization, B.Š. and D.C.; Methodology, B.Š. and J.S.; Software, B.Š.; Data curation, M.U.; Formal analysis, M.B.; Investigation, B.Š.; J.S. and I.T.; Resources, D.C.; Project administration, J.S. and I.T.; Supervision, S.S. and M.U.; Visualization, M.B.; S.S. and K.L.; Validation, J.S., I.T. and M.U.; Funding acquisition, D.C.; Writing — original draft, B.Š.; J.S. and I.T.; Writing — review & editing, J.S.; I.T. and K.L.

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GENETIČKA DIVERGENTNOST LINIJA LJUTIH PAPRIKA (*Capsicum spp.*) U HIBRIDNIM KOMBINACIJAMA

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Sažetak

U oplemenjivačkim programima se stavlja akcenat pri stvaranju novih genotipova na osobine kao što su ranostasnost, kvalitet ploda, visina prinosa, otpornost na ekonomski značajne bolesti, tolerantnost na abiotički stres, morfologiju biljaka. Prednosti hibrida u odnosu na sorte su mnogobrojne poput povećanja prinosa, uniformnosti plodova, otpornosti na biljne patogene, pojava heterozisa i dr. To su razlozi zbog kojih se započelo istraživanje sa hibridnim kombinacijama ljutih paprika. Cilj ovog rada bio je da se stvori hibrid ljute paprike, koji će moći da odgovori postavljenim zahtevima na tržištu. Korišćeno je sedam eksperimentalnih hibrida paprike stvorenih ukrštanjem *ms* linije HM5 (jedarna muška sterilnost) kao linije majke i sedam čistih linija kao linije oca (LHP 1-7) i proizvedena su semena hibridnih kombinacija. Tokom 2023. godine postavljen je ogled po randomiziranom blok sistemu u tri ponavljanja sa po 40 biljaka u plasteniku Instituta za povrtarstvo Smederevska Palanka. Berba je obavljena u tehnološkoj zrelosti plodova. Analizirane su najvažnije morfološke osobine ploda i sadržaj suve materije (SSM) na 10 plodova (tip kapije) od svake kombinacije. Rezultati pokazuju da su hibridne kombinacije ispoljile statistički vrlo značajan uticaj kod parametara masa, dužina i širina ploda, debljina perikarpa i SSM. Najbolje rezultate mase ploda je ostvario hibrid HM5xLHP6 (119.70 g), dok je najveća dužina izmerena kod HM5xLPH4 (18.26 cm). Najveću širina plodu od 6.02 cm je imao hibrid HM5xLHP1 i debljina perikarpa od 5.2 mm hibrid HM5xLHP3. Visok SSM je zabeležen kod HM5xLHP7 i HM5xLHP5 (10.5% i 9.40%). Broj plodova po biljci (11.40-16.80) i broj komora (2) je bio približan kod različitih hibrida i nije bilo statistički značajne razlike. Poređenjem sorti koje su prisutne na tržištu u odnosu na hibride, kojih ima veoma malo, na osnovu rezultata ovog rada možemo zaključiti superiornost hibrida za gajenje u zaštićenom prostoru sa opravdanim ekonomskim efektom, za svežu upotrebu i industrijsku preradu.

Ključne reči: metod ukrštanja, sterilne linije, morfološke osobine

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