

Development of *Phytophthora capsici*–resistant Kahramanmaraş pepper lines using marker-assisted selection and anther culture

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Abstract

Phytophthora root and crown rot, caused by *Phytophthora capsici*, is one of the most destructive diseases limiting pepper (*Capsicum annuum* L.) production, particularly in regions where local cultivars such as Kahramanmaraş pepper are widely grown. This study aimed to rapidly develop *P. capsici* resistant Kahramanmaraş pepper type by integrating marker-assisted selection (MAS) with in vitro anther culture. A total of 198 genotypes belonging to five backcross-derived GM2 populations were developed by crossing a susceptible Kahramanmaraş pepper genotype with five resistant donor parents. Molecular screening was performed using the Phyto.5.2 SCAR marker to identify resistant genotypes at the seedling stage. Phenotypic variation in plant morphology and fruit-related traits was evaluated using principal component analysis (PCA). Genotypes identified as resistant were subsequently used as donor plants for anther culture to generate homozygous double haploid (DH) lines. Molecular analyses revealed that 83 genotypes were resistant to *P. capsici*. PCA results demonstrated substantial variation among genotypes in terms of yield and morphological traits, with resistant individuals distributed across diverse phenotypic backgrounds. Anther culture efficiency varied among hybrid combinations, with the highest success observed in the KP × P1 combination. The resulting DH lines exhibited wide variation in yield and fruit quality traits relevant to dried pepper production. Overall, the combined application of MAS, multivariate phenotypic analysis, and anther culture proved to be an effective and time-saving breeding strategy for developing *P. capsici*–resistant and agronomically superior pepper lines. The DH lines obtained in this study represent valuable genetic resources for future cultivar development and sustainable pepper production.

Keywords: Anther Culture, Double Haploid, Marker Assisted Selection, Pepper breeding, *Phytophthora capsici*

INTRODUCTION

Due to increasing global warming, maintaining agricultural production under adverse soil conditions has become a major challenge. This issue has attracted increasing scientific and practical attention (Yuan et al., 2024). Vegetable cultivation, like other plants, is significantly affected by biotic and abiotic stress factors. Stress in plants is caused by biotic factors such as viruses, bacteria, fungi and harmful organisms, as well as environmental (abiotic) factors such as salt, water deficiency, high or low temperature, nutrient imbalances, heavy metal toxicity, air pollution and radiation (Aydın 2024; Aydın et al., 2025; Başak et al., 2025; Coşkun et al., 2025). These factors disrupt the growth and development processes of the plant, eventually causing yield and quality losses (Janmohammadi & Sabaghnia, 2023; Lesk et al., 2021; Li et al., 2022; Minoli et al., 2022; Zhang et al., 2023; Zhu et al., 2022).

Phytophthora root and crown rot, caused by the oomycete *Phytophthora capsici* (Leon.) (Pythiaceae, Peronosporales), is considered one of the most devastating pathogens in *Capsicum annuum* L. (Solanaceae) production, potentially leading to total yield loss (Bagheri et al., 2020; Barchenger et al., 2018; Mohammadbagheri et al., 2021; Sánchez-Rodríguez et al., 2016). *P. capsici* not only causes root rot but also causes a variety of disease syndromes such as stem, leaf and fruit blight, posing a constant threat throughout the plant's life cycle (Foster et al., 2013; Naegele & Hausbeck, 2014). Kahramanmaraş pepper, which is an important pepper production province and its surrounding regions, has experienced significant yield and quality losses due to *Phytophthora capsici*. Although several studies have addressed this pathogen, comprehensive efforts aimed at developing resistant varieties remain limited. As with many plant species, breeding a new variety, introducing

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desired traits, and yield trials before it can be commercially released requires a significant amount of time. Achieving this goal with traditional breeding methods is particularly time-consuming.

Marker-assisted selection (MAS) offers significant advantages in plant breeding, especially in the identification and selection of polygenic traits (Thabuis et al., 2003; Aydın et al., 2024). This method enables sensitive and rapid identification of complex traits controlled by multiple genes and influenced by environmental interactions. MAS significantly accelerates the breeding process and increases its efficiency compared to traditional selection methods. Integrating molecular markers into traditional breeding programs facilitates the development of high-yielding, high-quality and disease-resistant varieties by enabling early detection of desired traits. One of the widely used biotechnological approaches for rapid development of pure lines in pepper (*Capsicum annuum* L.) breeding is anther culture, a tissue culture-based technique. The anther is a unique generative tissue that enables haploid plant production and is widely used in plant tissue culture applications. Haploid plants obtained through anther culture can be converted into double haploid (DH) lines via chromosome doubling, allowing the rapid development of homozygous genotypes. (Şahin et al., 2025). This technique allows the production of haploid plants directly or indirectly from male gametophytes, and subsequent chromosome doubling allows the generation of homozygous lines in a significantly shorter timeframe than traditional methods (Parra-Vega & Seguí-Simarro, 2016).

This study aimed to develop breeding lines of the Kahramanmaraş pepper variety resistant to *Phytophthora capsici* using marker-assisted selection and in vitro anther culture. This integrated approach allows rapid development of improved lines for pepper-growing regions where the pathogen is a major problem.

MATERIALS AND METHODS

Seeds of hybrid plants (GM2) were used as the plant material. The hybrids were generated by crossing a Kahramanmaraş pepper genotype (female parent), previously characterized as susceptible to *Phytophthora capsici*, with five resistant genotypes (P1–P5) as male parents. The donor genotypes (P1–P5) belong to *Capsicum annuum* L. and were previously identified as resistant to *P. capsici*, exhibiting variation in fruit morphology and plant growth characteristics. The progeny was advanced through two successive backcrosses. The study included five hybrid combinations and the number of plants used in each combination is presented in Table 1.

Table 1. Hybrid Combinations and Number of Plants

Female Parent	Male Parent	Number of Plants
Kahramanmaraş pepper	P1 426	27
Kahramanmaraş pepper	P2 667	44
Kahramanmaraş pepper	P3 532	42
Kahramanmaraş pepper	P4 435	40
Kahramanmaraş pepper	P5 419	45
Total	-	198

Morphological and Fruit Traits of Backcross-Derived Plants

A total of 198 genotypes obtained from five different backcross populations were evaluated in terms of plant morphology and fruit-related characteristics. The measured parameters for each genotype are presented in Table 2.

Table 2. Measured Plant and Fruit Traits

Parameters	Measurement Methods
Plant Height (cm)	Measured from the base of the plant to the growing point using a ruler.
Plant Diameter (mm)	Measured at the stem base using a caliper and recorded in millimeters.
Branching Intensity	Scored as 3 = low, 5 = medium, 7 = high.
Average Fruit Diameter (mm)	Measured at the middle part of the fruit using a caliper.
Average Fruit Length (cm)	Measured from the calyx to the blossom end using a caliper.
Average Fruit Weight (g)	Determined using a precision scale (0.01 g sensitivity).
Average Pericarp Thickness (mm)	Measured at the middle part of the fruit using a caliper.
Total Red Fruit Weight per Plant (g)	Determined using a precision scale (0.01 g sensitivity).
Total Green Fruit Weight per Plant (g)	Determined using a precision scale (0.01 g sensitivity).
Number of Red Fruits per Plant	Red fruits on each plant were counted individually.
Number of Green Fruits per Plant	Green fruits on each plant were counted individually.

Determination of Resistance to *Phytophthora capsici* in Backcross Populations

Seeds were germinated in a growth medium composed of 2:1 peat to perlite. DNA extraction was performed at the first true leaf stage. The CTAB (Cetyl Trimethyl Ammonium Bromide) method described by Doyle & Doyle (1990) was used for DNA isolation. DNA concentrations were determined via 1% agarose gel electrophoresis and DNA samples were diluted to a final concentration of 10 ng/µl for PCR analysis. Molecular analysis was performed using the dominant SCAR marker OpD04.717, which is tightly linked to the Phyto.5.2 quantitative trait locus (QTL) located on chromosome 5 and associated

with resistance to *Phytophthora capsici* in pepper. The SCAR primer pair (OpD04.717-F: 5'-CCA TAA GGG TTG GTA AAT TTA CAA AG-3' and OpD04.717-R: 5'-TCG AGA GAT AAT TCA GAT AGT ATA ATC-3') amplifies an 717 bp fragment in resistant genotypes (Quirin et al., 2005). PCR amplification was performed in a total reaction volume of 15 µL. The reaction mixture contained 1.5 µL of 10× PCR buffer, 1.5 µL of MgCl₂ (25 mM), 1 µL of dNTP mix (10 mM), 0.2 µL of Taq DNA polymerase (5 U/µL), 1 µL each of forward and reverse primers (10 µM), and 1.5 µL of genomic DNA (5 ng/µL). Nuclease-free water was added to adjust the final reaction volume to 15 µL. The PCR thermal cycling conditions consisted of an initial denaturation at 94 °C for 2 min, followed by 32 cycles of denaturation at 94 °C for 30 s, annealing at 45 °C for 30 s, and extension at 72 °C for 1 min. A final extension was performed at 72 °C for 5 min. Following molecular analysis, all plants identified as either resistant or susceptible were transferred to the greenhouse, where standard agronomic practices were carried out until harvest.

Anther Culture Studies

For the purpose of anther culture, flower buds were collected from plants previously identified as resistant to *Phytophthora capsici* during the months of April, May, and June, which are characterized by mild climatic conditions in the Kahramanmaraş region. Flower buds were selected at a developmental stage where the petals did not exceed the sepals and were at the same level. Prior to surface sterilization, the buds were subjected to a cold pretreatment at 4°C for 24 h. A Murashige and Skoog (MS) basal medium was prepared for anther culture, supplemented with 4 mg/L NAA (naphthaleneacetic acid), 0.5 mg/L BAP (benzylaminopurine), 0.25% activated charcoal, 30 g/L sucrose, and 15 mg/L AgNO₃ (Buyukalaca et al., 2004). Following embryo formation in the anthers, embryo conversion into plants was carried out using an MS medium devoid of growth regulators and containing 30 g/L sucrose. After the addition of all necessary chemicals, the pH of the medium was adjusted to 5.8 using 1 N HCl and 1 N KOH. Agar at a concentration of 8 g/L was used as the gelling agent. The media were sterilized in an autoclave at 121°C and 1.2 kg/cm² pressure for 15 minutes. After sterilization, under aseptic conditions, the media were distributed into sterile 6 cm Petri dishes and 15 cm test tubes at volumes of 10 mL per Petri dish and 10 mL per tube. Each Petri dish contained five anthers, with a total of 100 anthers cultured per backcross population. Once embryo development was observed, the embryos were transferred to hormone-free media (jars) for plant regeneration. Regenerated plantlets were first transferred to seedling trays (trays with cells) and then to pots for further development. Since doubled haploid plants were directly obtained in this study, no colchicine treatment was applied to haploid plants, and such individuals were excluded from further evaluation. The homozygous pepper lines obtained were planted in a greenhouse in order to evaluate plant growth, yield performance, and certain fruit quality traits relevant to powdered pepper production. The evaluations were conducted based on the morphological and yield parameters listed in Table 2.

Statistical analysis

Principal component analysis (PCA) was conducted using Minitab Statistical Software version 22 (Minitab LLC, State College, PA, USA). Z-score normalization and heatmap analysis were performed using GraphPad Prism version 8 (GraphPad Software, Boston, MA, USA).

RESULTS

A total of 198 genotypes derived from five backcross populations (KP × P1–P5) were evaluated using molecular and phenotypic analyses. Screening with the Phyto.5.2 SCAR marker identified 83 genotypes carrying the 717 bp amplification band associated with *Phytophthora capsici* resistant. Principal component analysis (PCA) revealed substantial phenotypic variation among genotypes within each hybrid combination. In the KP × P1 population, the first two principal components (PC1 and PC2) explained 42% and 20% of the total variation, respectively, accounting for 62% of overall diversity. Resistant genotypes were distributed across the PCA space without clustering into a single phenotypic group. Eleven genotypes were classified as resistant based on marker analysis (Figure 1).

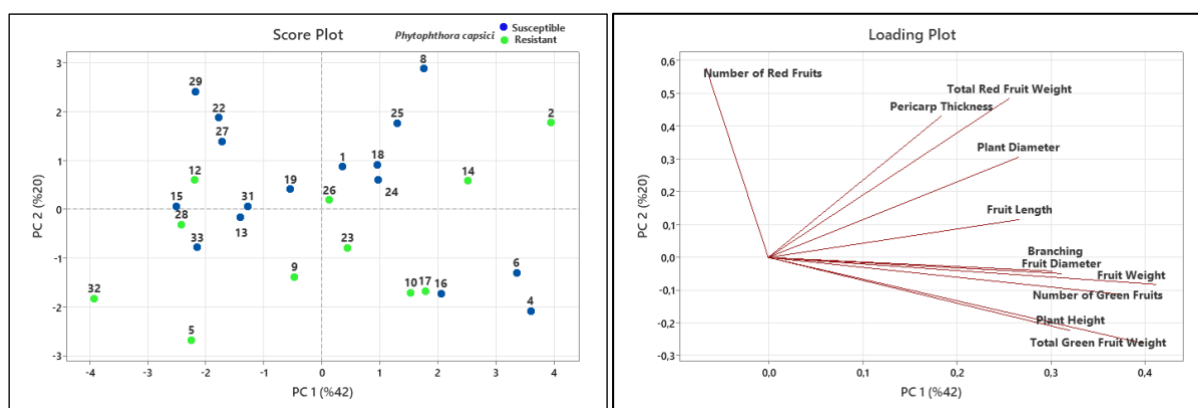


Figure 1. Principal component analysis plot representing the variation in plant and fruit characteristics among Kahramanmaraş pepper × P1 hybrid (GM2) genotypes.

In the KP × P2 population, PC1 and PC2 explained 33% and 21% of the total variation, respectively, representing 54% of cumulative variation. Eighteen genotypes were identified as resistant. Genotypes exhibited a broad distribution across the PCA plane, indicating variability in morphological and yield-related traits (Figure 2).

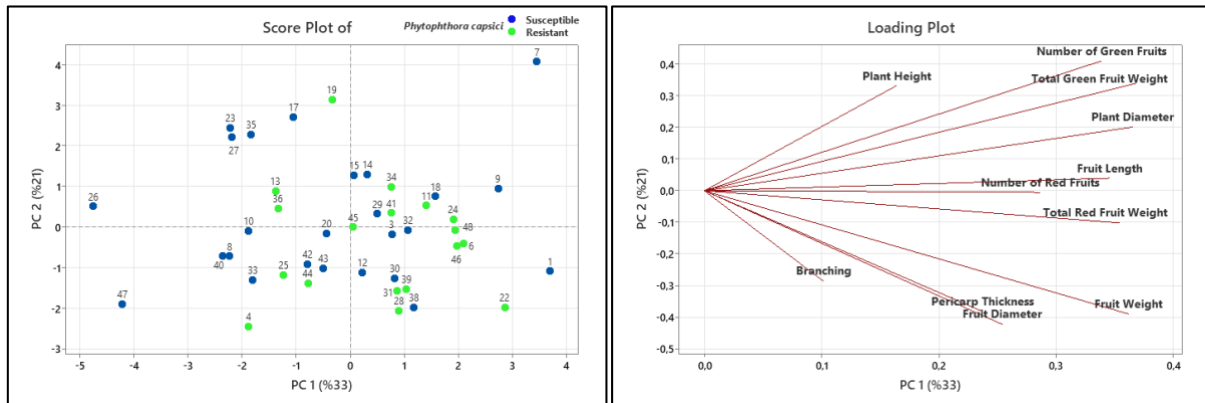


Figure 2. Principal component analysis plot representing the variation in plant and fruit characteristics among Kahramanmaraş pepper × P2 hybrid (GM2) genotypes.

For the KP × P3 population, PC1 (28%) and PC2 (21%) together accounted for 49% of total variation. Resistant and susceptible genotypes showed separation along both principal components. Variation was observed for vegetative growth and yield-related parameters (Figure 3).

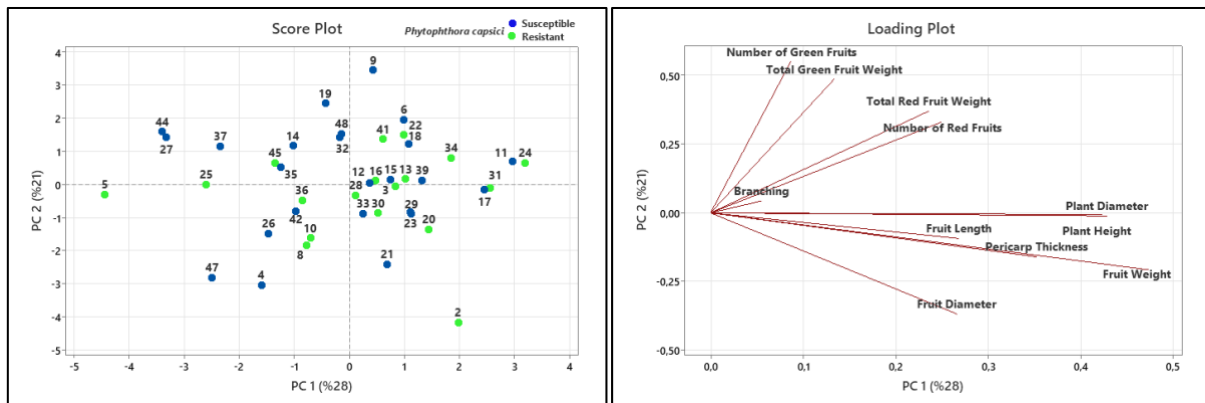


Figure 3. Principal component analysis plot representing the variation in plant and fruit characteristics among Kahramanmaraş pepper × P3 hybrid (GM2) genotypes.

In the KP × P4 population, PC1 and PC2 explained 50% and 15% of total variation, respectively, accounting for 65% of cumulative variation. Fifteen genotypes were identified as resistant. Genotype 20 exhibited an extreme position along both principal components (Figure 4).

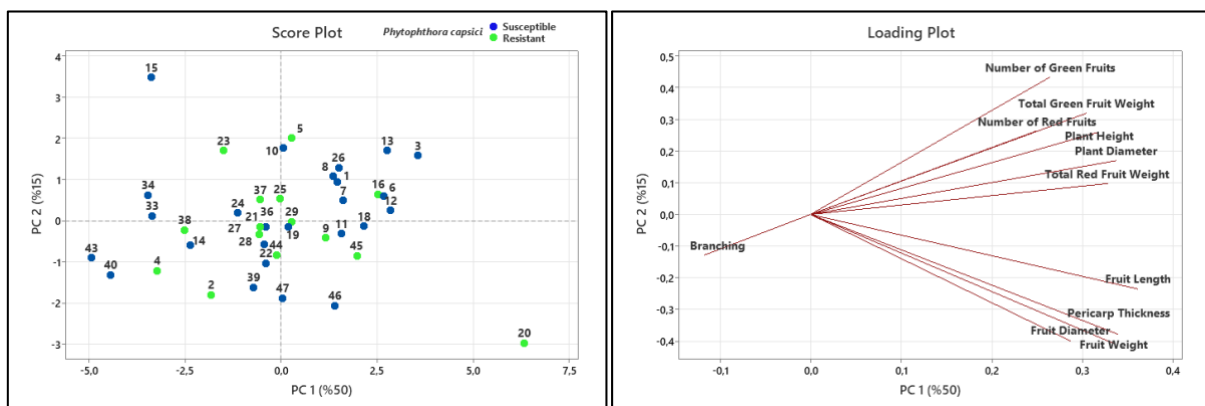


Figure 4. Principal component analysis plot representing the variation in plant and fruit characteristics among Kahramanmaraş pepper × P4 hybrid (GM2) genotypes.

For the KP × P5 population, PC1 (33%) and PC2 (20%) explained 53% of total phenotypic variation. Twenty-one genotypes were classified as resistant. Genotypes displayed dispersion across the PCA space, reflecting variation in fruit morphology and yield components (Figure 5).

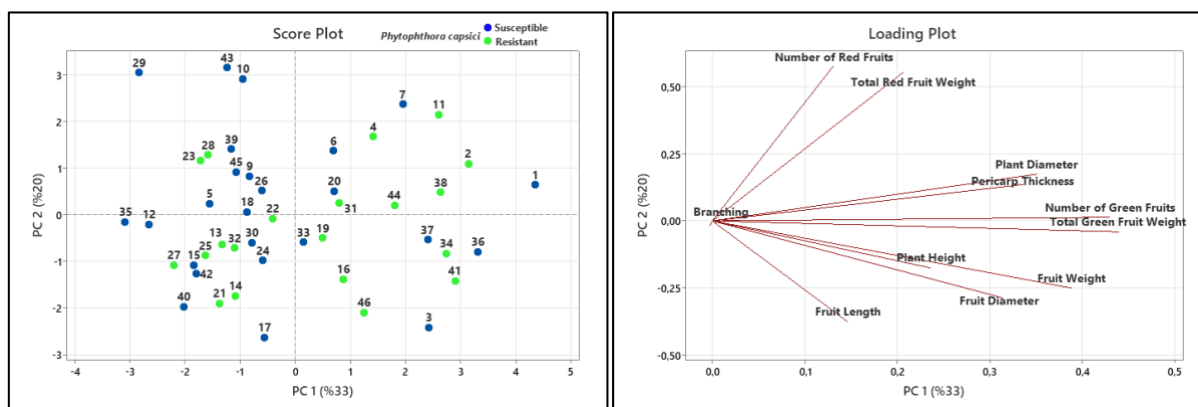


Figure 5. Principal component analysis plot representing the variation in plant and fruit characteristics among Kahramanmaraş pepper × P5 hybrid (GM2) genotypes.

Genotypes identified as resistant were used as donor plants for anther culture. Anther development rates varied among hybrid combinations, ranging from 46% to 65%. The highest anther development rate was observed in the KP × P1 combination (65%), followed by KP × P3 (57%), KP × P2 (55%), KP × P5 (54%), and KP × P4 (46%). Embryo formation ranged from 35 to 55 embryos per combination. Plant regeneration ranged between 25 and 41 plants. The target number of double haploid (DH) plants (20 per combination) was achieved in all combinations except KP × P4, where 10 DH plants were obtained (Table 3). All regenerated plants were identified as spontaneous double haploids, and no artificial chromosome doubling treatment was applied.

Table 3. Cultured anthers, developed anthers, development rate (%), number of embryos, and plant regeneration in hybrid combinations.

Hybrid combinations	Number of anthers cultured (pcs)	Number of developed anthers (pcs)	Anther development rate (%)	Number of embryos (pcs)	Number of regenerated plants (pcs)	Number of double haploid plants (pcs)
KP X P1	100	65	65	55	41	20
KP X P2	100	55	55	52	38	20
KP X P3	100	57	57	45	33	20
KP X P4	100	46	46	35	25	10
KP X P5	100	54	54	51	36	20

Z-score normalization of morphological and fruit-related traits revealed pronounced variability among DH genotypes derived from the KP × P1 combination. Several genotypes exhibited positive deviations across yield-related parameters, particularly total red fruit weight and number of red fruits per plant. Genotype 5 displayed consistently high standardized values for red fruit yield components. In contrast, Genotypes 1 and 2 showed negative deviations across multiple evaluated traits. Genotype 12 exhibited positive deviations primarily in fruit morphological traits, including fruit diameter and fruit length. Genotypes 10 and 11 demonstrated intermediate standardized values across both yield- and morphology-related parameters (Figure 6).

Standardized trait distribution indicated substantial variation among DH genotypes within the KP × P2 population. Genotype 12 exhibited positive deviations for average fruit weight, pericarp thickness, and total green fruit weight per plant. Genotype 20 showed positive deviations associated with fruit size-related parameters, particularly fruit diameter and fruit length. Genotype 8 presented negative deviations in red fruit yield components. Genotype 6 demonstrated positive deviations in green fruit-related traits but comparatively lower standardized values for red fruit yield parameters (Figure 7).

Z-score normalization highlighted variability in both yield- and morphology-related traits among DH genotypes derived from the KP × P3 combination. Genotypes 12 and 13 displayed positive deviations for red fruit weight and number of red fruits per plant. Negative deviations across red fruit-related traits were observed in Genotypes 7 and 17. Genotype 7, however, exhibited a positive deviation in the number of green fruits per plant. The remaining genotypes showed a heterogeneous distribution of standardized trait values (Figure 8).

Considerable phenotypic variation was detected among DH genotypes in the KP × P4 population. Genotype 9 exhibited positive deviations across multiple fruit-related parameters, including fruit diameter, fruit length, fruit weight, and red fruit weight per plant. Genotype 4 showed positive deviations primarily associated with fruit weight and pericarp thickness. Genotypes 2 and 7 displayed negative deviations across several yield- and quality-related traits. Genotype 10 demonstrated positive deviations for fruit diameter, fruit weight, and red fruit weight, while maintaining intermediate values for other traits (Figure 9).

Z-score standardized data revealed wide variation among DH genotypes derived from the KP × P5 combination. Genotype 10 displayed positive deviations for red fruit weight, number of green fruits, and total yield-related parameters. Genotype 9

exhibited positive deviations primarily associated with fruit size and fruit weight. Negative deviations across multiple traits were observed in Genotypes 4, 7, and 11. Genotype 20 showed positive deviations for plant height, pericarp thickness, and green fruit-related parameters (Figure 10).

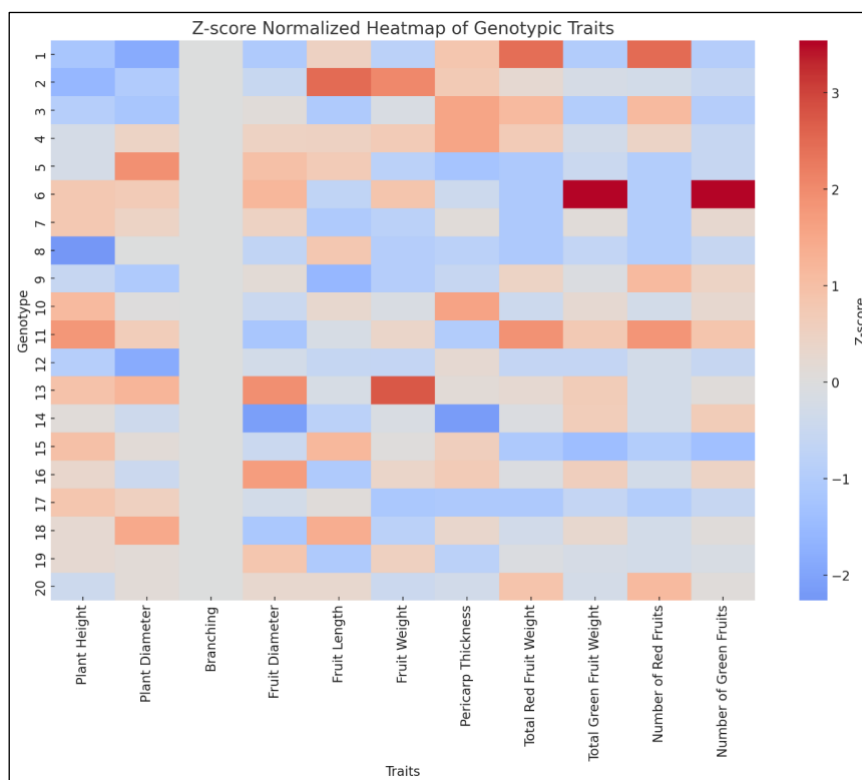


Figure 6. Heatmap of Z-Score Normalized Morphological and Fruit Traits of Double Haploid Genotypes Derived from the Kahramanmaraş Pepper × P1 Cross (GM2)

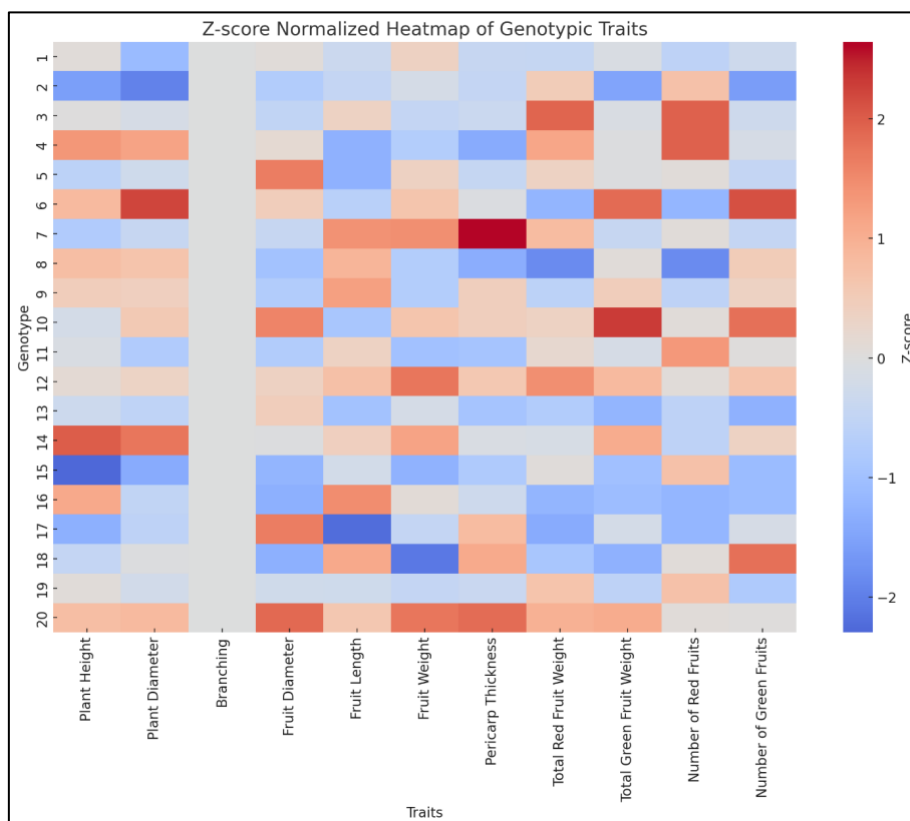


Figure 7. Heatmap of Z-Score Normalized Morphological and Fruit Traits of Double Haploid Genotypes Derived from the Kahramanmaraş Pepper × P2 Cross (GM2)

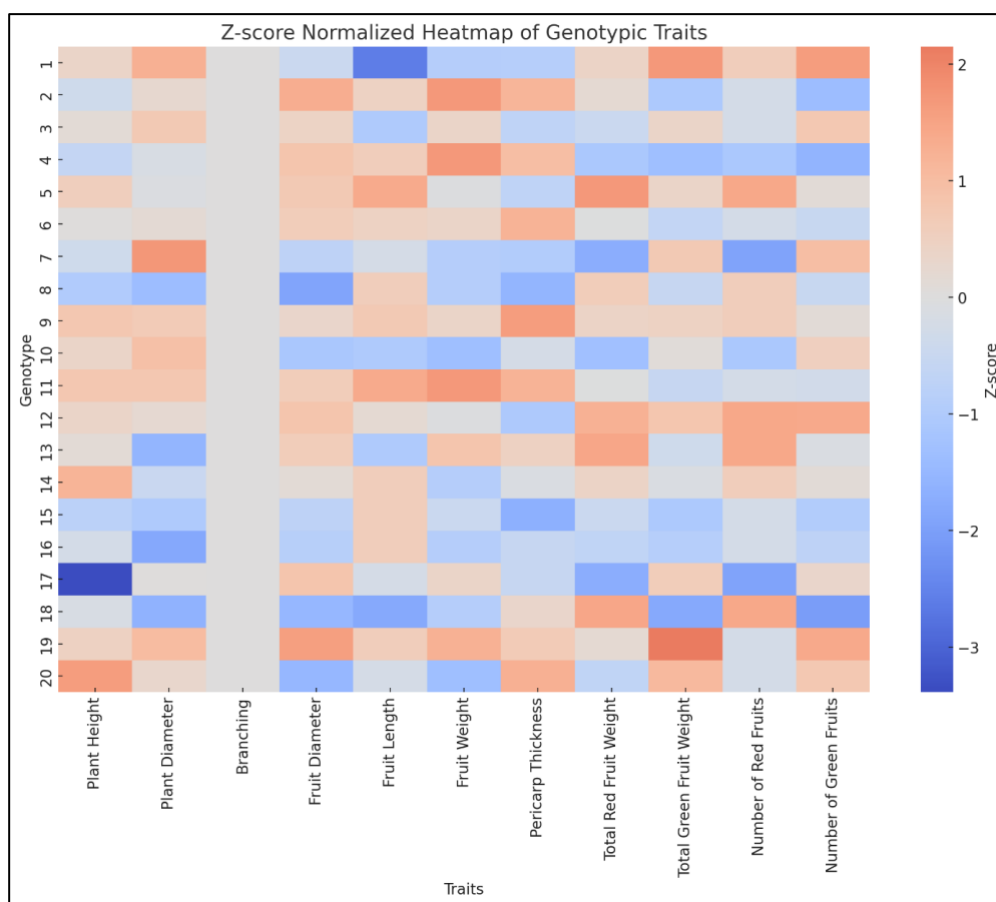


Figure 8. Heatmap of Z-Score Normalized Morphological and Fruit Traits of Double Haploid Genotypes Derived from the Kahramanmaraş Pepper × P3 Cross (GM2)

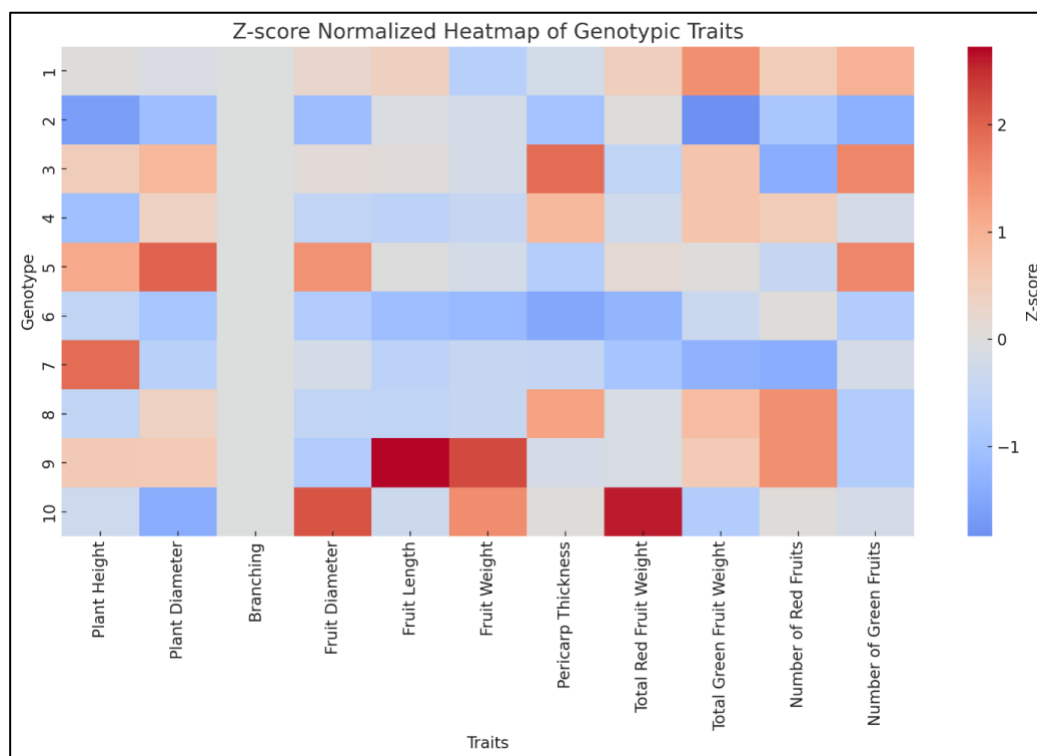


Figure 9. Heatmap of Z-Score Normalized Morphological and Fruit Traits of Double Haploid Genotypes Derived from the Kahramanmaraş Pepper × P4 Cross (GM2)

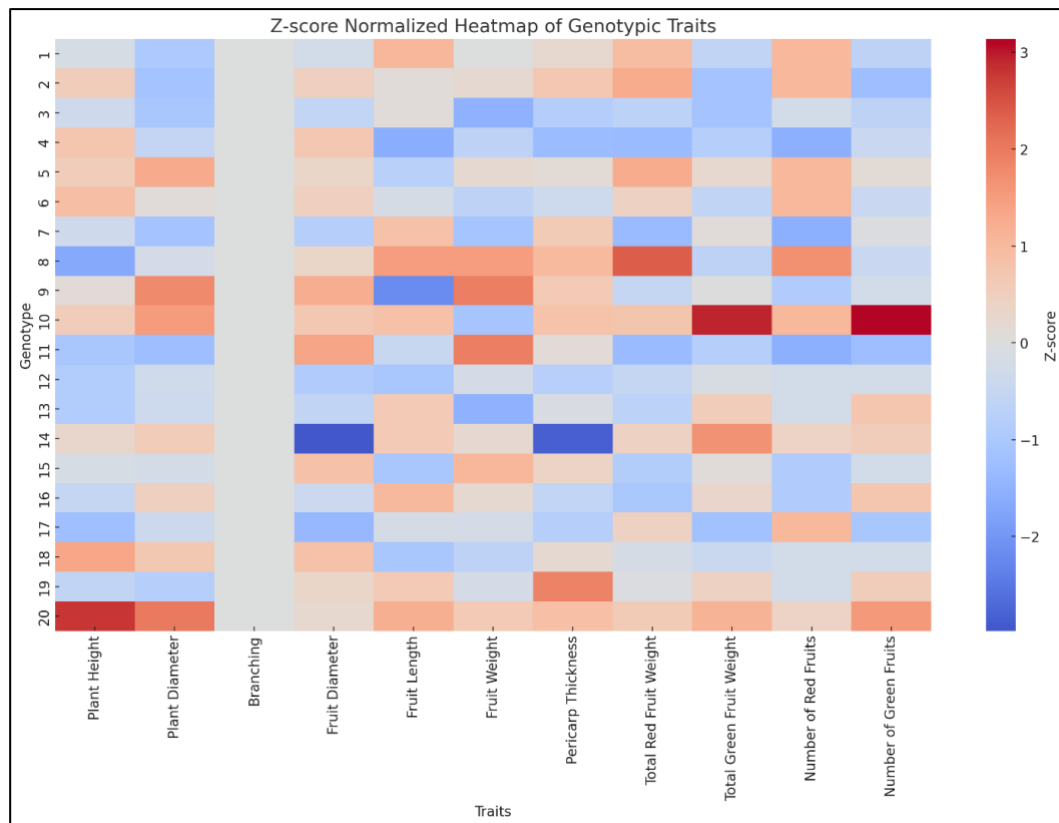


Figure 10. Heatmap of Z-Score Normalized Morphological and Fruit Traits of Double Haploid Genotypes Derived from the Kahramanmaraş Pepper $\times$ P5 Cross (GM2)

DISCUSSION

A total of 198 genotypes belonging to the GM2 population, obtained from crosses between Kahramanmaraş pepper and five different parents (P1–P5), were evaluated and 83 of them were identified as resistant to *Phytophthora capsici*. Similarly, in a study conducted on 95 pepper genotypes, Ateş & Yılmaz (2020) identified seven genotypes as resistant to *P. capsici* using the SCAR marker (OpD04.717) and this finding was also confirmed by classical seedling dip tests. This result indicates the presence of genetic variation for disease resistance within the population and demonstrates that resistant individuals represent valuable genetic resources that can be utilized in breeding programs. Genetic sources of resistance to *Phytophthora capsici* have been reported in a limited number of registered genotypes worldwide. Among these resistance sources, lines such as Criollo de Morelos 334 (CM334), PI 201232, PI 201234, PI 201237, and PI 640532, which originate from southern Mexico and belong to the species *Capsicum annuum*, are particularly prominent (McGregor et al., 2011). Among these genotypes, the CM334 line in particular is widely used in international pepper breeding programs because it exhibits a high level of resistance not only to *P. capsici* but also to certain viruses and other fungal pathogens (Quirin et al., 2005). In addition, AC2258 of Central American origin (Ares et al., 2005), the Indian-origin cultivar ‘Perennial’ (Thabuis et al., 2004) and the local genotype KM211 reported from Türkiye (Göçmen et al., 2019) are also considered important materials in terms of resistance genes.

The results of the Principal Component Analysis (PCA) revealed that the genotypes derived from crosses between Kahramanmaraş pepper and P1–P5 exhibited diversity in terms of morphological and yield-related traits. For each hybrid combination, the first two principal components obtained from the PCA explained a large proportion of the total variation and were effective in discriminating differences among the genotypes. In particular, the PC1 and PC2 components explained a total of 62% of the variation in the Kahramanmaraş pepper $\times$ P1 hybrid, while this proportion was determined as 54% in the P2 hybrid, 49% in the P3 hybrid, 65% in the P4 hybrid, and 53% in the P5 hybrid. This indicates that each hybrid combination contains a certain level of phenotypic diversity within itself and that the genotypes are distinctly positioned on the PCA plane. It has been stated that the most important factor affecting the response to androgenesis is the genetic constitution of the donor plants, and that this effect can show significant variation not only among species but also among genotypes within the same species (Irikova et al., 2011; Phippen & Ockendon, 1990; Taşkın et al., 2011). Therefore, the identification of donor plants that are both highly productive and highly responsive to tissue culture is of great importance for the efficiency of androgenetic haploid production.

It has been clearly demonstrated, based on both the findings of this study and similar studies reported in the literature, that success rates in the anther culture process are significantly influenced by genetic background. This situation is also consistent with the study conducted by Şahin et al. (2025), in which Besni pepper genotypes belonging to *Capsicum annuum* were evaluated. In that study, embryo formation rates among genotypes were reported to ranged from 0.0% to 45.3%. Similarly,

in the present study, marked differences were observed in anther development, embryo formation, and regeneration rates depending on the hybrid combinations; in particular, the KP × P1 combination stood out with high success, whereas KP × P4 exhibited low performance. The superior androgenic response observed in the KP × P1 combination can be explained by the well-documented genotype dependency of anther culture. Numerous studies have demonstrated that androgenesis efficiency is strongly influenced by genetic background, even when donor plants are grown under identical environmental conditions (Keles et al., 2015; Durna, 2016). Genotype has been consistently identified as the most critical factor affecting embryo formation frequency and subsequent plant regeneration (Parra-Vega et al., 2013). Furthermore, it has been reported that anthers cultured within the same period may exhibit markedly different developmental responses depending on genotype (Taşkın et al., 2011). In this context, the enhanced performance of KP × P1 may reflect a genetic constitution more favorable for microspore embryogenesis and regeneration competence. Considering both the present findings and previous reports, the observed variation in anther culture response can be primarily attributed to genotype-dependent effects. The double haploid (DH) individuals obtained from different hybrid combinations clearly exhibited significant variation in morphological and yield-related traits. Within each combination, some genotypes showed above-average performance for specific traits, whereas others exhibited limited values in terms of yield and quality. The high level of variation observed represents considerable potential not only for the Kahramanmaraş region but also for other major dried pepper production areas in Türkiye. These developed pure DH lines may enable the development of dried pepper cultivars adapted to diverse ecological conditions in the coming years. Consequently, it may be possible to develop cultivars with both high regional adaptability and suitability for industrial use.

CONCLUSION

This study demonstrates that the integration of marker-assisted selection (MAS) with in vitro anther culture is an effective and time-efficient strategy for the development of *Phytophthora capsici*-resistant breeding lines in Kahramanmaraş pepper (*Capsicum annuum* L.). Molecular screening using the Phyto.5.2 SCAR marker enabled the reliable identification of resistant genotypes at an early developmental stage, significantly reducing the time and resources required for conventional phenotypic screening. The evaluation of backcross-derived populations revealed substantial phenotypic variation in morphological and yield-related traits, as confirmed by principal component analysis and heatmap clustering. Importantly, *P. capsici* resistant was observed across a wide range of phenotypic backgrounds, indicating that disease resistance was not associated with a single morphological or yield profile. This diversity provides valuable opportunities for selecting genotypes that combine disease resistant with superior agronomic performance. Anther culture efficiency varied among hybrid combinations, highlighting the strong influence of genotypic background on androgenic response. Nevertheless, the successful production of homozygous double haploid lines from resistant genotypes demonstrates the practical applicability of this approach for rapid line fixation. The resulting double haploid populations exhibited considerable variation in fruit yield and quality traits, allowing the identification of promising candidates for dried pepper production. Overall, the combined application of MAS, multivariate phenotypic analysis, and anther culture proved to be a powerful breeding framework for accelerating the development of disease-resistant and agronomically superior pepper lines. The double haploid lines generated in this study constitute valuable genetic material for future cultivar development and may contribute to sustainable pepper production in regions severely affected by *Phytophthora capsici*. In subsequent years, the genotypes developed in this study will be further evaluated under field conditions for their resistance to *Phytophthora capsici*, as well as for yield and fruit quality traits.

Conflict of interest

The authors state there is no competing interest.

Author contribution

Authors' individual contributions to the article are equal.

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Thesis Information

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REFERENCES

- Ares, J. L. A., Martínez, A. R., & Paz, J. F. (2005). Resistance of pepper germplasm to *Phytophthora capsici* isolates collected in Northwest Spain. *Spanish Journal of Agricultural Research*, 3(4), 429–436. <https://doi.org/10.5424/SJAR/2005034-170>
- Ateş, A. Ç., & Yilmaz, N. (2020). Molecular Marker Assisted Selection for *Phytophthora Capsici* Leon. Resistance Lines in Pepper (*Capsicum annuum* L.). *Acta Scientiarum Polonorum Hortorum Cultus*, 19(3), 179–188. <https://doi.org/10.24326/ASPHC.2020.3.16>
- Aydin, A. (2024). The Growth, Leaf antioxidant enzymes and amino acid content of tomato as affected by grafting on wild tomato rootstocks 1 (*S. Pimpinellifolium* and *S. Habrochaites*) under salt stress. *Scientia Horticulturae*, 325. <https://doi.org/10.1016/j.scienta.2023.112679>

- Aydın, A., Başak, H., Aydın, H., & Güngör, R. (2024). Characterization of F2 generation tomato plants and marker assisted selection against tomato spotted wilt virus (tswv) and tomato yellow leaf curl virus (tylcv). *International Journal of Agriculture Environment and Food Sciences*, 8(3), 618–628. <https://doi.org/10.31015/jaefs.2024.3.15>
- Aydın, A., Pinar, H., Ulaş, F., Yetişir, H., Şahin, M., & Aydın, H. (2025). Comparison of In Vitro and Hydroponic Screening Methods for Nitrogen Efficiency in Pepper (*Capsicum annuum* L.). *Black Sea Journal of Engineering and Science*, 8(3), 863–874. <https://doi.org/10.34248/bsengineering.1640556>
- Bagheri, L. M., Nasr-Esfahani, M., Abdossi, V., & Naderi, D. (2020). Analysis of candidate genes expression associated with defense responses to root and collar rot disease caused by *Phytophthora capsici* in peppers *Capsicum annuum*. *Genomics*, 112(3), 2309–2317. <https://doi.org/10.1016/J.YGENO.2020.01.002>
- Barchenger, D. W., Lamour, K. H., & Bosland, P. W. (2018). Challenges and strategies for breeding resistance in *Capsicum annuum* to the multifarious pathogen, *Phytophthora capsici*. *Frontiers in Plant Science*, 9, 338923. <https://doi.org/10.3389/FPLS.2018.00628/XML/NLM>
- Başak, H., Aydın, A., Yetişir, H., & Turan, M. (2025). Salt stress effects on hybrid bottle gourd (*Lagenaria siceraria*) rootstock candidates plant growth, hormones and nutrient content. *Journal of Crop Health*, 77(1), 28. <https://doi.org/10.1007/s10343-024-01087-4>
- Buyukalaca, S., Comlekcioglu, N., Abak, K., Ekbic, E., & Kilic, N. (2004). Effects of Silver Nitrate and Donor Plant Growing Conditions on Production of Pepper (*Capsicum annuum* L.) Haploid Embryos via Anther Culture. *Europ.J.Hort.Sci*, 5(5), 206–209.
- Coşkun, Ö. F., Aydın, A., Başak, H., Mavi, K., Yetişir, H., & Toprak, S. (2025). Perspectives on morphology, physiology, genetic polymorphism and machine learning in cucumber grafting under zinc toxicity. *BMC Plant Biology*, 25(1), 1647. <https://doi.org/10.1186/s12870-025-07709-x>
- Durna, P., (2016). Bazı Biber Genotiplerinde İn Vitro Androgenesis Uygulamalarının Dihaploid Hat Geliştirmeye Etkisi ve Dihaploid Hatların Morfolojik Karakterizasyonu. Doktora Tezi, Tokat Gazi Osmanpaşa Üniversitesi, TOKAT
- Doyle & Doyle. (1990). Isolation of plant DNA from fresh tissue. *Focus*, 12, 13–15. <https://cir.nii.ac.jp/crid/1573950400018579968>
- Foster, J. M., Naegle, R. P., & Hausbeck, M. K. (2013). Evaluation of Eggplant Rootstocks and Pepper Varieties for Potential Resistance to Isolates of *Phytophthora capsici* from Michigan and New York. *Plant Disease*, 97(8), 1037–1041. <https://doi.org/10.1094/PDIS-06-12-0562-RE>
- Göçmen, M., & Abak, K. (2019). *Phytophthora capsici*'ye dayanıklı bazı biber genotiplerinin SRAP ve SSR belirteçlerle genetik farklılıklarının belirlenmesi. *Derim*, 36(2), 124–134. <https://doi.org/10.16882/derim.2019.557877>
- Irikova, T., Grozeva, S., & Rodeva, V. (2011). Anther culture in pepper (*Capsicum annuum* L.) in vitro. *Acta Physiologiae Plantarum* 2011 33:5, 33(5), 1559–1570. <https://doi.org/10.1007/S11738-011-0736-6>
- Janmohammadi, M., & Sabaghnia, N. (2023). Strategies to alleviate the unusual effects of climate change on crop production: a thirsty and warm future, low crop quality. A review. *Biologija*, 69(2), 121–133. <https://doi.org/10.6001/BIOLOGIJA.2023.69.2.1>
- Keleş, D., Pinar, H., Ata, A., Taşkın, H., Yıldız, S., & Büyükcalaca, S. (2015). Effect of pepper types on obtaining spontaneous doubled haploid plants via anther culture. *HortScience*, 50(11), 1671–1676. <https://doi.org/10.21273/HORTSCI.50.11.1671>
- Lesk, C., Coffel, E., Winter, J., Ray, D., Zscheischler, J., Seneviratne, S. I., & Horton, R. (2021). Stronger temperature–moisture couplings exacerbate the impact of climate warming on global crop yields. *Nature Food*, 2(9), 683–691. <https://doi.org/10.1038/S43016-021-00341-6>
- Li, X., Tan, L., Li, Y., Qi, J., Feng, P., Li, B., Li Liu, D., Zhang, X., Marek, G. W., Zhang, Y., Liu, H., Srinivasan, R., & Chen, Y. (2022). Effects of global climate change on the hydrological cycle and crop growth under heavily irrigated management – A comparison between CMIP5 and CMIP6. *Computers and Electronics in Agriculture*, 202, 107408. <https://doi.org/10.1016/J.COMPAG.2022.107408>
- McGregor, C., Waters, V., Nambeesan, S., Maclean, D., Candole, B. L., & Conner, P. (2011). Genotypic and Phenotypic Variation among Pepper Accessions Resistant to *Phytophthora capsici*. *HortScience*, 46(9), 1235–1240. <https://doi.org/10.21273/HORTSCI.46.9.1235>
- Minoli, S., Jägermeyr, J., Asseng, S., Urfels, A., & Müller, C. (2022). Global crop yields can be lifted by timely adaptation of growing periods to climate change. *Nature Communications*, 13(1). <https://doi.org/10.1038/S41467-022-34411-5>
- Mohammadbagheri, L., Nasr-Esfahani, M., Abdossi, V., & Naderi, D. (2021). Genetic diversity and biochemical analysis of *Capsicum annuum* (Bell pepper) in response to root and basal rot disease, *Phytophthora capsici*. *Phytochemistry*, 190, 112884. <https://doi.org/10.1016/J.PHYTOCHEM.2021.112884>
- Naegle, R. P., & Hausbeck, M. K. (2014). Evaluation of Pepper Fruit for Resistance to *Phytophthora capsici* in a Recombinant Inbred Line Population, and the Correlation with Fruit Shape. *Plant Disease*, 98(7), 885–890. <https://doi.org/10.1094/PDIS-03-13-0295-RE>
- Parra-Vega, V., Renau-Morata, B., Sifres, A., & Seguí-Simarro, J. M. (2013). Stress treatments and in vitro culture conditions influence microspore embryogenesis and growth of callus from anther walls of sweet pepper (*Capsicum annuum* L.). *Plant Cell, Tissue and Organ Culture (PCTOC)*, 112(3), 353–360. <https://doi.org/10.1007/s11240-012-0242-6>
- Parra-Vega, V., & Seguí-Simarro, J. M. (2016). Anther Culture in Pepper (*Capsicum annuum* L.). *Methods in Molecular Biology*, 1359, 467–474. https://doi.org/10.1007/978-1-4939-3061-6_26

- Phippen, C., & Ockendon, D. J. (1990). Genotype, plant, bud size and media factors affecting anther culture of cauliflowers (*Brassica oleracea* var. *botrytis*). *Theoretical and Applied Genetics*, 79(1), 33–38. <https://doi.org/10.1007/BF00223783/METRICS>
- Quirin, E. A., Ogundiwin, E. A., Prince, J. P., Mazourek, M., Briggs, M. O., Chlanda, T. S., Kim, K. T., Falise, M., Kang, B. C., & Jahn, M. M. (2005). Development of sequence characterized amplified region (SCAR) primers for the detection of Phyto.5.2, a major QTL for resistance to *Phytophthora capsici* Leon. in pepper. *Theoretical and Applied Genetics*, 110(4), 605–612. <https://doi.org/10.1007/S00122-004-1874-7>
- Şahin, M., Yetişir, H., Pinar, H., & Aydin, A. (2025). Genotypic Responses of Some Besni Pepper (*Capsicum annuum* L.) Genotypes to Anther Culture. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi*, 28(1), 114–131. <https://doi.org/10.18016/KSUTARIMDOGA.VI.1555480>
- Sánchez-Rodríguez, E., Romero, L., & Ruiz, J. M. (2016). Accumulation of free polyamines enhances the antioxidant response in fruits of grafted tomato plants under water stress. *Journal of Plant Physiology*, 190, 72–78. <https://doi.org/10.1016/J.JPLPH.2015.10.010>
- Taşkın, H., Büyükalaca, S., Keleş, D., & Ekbiç, E. (2011). Induction of microspore-derived embryos by anther culture in selected pepper genotypes. *African Journal of Biotechnology*, 10(75), 17116–17121. <https://doi.org/10.5897/AJB11.2023>
- Thabuis, A., Lefebvre, V., Bernard, G., Daubèze, A. M., Phaly, T., Pochard, E., & Palloix, A. (2004). Phenotypic and molecular evaluation of a recurrent selection program for a polygenic resistance to *Phytophthora capsici* in pepper. *Theoretical and Applied Genetics* 2004 109:2, 109(2), 342–351. <https://doi.org/10.1007/S00122-004-1633-9>
- Thabuis, A., Palloix, A., Pflieger, S., Daubèze, A. M., Caranta, C., & Lefebvre, V. (2003). Comparative mapping of *Phytophthora* resistance loci in pepper germplasm: Evidence for conserved resistance loci across Solanaceae and for a large genetic diversity. *Theoretical and Applied Genetics*, 106(8), 1473–1485. <https://doi.org/10.1007/S00122-003-1206-3>
- Yuan, X., Li, S., Chen, J., Yu, H., Yang, T., Wang, C., Huang, S., Chen, H., & Ao, X. (2024). Impacts of Global Climate Change on Agricultural Production: A Comprehensive Review. *Agronomy* 2024, Vol. 14, Page 1360, 14(7), 1360. <https://doi.org/10.3390/AGRONOMY14071360>
- Zhang, D., Liu, J., Li, D., Batchelor, W. D., Wu, D., Zhen, X., & Ju, H. (2023). Future climate change impacts on wheat grain yield and protein in the North China Region. *Science of the Total Environment*, 902. <https://doi.org/10.1016/j.scitotenv.2023.166147>
- Zhu, P., Burney, J., Chang, J., Jin, Z., Mueller, N. D., Xin, Q., & Ciais, P. (2022). Warming reduces global agricultural production by decreasing cropping frequency and yields. *Nature Climate Change*, 12(11), 1016–1023. <https://doi.org/10.1038/s41558-022-01492-5>