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Expansion of the Genetic Base by Interspecific Hybridization in *Capsicum annuum* and *Capsicum chinense*

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ABSTRACT

In Turkey, there is huge diversity in terms of pepper genotypes and varieties. However, in recent years, using standard and hybrid varieties in production caused the reduction of genetic diversity over time. While creating breeding programs for different purposes, the existing gene pool is needed to be well known and it should be enriched according to the breeding targets. In recent years, interspecific hybridization is intensively carried out to increase the existing genetic variation and to extend of genetic bases of varieties that have biotic and abiotic stresses tolerance in pepper breeding programs. The objective of the study was to broaden the existing genetic base by crossing *C. annuum* (253A and İnan3363) and *C. chinense* (PI 159236) varieties. The study involves the evaluation of 54 morphological characters. The first three eigen values showed 56% total variance in the F_2 population obtained from 253A x PI 159236 crossing (110 offspring), whereas in the F_2 population obtained from İnan3363 x PI 159236 crossing (150 offspring), it was 87%.

Keywords**:** Pepper, interspecific hybridization, genetic base, morphological characterization

Introduction

Pepper is grown in all regions of Turkey and is one of the vegetable species having high commercial potential. Turkey has been ranked third after China and Mexico with 7% of total world pepper (*Capsicum annuum*) production (FAOSTAT, 2021). Although Turkey is not the origin of pepper, it contains a great variety in terms of genetic resources (Bozokalfa et al. 2009). However, the use of standard and especially hybrid varieties in production in recent years leads to a decrease in the genetic diversity existing.

The use of wild species in breeding allows more widely and effectively use their valuable properties to improve cultivars by many characteristics, such as resistance to biotic and abiotic factors, cytoplasmic male sterility, and restoration of fertility (Shmykova et al. 2014). Plant breeding focuses on increasing crop yield to meet the needs of the developing world population, improving food quality to ensure a healthy life, developing new biofuels and addressing global warming and environmental pollution issues. As creating breeding programs for different purposes, the available gene pool should be well known and enriched with the goals aiming. It is very important to design breeding programs by creating a large gene pool where wild and cultural forms are evaluated together.

With breeding studies, intraspecific or interspecific crossings are carried out between parents having desired characters to create variations in the population (Sunil and Rasheed, 1998). The use of wild species in breeding makes it possible to more widely and effectively use their valuable properties to improve cultivars by many characteristics, such as resistance to biotic and abiotic factors, cytoplasmic male sterility, and restoration of fertility (Shmykova et al. 2014).

In this study, the pepper genetic base has been expanded through interspecific crossings among a genotype (PI 159236) belonging *Capsicum chinense* and a genotype (253A) and a variety (İnan3363) belonging *Capsicum annuum* obtained from Alata Horticultural Research Institute (Mersin, Turkey).

Materials and Methods

253A (a Charleston type inbred line with high heterosis strength) and İnan3363 (bell pepper type) belonging to the *Capsicum annuum* species and genotype PI 159236 (obtained from AVRDC-Taiwan and *Capsicum chinense* species) was used as parents. Seeds of pepper used as a parent were sown into trays containing peat: perlite (2 w/1 w). Seedlings having 6-7 leaves were planted in a plastic greenhouse at intervals of 40 x 100 cm as 5 plants from each genotype and necessary agricultural practices such as irrigation and fertilization were carried out. During the full anthesis phase, when stigma is active, crossings were performed between the parents reciprocally. To prevent embryo abortion due to interspecies crossing, fruits were harvested 28-40 days later from pollination and the embryo recovery method was applied *in vitro* conditions (Hossain et al. 2003; Yoon et al. 2006). To ensure embryo development; MS (Murashige and Skoog, 1962) nutrient medium including 0.5 mg L^{-1} Gibberellic acid (GA_3), 0.05 mg L^{-1} Naphthaleneacetic acid (NAA), 15 mg L^{-1} silver nitrate ($AgNO_3$) and 0.25% activated charcoal was used (Pınar et al. 2011). The plants developed from embryos were acclimated and, 20 plants from each hybrid combination were planted into the greenhouse at 80 x 100 cm intervals and necessary agricultural practices such as irrigation and fertilization were performed. F_2 populations were obtained by selfing of F_1 plants at the full anthesis stage. In order to evaluate the morphological properties of pepper plants of F_2 populations, 62 criteria requested in the pepper descriptor list prepared on the basis of IPGRI feature documents were examined (IPBGRI, 1995). Trees showing the degree of relationship related to the morphological features of the pepper genotypes obtained were drawn by using the NTSYSpc 2.1 computer package program.

Results and Discussion

Embryo abortion, which is common in crosses between *Capsicum* species, occurs as a result of the effect of barriers existing before and after fertilization (Pickersgill, 1992). Endosperm development is much slower than normal after fertilization, which delays embryo development, and the embryonic sac collapses after about two weeks (Pickersgill, 1997). In the present study, plants were crossed reciprocally during the flowering period and embryo rescue was carried out 28-40 days later after pollination to prevent embryo abortion. The plants developed from embryos were acclimated and transferred to greenhouse conditions (Figure 1).

The number of embryos rescued from the fruits opened varied in crossing combinations. When the İnan3363 and PI 159236 were used as female and male parents respectively, the highest viability rate of the embryos was recorded with 82%. On the contrary, the lowest rate was observed with 13%. It was the same in the hybrids of 253A and PI 159236. The viability rate was high (66%) and low (34%), when 253A and PI 159236 were used as the female parent (Table 1). In the combinations obtained by crossing two different *C. annuum* with PI 159236, it was observed that the viability rate was higher in the hybrids when *C. annuum* was used as a female parent. Casali (1970) reported that the germination rate of seeds varies between 0-6.6% and 0-47% when *C. chinense* and *C. annuum* are used as the female parent, respectively.

In this study, fruits were obtained from each combination in interspecific crossing and different rates of viability were observed in embryos rescued from seeds in these fruits. Similarly, Saccardo and Ramulu (1977) observed 70-76% fruits and 7-14% fertile seeds in *C. chinense* and *C. annuum* hybrids. Costa et al. (2009) performed crossings between twenty different *C. chinense* genotypes and one *C. annuum* variety and they could obtain fruits from all hybrid combinations. Fruit ratios and seed germination were found between 8.9-40.0% and 0-87.5%, respectively, regarded with combinations. It was reported that fruits and fertile seeds are observed in the *C. chinense* and *C. annuum* hybrids. Similar results were obtained in this study presented and less number of fruits and seeds were recorded from crossing between *C. chinense* and *C. annuum* compared to reciprocal hybrids.

Among the obstacles after fertilization in interspecific hybrids, hybrid weakness or necrosis can be mentioned. This anomaly is defined by a series of phenotypic features that are similar to those associated with a response to environmental stress, such as the attack of pathogens and viral diseases (Bomblies and Weigel, 2007). Martins et al. (2015) observed this type of expression in the hybrids of the *C. frutescens* × *C. baccatum* combination in the seedling and later stages and reported that some undeveloped seedlings die at a young period and those that develop stunted and have deformed cotyledons, plants do not produce flowers and therefore fruit cannot be obtained. In this study, a similar situation was observed in the hybrids of *C. chinense* × *C. annuum* combinations such as death at the seedling stage and deformed leaves, flowers and fruits with stunted development (Figure 2).

Although these plants were left open-pollinated, no seeds were formed in any fruit.

When F_1 plants obtained from 253A × *C. chinense* and İnan3363 × *C. chinense* crossings were at full anthesis stage, F_2 populations were created by selfing. The plants were isolated with nets for obtaining F_2 populations and allowed to be pollinated with their pollens. In order to create back-cross populations, manual pollination was performed.

In the study, 110 plants from 253A × PI 159236 F_2 population and 150 plants from Inan3363 × PI 159236 F_2 population represented the transgressive segregation from each F_2 population. Accordingly, the measurements and observations were carried out according to 54 morphological criteria and cluster analysis of the data obtained and trees showing the degree of relationship related to morphological features were drawn using NTSYS-PC 2.1 computer package program.

As a result of the analysis, when the dendrogram of 253A × PI 159236 (*C. chinense*) F_2 population was examined in Figure 3, it was consisted of 2 main groups. In general, it was determined that both main groups were divided into 4 side groups. In this F_2 population, the total number of plants was 110. The coefficient average of this dendrogram drawn according to the correlation matrix using UPGMA coefficient in SAHN was 0.48 and consisted of four groups according to this average. The value that the similarity index represents the dendrogram was r = 0.99. According to the first three eigen values, the cumulative variance was 87.7 (Table 2).

When the dendrogram of Inan3363 × PI 159236 (*C. chinense*) F_2 population presented in Figure 4 was examined, it was consisted of 150 pepper plants in total. According to the correlation matrix, the cluster mean (similarity average) of this dendrogram drawn using the UPGMA coefficient in SAHN was 0.48 and when the dendrogram was examined, it was consisted of 2 main groups. It was determined that the first main group was divided into 2 side groups and the second main group was divided into 6 side groups. The value that the similarity index represents the dendrogram was r = 0.89. According to the first three eigen values, the cumulative variance was 56.46 (Table 3).

In the study, according to the dendograms obtained from the morphological characterization of F_2 populations, it was seen that while the parents were in different groups, a separate group formed apart from the groups involved both parents. Based on these findings, the populations appeared to be segregated to represent transgressive segregation.

Genetic variations, which have an important role in the success of plant breeding, have been continued to be reduced by modern plant breeding following cultivation (Tanksley and McCouch, 1997). It is known that interspecific crosses increase genetic diversity and heterosis. In the study carried out for this purpose, genetic variation due to interspecific crosses was calculated. While modern molecular methods are preferred to reveal genetic diversity among genotypes, agro-morphological characterization constitutes the basis and the first step of identification (Smith and Smith, 1989). The genetic diversity of *Capsicum* species can be evaluated as a whole, including agronomic, morphological and molecular features (Costa et al. 2016). Despite the accuracy in predicting the genetic deviation between accessions by molecular markers, phenotype knowledge by morphological and agronomic identifiers is still important. The collection of morphological data is practical and economical compared to the collection of quantitative and molecular data (Sudré et al. 2010).

Cluster analysis is widely used in comparing the plant and fruit characteristics of the varieties, and with the dendograms created using this data, it is possible to determine groups of the cultivars (Panayotov et al. 2000). In the study, a total of 54 morphological features were evaluated and the genetic variation in the F_2 populations obtained for these features was found to be 58% in the F_2 population obtained from 253A × PI 159236 hybrids and 87% in the F_2 population obtained from İnan3363 × PI 159236 hybrids. Keleş (2007) carried out the morphological characterization of 562 pepper genotypes of *C. annuum* and obtained a collection containing 96 genotypes by evaluating the dendograms drawn as a result of the analyses. Total variance of three eigen value in terms of 53 morphological features in PCA was found to be 30%. When the study results are compared with this study, it is seen that interspecific crossing increases the genetic variation in *C. annuum* existed. Olszewska et al. (2011) analyzed biometrically F_1 hybrids (*C. annuum* × *C. frutescens, C. frutescens* × *C. annuum, C. frutescens* × *C. chinense* ve *C. chinense* × *C. frutescens*) obtained as a result of interspecific crossing. The characters evaluated in the hybrids tested were found to produce large variations. They described that the hybrids obtained from crossing as the most valuable material for future pepper breeding projects. They also reported that these materials contained valuable features that will be used as a parent in the genetic recovery of pepper.

Product development depends on the presence of genetic diversity and the degree of genetic variation that existed, and the degree of development also depends on the size of useful genetic variability (Uma Jyothi et al. 2011). Interspecific hybridization enables transferring of genes between different species and allows them to develop genetically superior genotypes (Bosland and Votava, 1999). The characterization and evaluation of the cultivated *Capsicum* species are especially interesting for gene banks. Because, a wide variation, which is not yet fully known and used, exists in these species (İnce et al. 2009). Despite the accuracy in predicting genetic deviations between accessions by molecular markers, phenotype knowledge obtained from morphological and molecular identifiers is still important. Some studies have concluded that the relationship among morphological, agronomic and molecular data of *Capsicum* accessions is the most appropriate approach to estimate *Capsicum* genetic segregation (Costa et al. 2009; Moura et al. 2010) or that joint analysis of qualitative and quantitative data results in greater efficiency. Sudré et al. (2010) observed that only morphological descriptors can effectively distinguish between *Capsicum* species and botanical varieties.

Conclusions

Increasing the genetic diversity in the *Capsicum* genus provides parameters for identifying parents that produce more heterotic effects and increases the likelihood of obtaining superior genotypes in separated generations. In this study, genetic variation existing in *C. annuum* however decreasing gradually during the breeding process was increased by using populations obtaining through the crossing of *C. anuum* with *C. chinense*. Plants obtained from interspecific hybrids are very valuable materials for future breeding projects. As a result of this study, gene flow to the shrinking pepper genetic basis was provided and the variation was increased. Since the rich material basis needed for different pepper breeding projects (heterosis, biotic/abiotic stress resistance, QTL mapping, variety breeding having different biochemical content) was created, homozygous lines having genetically valuable properties can be produced and used as parents in breeding studies.

Table 1. Number of fruits opened, number of seeds, number of embryos recovered, % viability rate and number of alive plants in crossing combinations.

Crossing Combination	**Opened Fruit Number**	**Seed Number**	**Recovered Embryo Number**	**Viability (%)**	**Alive Plant Number**
253A × PI 159236	10	915	607	66	35
İnan3363 × PI 159236	8	1095	895	82	55
PI 159236 × 253A	10	558	195	34	58
PI 159236 × İnan3363	10	714	94	13	42

Table 2. First three eigen values of the correlation matrix in 253A × PI 159236 F_2 population.

	Eigen Value	**Variance (%)**	**Cumulative Variance (%)**
1	63.90	56.55	56.55
2	30.62	27.10	83.66
3	4.56	4.04	87.70

Table 3. First three eigen values of the correlation matrix in İnan3363 × PI 159236 F_2 population.

	Eigen Value	**Variance (%)**	**Cumulative Variance (%)**
1	29.79	19.60	19.60
2	23.71	15.60	35.20
3	18.46	12.14	56.46

Figure 1. Embryo rescue processes and acclimation of plants (a: the fruits harvested, b: the seeds opened, c: the plants developed from embryos, d: the plants transferred to trays, e: the plants transferred to pots, f: the plants transferred to greenhouse). (Original)

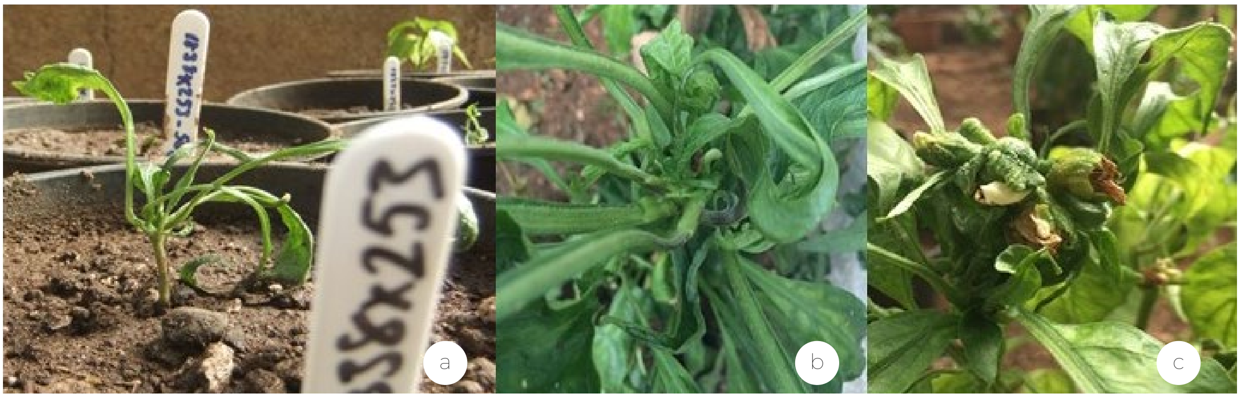

Figure 2. In the *C. chinense* × *C. annuum* hybrid combinations, the plants developed by the effects of hybrid necrosis due to post-zygotic barriers, however deformed (a: 4-5 seedling having leaves, b: the abnormal appearance of hybrid plants, c: deformed flowers). (Original)

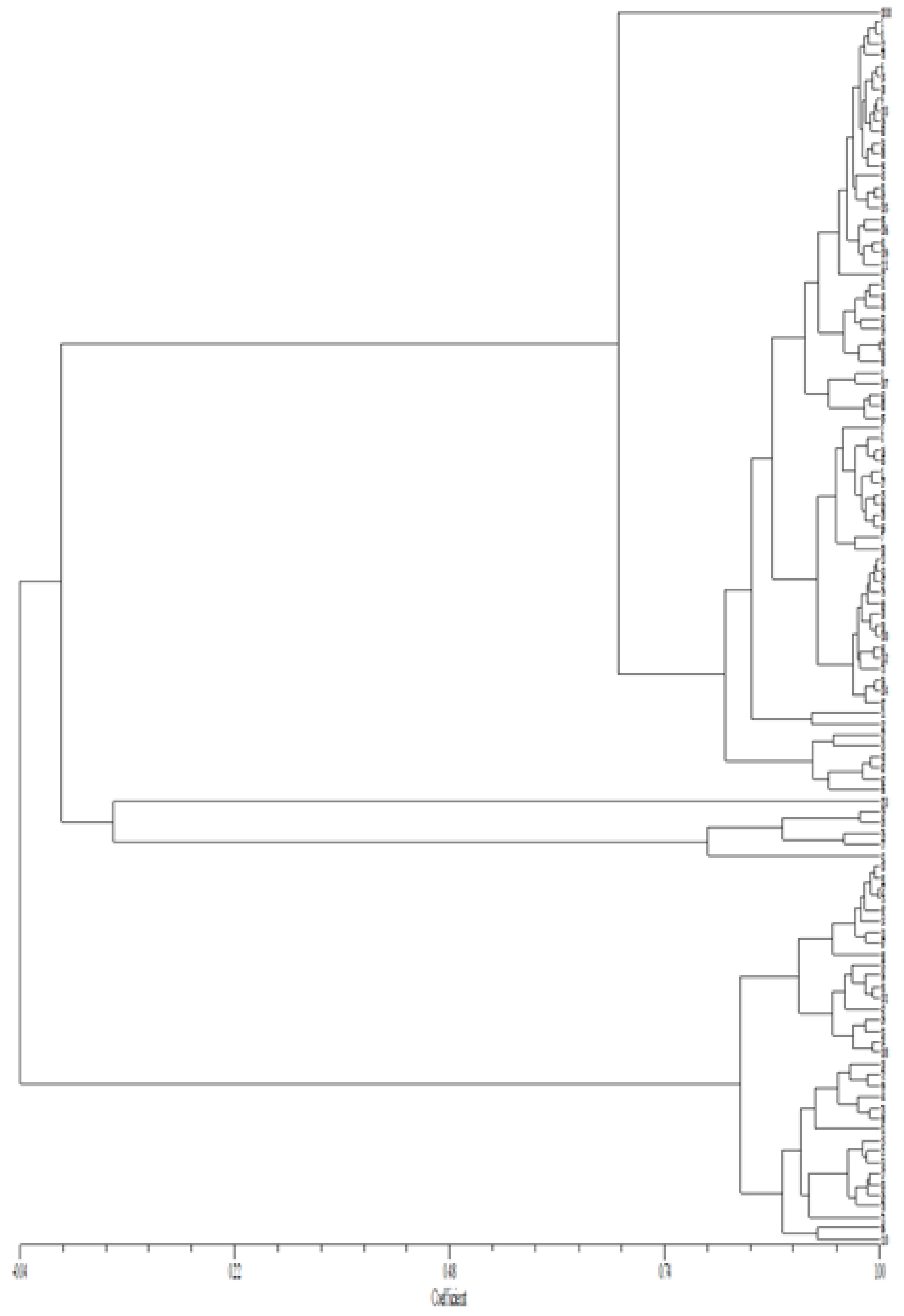

Figure 3. Dendrogram of pepper plants of 252A × PI 159236 (*C. chinense*) F_2 population obtained according to UPGMA using the correlation matrix.

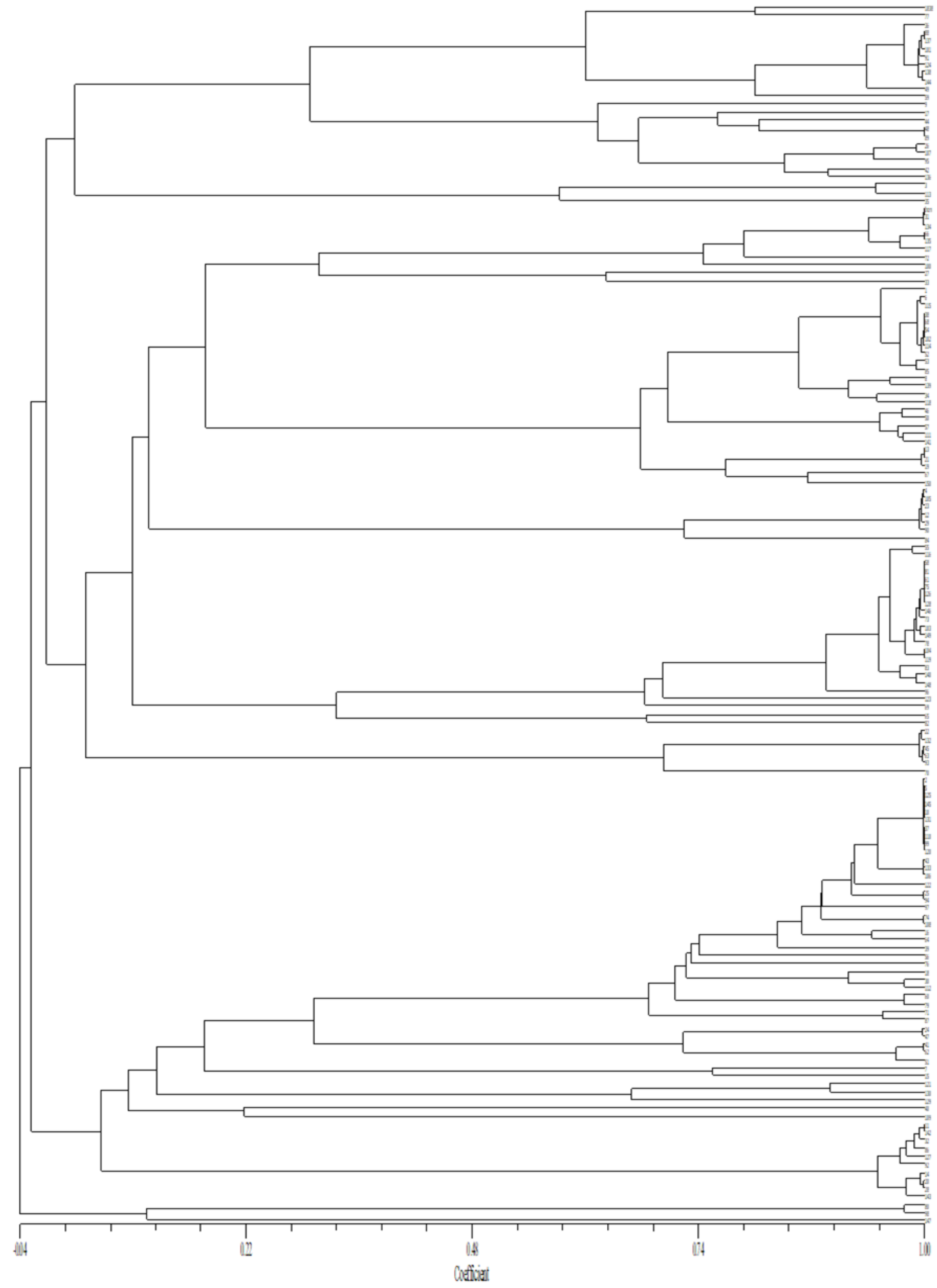

Figure 4. Dendrogram of pepper plants of İnan3363 × PI 159236 (*C. chinense*) F_2 population obtained according to UPGMA using the correlation matrix.

References

Bomblies K and Weigel D, (2007). Hybrid necrosis: Autoimmunity as a potential gene-flow barrier in plant species. Nat Rev Genet., 8(5):382-393.

Bosland and Votava E, (1999). Peppers: Vegetable and spice capsicums. United Kingdom, CAB International, p. 230.

Bozokalfa MK, Eşiyok D and Turhan K, (2009). Patterns of phenotypic variation in a germplasm collection of pepper (*Capsicum annuum* L.) from Turkey. Spanish Journal of Agricultural Science, 7(1):83-95.

Casali VWD, (1970). Cruzamentos interespecíficos no gênero *Capsicum*. Dissertação de mestrado, Universidade Federal de Viçosa, Viçosa, p. 58.

Costa FR, Pereira TNS, Sudré CP and Rodrigues R, (2009). RAPD markers and morpho-agronomic characters' determination of genetic diversity between accessions of pimentes and pimentões. Cienc. Rural, 39:696-704.

Costa MPSD, Rêgo MM, Da Silva APG, Rêgo ER and Barroso PA, (2016). Characterization and genetic diversity of pepper (*Capsicum* spp.) parents and interspecific hybrids. Genetics and Molecular Research 15 (2):gmr.15027652.

FAOSTAT, 2021. http://www.fao.org/faostat/en/

Hossain MA, Minami M and Nemoto K, (2003). Immature embryo culture and interspecific hybridization between *Capsicum annuum* L. and *C. frutescens* L. via embryo rescue. Japanese Journal of Tropical Agriculture, 47(1):9-16.

IPBGRI, (1995). Descriptors fir capsicum (*Capsicum* spp.). International Plant Genetic Resources Instit., (IPGRI), Rome (Italy). 114p.

İnce AG, Karaca M and Onus AN, (2009). Genetic relationships within and between *Capsicum* species. Biochem. Genet., 48:83-95.

Keleş D, (2007). Farklı biber genotiplerinin karakterizasyonu ve düşük sıcaklığa tolerans. Doktora Tezi. Çukurova Üniversitesi Fen Bilimleri Enstitüsü, Adana.

Martins KC, Pereira TNS, Souza SAM, Rodrigues R and Junior ATA, (2015). Crossability and evaluation of incompatibility barriers in crosses between *Capsicum* species. Crop Breeding and Applied Biotechnology, 15:139-145.

Moura MCCL, Gonçcalves LSA, Sudré CP and Rodrigues R, (2010). Gower algorithm estimates the genetic divergence in pimenta germplasm. Hort. Bras., 28:155-161.

Murashige T and Skoog F, (1962). A revised medium for rapid growth and bioassy with tobacco tissue cultures. Physiol. Plant., 15:473-497.

Olszewska D, Jedrzejczyk I and Nowaczyk P, (2011). Biometrical assessment of interspecific hybrids of *Capsicum* Genus. Vegetable Crops Research Bulletin, 75:21-30.

Panayotov N, Gueorguiev V and Ivanova I, (2000). Characteristics and grouping of F_1 pepper (*Capsicum annuum* L.) hybrids on the basis of cluster analysis by morphological characteristics of fruits. Capsicum Eggplant Newsletter, 19:62-65.

Pınar H, Denli N, Ata A, Keleş D, Büyükalaca S and Mutlu N, (2011). "Biberde türler arası melezlemede farklı tarihlerde yapılan embriyo kurtarmanın embriyo sayısı ve bitkiye dönüşüme etkileri", Türkiye IV. Tohumculuk Kongresi, Samsun.

Pickersgill B, (1992). Barriers to interspecific gene exchange in *Capsicum*. VIIIth Meeting "Genetics and Breeding on Capsicum and Eggplant" Rome, Italy, 7-10 September 1992, pp. 57-60

Pickersgill B, (1997). Genetic resources and breeding of *Capsicum* spp. Euphytica, 96:129-133.

Saccardo F and Ramulu KS, (1977). Cytological investigatins in the genus *Capsicum* (E. Pochard editor). Capsicum 77: Comptes Rendus. Congrès Eucarpia sur la Genetique et la Selection du Piment, 3, INRA, Avignon-Montfavet. pp. 51-66.

Shmykova NA, Pyshnaya ON, Shumilina DV and Dzhos EA, (2014). Morphological characteristics of doubled haploid plants of pepper produced using microspore/anther *in vitro* culture of the interspecies hybrids of *Capsicum annum* L. and *C. chinense* Jacq. Russian Agricultural Sciences, 40(6):417-421.

Smith JSC and Smith OS, (1989). The description and assessment of distances between lines of maize: The utility of morphological, biochemical and genetic descriptors and a scheme for the testing of distinctiveness between inbred lines. Maydica 34: 151-161

Sudré CP, Gonçalves LS, Rodrigues R, Amaral Júnior AT, Riva-Souza EM and Bento Cdos S, (2010). Genetic variability in domesticated *Capsicum* spp. as assessed by morphological and agronomic data in mixed statistical analysis. Genet. Mol. Res., 9(1):283-94.

Sunil KP and Rasheed A, (1998). Cross compatibility in five species of *Capsicum*. J. Spices Aromat. Crop, 7(1):35-38.

Tanksley SD and Mccouch SR, (1997). Seed banks and molecular maps: Unlocking genetic potential from the wild. Science, 277:1063-106.

Uma Jyothi K, Surya Kumari S and Venkata Ramana C, (2011). Variability studies in chilli (*Capsicum annuum* L.) with reference to yield attributes. J. Hortl. Sci., 6(2):133-135.

Yoon BJ, Jang CD, Do JW and Park GH, (2006). Over coming two postfertilisation genetic barriers in inter specific hybridization of anthracnose resistance. Breed. Sci., 56:31-38.