UDC 575. 630
https://doi.org/10.2298/GENSR2203171B
Original scientific article

GENETIC CHARACTERIZATION OF PEPPER (*Capsicum annuum* L.) GENOTYPES FROM CENTRAL ANATOLIA WITH SSR AND SCAR MARKERS

Hakan BAŞAK[1]*, Gölge SARIKAMIŞ[2], Gamze ÇAKIRER[2], Mevlüde Alev ATEŞ[3]

[1]Department of Horticulture, Faculty of Agriculture, Kirsehir Ahi Evran University, 40200 Kırşehir, Turkey
[2] Department of Horticulture, Faculty of Agriculture, Ankara University, 06110 Ankara, Turkey
[3]Department of Agricultural Biotechnology, Faculty of Agriculture, Kirsehir Ahi Evran University, 40200 Kırşehir, Turkey

Başak H., G. Sarikamiş, G. Çakirer, M. A. Ateş (2022). *Genetic characterization of pepper (Capsicum annuum L.) genotypes from central Anatolia with SSR and SCAR markers*. - Genetika, Vol 54, No.3, 1171-1182.

The major objective in pepper breeding programs is to generate high yielding novel varieties resistant to pests and diseases, tolerant to abiotic stress conditions with improved fruit quality traits including capsaicin content. Germplasm collections are important sources of variability for breeding studies. Molecular markers are important tools to evaluate genetic relationships among germplasm collections. Moreover, markers are used to select the genotypes conferring the desired traits via marker-assisted selection (MAS) as a powerful approach accelerating breeding programs. In the current study, 56 pepper genotypes selected among 313 pepper genotypes collected from Kırşehir province in the Central Anatolian region of Turkey according to their agronomic and morphological characteristics were used for molecular assays. Six SSR markers two of which were linked to fruit morphology were selected to characterize pepper genotypes according to their high polymorphism information content. Three SCAR markers associated with capsaicinoid synthesis and resistance to *Phytophtora capsici* (Phyto.5.2) in pepper were used to assess pungency and resistance among genotypes. According to the results obtained with SSR markers, the total number of alleles ranged from 1 to 8 among genotypes. The most polymorphic SSR markers were CaeMS015 and CAMS452 within the pepper population. The genetic distance among genotypes was determined ranging between 0.75-1.00. The segregation of the SCAR marker BF6-BF8 linked to pungency in pepper was determined relative to pungent and sweet reference cultivars. The OP004.717 SCAR marker linked to *Phytophtora capsici* was tested among genotypes relative to CM334 pepper variety, known as a source of resistance to *Phytophtora*.

Key words: Capsicum annuum, Capsaicin, *Phytophtora capsici*, SSR, SCAR

Corresponding author: Hakan Başak, Department of Horticulture, Faculty of Agriculture, Kirsehir Ahi Evran University, 40200 Kırşehir, Turke, E-mail: hbasak@ahievran.edu.tr Phone: +905338147373

INTRODUCTION

Pepper (*Capsicum annuum* L.) is an important vegetable species grown and consumed worldwide. *Capsicum* species are originated in Central and South America. *Capsicum annuum* is probably originated in southern Mexico and was then introduced to Europe and to Asia as the secondary regions of diversity where large phenotypic variation exists (MORRIS *et al*., 2017). Natural variation is important to select plants carrying the desired traits and to design breeding programs to generate improved novel varieties. The major objectives in pepper breeding programs are the development of high yielding varieties resistant to pests and diseases and tolerant to abiotic conditions that limit plant growth and development. Fruit quality traits including fruit morphology, phytonutrient and capsaicin contents are important characters in pepper breeding (*NICOLAI* et al*., 2012)*.

Molecular markers have proved valuable tools in plant breeding (UPOV-BMT, 2002). One of the main areas of applications of molecular markers includes germplasm characterization. Characterization of germplasm collections reveals the genetic relationships among genotypes and guide breeding studies. In *Capsicum*, restriction fragment length polymorphism (RFLP) (LEFEBVRE *et al*., 1993), amplified fragment length polymorphism (AFLP) (AKTAŞ *et al*., 2009), random amplified polymorphic DNA (RAPD) (ADETULA, 2006), cleaved amplified polymorphic sequence (CAPS) (MIMURA *et al*., 2012) and simple sequence repeat (SSR) (PORTIS *et al*., 2007; STAGEL *et al*., 2009; MIMURA *et al*., 2012; RAI *et al*., 2013; PARAN and MICHELMORE, 1993) markers were used to identify genetic relationships. SSR markers are highly polymorphic, reproducible, co-dominant markers (BECHER *et al*., 2000). They are mostly used in the estimation of genetic diversity, genome mapping, gene tagging, variety identification, hybrid purity testing and marker-assisted selection (MAS) (MCCOUCH *et al*., 1997).

MAS, improves the efficiency and precision of conventional plant breeding enabling the selection of genotypes carrying the traits of interest *(*LEE *et al*., 2004). Several genetic linkage maps have been developed in pepper (MINAMIYAMA *et al*., 2006; WU *et al.*, 2009). These maps are mainly based on RFLPs, RAPDs, AFLPs, and SSRs (PRINCE *et al*., 1992; KANG *et al*., 1997; 2001; PARAN *et al*., 1998; LEFEBVRE *et al*., 2001; MOON *et al*., 2003; JANG *et al*., 2004; LEE *et al*., 2004). Moreover, QTLs linked to important traits and markers associated with these QTLs have been determined (MIMURA *et al.,* 2012). MINAMIYAMA *et al*. (2006, 2007) have also constructed a pepper map mainly by using SSR markers. An SSR-based genetic map of cultivated *C. annuum* demonstrating several QTLs related to economically important fruit traits were generated (MIMURA *et al*., 2012).

Sequence-characterized amplified region (SCAR) markers are valuable tools that can be used for MAS to detect genotypes carrying the traits of interest in breeding programs. SCAR markers associated with capsaicin biosynthesis genes in pepper are developed (LEE *et al*., 2005). Phytochemicals found in *C. annuum* include capsaicinoids (capsaicin and its analogs) and capsaicin was found to be responsible for the pungency in pepper. Therefore, SCAR markers were developed for early detection of pungency in pepper in breeding programs designed to control the pungency of the fruit.

MAS facilitate the detection of complex traits such as resistance to Phytophtora capsici in pepper. SCAR markers linked to Phyto.5.2, a major QTL for resistance to P. capsici was developed (QUIRIN et al., 2005). *Phytophtora capsici is a fungal disease that cause serious*

damage in *Solanaceae* family including pepper. The disease can infect all parts of the plant such as roots, stems, leaves, and fruits at any stages of development (OELKE *et al*., 2003). Resistance to *P. capsici* is a very complex trait and therefore, it is difficult to develop resistant varieties with classical breeding methods. *Capsicum annuum* L. Criollo de Morelos 334 (CM334), which is a small-fruited, hot pepper variety, was determined to show a high degree of resistance to *Phytophthora capsici*. Therefore, it has been the most promising source of resistance for most of the breeding programs in pepper (WALKER and BOSLAND, 1999; THABUIS *et al*., 2003). In a recent work, thirty-two pepper landraces from Mexico were evaluated as sources of resistance for *P. capsici* among these landraces, six showed high level of resistance and were reported as promising sources of resistance to *P. capsici* (RETES-MANJARREZ *et al*., 2020). Studies on the resistance to *P. capsici* were related with the source of germplasm consisting of *C. annuum* and *C. chinense* species which were pathogen-resistant, sensitive and tolerant to *P.capsici* (OELKE *et al*., 2003). The inheritance of resistance found in CM334 has been, first determined by GUERRERO-MORENO and LABORDE (1980) and they reported that the resistance was provided by two recessive genes in CM334. Also, ORTEGA *et al*. (1991) reported 3 main QTL regions. In subsequent studies, it was shown that there was a 107cM main QTL in the pepper chromosome number 5 (PFLIEGER *et al*., 2001; LEFEBVRE *et al*., 2002) and the other two QTL regions are in the pepper chromosome number 11. Six chromosomal regions, Phyto.4.1, Phyto 5.1 and Phyto.5.2, Phyto.6.1, Phyto.11.1 and Phyto.12.1, have been identified on chromosomes 4, 5, 6, 11 and 12 by THABUIS *et al*. (2003). A SCAR marker linked to Phyto.5.2 QTL were generated in *C. annuum* (Quirin et al. 2005) and in a recent work, three RAPD primers and a SCAR marker SA133_4 linked to resistance to *P. capsici* were determined on chromosome 5 by TRUONG *et al.* (2013).

With the inclusion of molecular methods in breeding studies, the breeding process can be more effective and faster in cases where there is the possibility of selection based on markers. However, few studies were performed on Turkish local pepper genotypes by using molecular methods. AKTAŞ *et al*. (2009) studied the genetic diversity among Turkish *Capsicum* species based on AFLP analysis and AKYAVUZ *et al*. (2018) studied the genetic variations and population structure of Turkish pepper genotypes based on peroxidase gene markers. YILDIZ *et al*. (2020) worked on genetic diversity analysis in Turkish pepper germplasm via iPBS retrotransposon based markers.

The main objectives of the current study were to determine the genetic variation among promising local pepper genotypes at the selected SSR (Simple Sequence Repeat) loci and to provide an insight into the inheritance of genes related with capsaicinioid synthesis and resistance of *Phytophthora capsici* using SCAR markers to guide for future breeding programs.

MATERIAL AND METHODS

Plant Material

In the current study a total of 69 pepper genotypes including 56 genotypes collected from Kırşehir province in Central Anatolia, and 13 reference cultivars were used. Within local genotypes 22 were of bell pepper, 25 of long pepper and 9 of Capia pepper. Bell pepper reference cultivars were namely Kandil Dolma, Doru 16, 11B 14; long pepper cultivars were

namely Çetinel, Ilıca, Ege 91, Kıl Acı, Bafra F_1, CM334, Çarliston Bağcı, Sera Demre 8; Capia cultivar was namely Yalova Yağlık 28.

DNA Extraction

Molecular studies were performed at Ankara University, Faculty of Agriculture, Department of Horticulture. Genomic DNA was extracted from young leaf tissue by using Wizard® Genomic DNA Purification Kit (Promega, Madison, WI). RNAse treatment was performed. The DNA samples were visualized on 1 % (w/v) agarose gel. The purity and concentration of the DNA were determined using NanoDrop® ND-1000 Spectrophotometer.

SSR Analysis

For the genetic analysis of pepper genotypes, six genomic SSR primers were selected within pepper SSRs presented by MIMURA *et al*. (2012) according to their high polymorphism information content (Table 1).

Table 1. SSR and SCAR primer list used in the study

Primer	Sequences	Size (bp)	Motif	Related features	References
	SSR Primer List				
CAMS493	F 5'- TCGATGACGAAAAAGTGT GAA-3' R 5'- AGGGCAAAAGACCCATTCTT-3'	223-225	(ag)6	Fruit shape	MİMURA *et al*. (2012)
CAMS452	F 5'- GAAGTCTGGGACCTCTTTT GG-3' R 5'- TTCATTTTGATCTTCACGAAC G-3'	161- 163	(ga)11	Fruit diameter	
CAeMS009	F 5'- ACGCACCAACGAATATCTA TCTCA-3' R 5'- GTTTCCGTCCAGATCTACTTT TCCTGC-3'	246 -232	(ag)4… (ag)8	-	
CAMS406	F 5'- TAAAAATCGCGGAAAGTT GC-3' R 5'- GTCGTTCTATGCGGCATTTT- 3'	184 -182	(ga)8	-	
CAeMS015	F 5'- ATGCCTTGGTGGTGGTTAA ATCTG-3' R 5'- GTTTAGCGGTATGGACTGC GTACATCTT-3'	273 -270	(caa)7	-	
CAeMS144	F 5'- ATAACTTTGATTCCTAGTTC GGCG-3' R 5'- GTTTGAACCCCCAATCATCA TATCCTCA-3'	222- 219	(gaa)5		

Table 1cont. SSR and SCAR primer list used in the study

	SCAR Primer List			
BF6-BF8	F 5'-GAA AGA TCC GAC CTC GTC AA-3' R 5'-TGA CAC CAA TAA GTG GAG TGC T-3'	2800bp & 272 bp	Pungency (capsaicin synthesis gene)	LEE *et al.* (2005)
BF7-BF9	F 5'-GGG GTT GGG TAG AGG TTG TT-3' R 5'-GAC AAA CAA TAA TGG ACG ATG G-3'	896bp		
OpD04.717	F 5'-CCA TAA GGG TTG GTA AAT TTA CAA AG-3' R 5' TCG AGA GAT AAT TCA GAT AGT ATA ATC-3'	-	Phyto.5.2	QUİRİN *et al.* (2005)

The polymerase chain reactions (PCRs) were performed in a 10 µl reaction mixture containing 15 ng template DNA, 5 pmol each primer pair, 0.5 mM dNTP, 1.5 mM $MgCl_2$, 2 µl 5X buffer and 0.5 U GoTaq DNA polymerase enzyme (Promega, Madison, WI, USA). PCR amplification was performed by Biometra® PCR system. Reactions without DNA were included as negative controls. The PCR reaction had an initial denaturation step at 94°C for 3 min followed by 35 cycles of 1 min at 94°C (denaturation), 1 min at 52–56 °C (annealing), and 2 min at 72°C (extension), with a final extension at 72°C for 10 min. The amplicons were separated on a 2 % (w/v) agarose gel stained with ethidium bromide at (10 mg/ml) and run at 80 volts for 1 h. DNA Ladder (100 bp) (Promega, Madison, WI, USA) was used for the approximate quantification of the bands. The amplification products were visualized under UV light and sized relative to the ladder.

Genetic Analysis of SSR Markers

IDENTITY 1.0 (WAGNER and SEFC, 1999) genetic analysis program was used to calculate the number of alleles, allele frequency, He, Ho, estimated frequency of null alleles and PI per locus (PAETKAU *et al*., 1995) (Table 3). MICROSAT version 1.5 program (MINCH *et al*., 1995) program was used for the genetic dissimilarity via getting proportion of shared alleles which were considered applying ps (option 1-(ps)) as mentioned by BOWCOCK *et al*. (1994). The similarity matrix was practiced constructing a dendrogram with UPGMA (Unweighted Pair Group Method with Aritmetic Mean) via NTSYS-pc (version 2.01) (ROHLF, 1988) program.

SCAR Analysis

SCAR markers linked to capsaicinoid synthesis (BF6-BF8 and BF7-BF9) (LEE *et al*., 2005) and resistance to *Phytophthora capsici* in pepper (OpD04.717) (QUIRIN *et al*., 2005) were

used in the study (Table 1). The PCR reaction was performed in a 25 µl total volume containing 50 ng template DNA, 2.5 µl of 10 pmol each primer pair, 2.5 µl of 2.5 mM dNTP, 2.5 mM $MgCl_2$, 5 µl of 5X buffer and 0.1 µl Promega® Taq DNA polymerase enzyme. The amplification conditions had an initial denaturation step at 94°C for 3 min followed by 35 cycles at 94°C for 30 sec of denaturation, 50°C to 56°C for 1 min of annealing, 72°C for 1 min of extension, followed by 72°C for 10 min of final extension. The amplicons were separated on a 2 % (w/v) agarose gel run at 80 volts and visualized under UV light. DNA Ladder (100 and 1000 bp) was used to estimate band sizes.

RESULTS AND DISCUSSIONS

Genetic Characterization of pepper genotypes with SSR markers

The genetic relationships among pepper genotypes were determined by SSR analysis. Six microsatellite markers selected on the basis of high polymorphism information content (MIMURA *et al*., 2012) revealed successful amplifications of expected allele sizes. A total of 19 polymorphic alleles were obtained among genotypes and reference cultivars. The number of alleles per locus ranged from 1 (CAMS493 & CAMS406) to 8 (CaeMS015). Probability of identity (PI) values were between 0.625 to 1 (Table 2). The most informative loci were CaeMS015 and CAMS452 according to the number of alleles generated and probability of identity (PI) values (Table 2). The SSR markers CAeMS009 and CAeMS144 were monomorphic revealing a single band of expected size among genotypes and reference cultivars.

Table2. Allele range (bp), number of alleles, expected heterozygosity (He), observed heterozygosity (Ho), estimated frequency of null alleles, and probability of identity (PI) values of pepper genotypes and reference cultivars at six SSR loci

Locus	Allele range (bp)	Number of alleles	He (Expected heterozygosity)	Ho (Observed heterozygosity)	Estimated frequency of null alleles	PI (Probability of identity)
CAMS493	223-225	1	0	0	0	1
CAMS452	161-163	5	0.161	0.159	0.002	0.724
CaeMS015	270-273	8	0.215	0.159	0.046	0.629
CAMS144	219-222	2	0.277	0.333	-0.043	0.637
CAMS406	182-184	1	0	0	0	1
CaeMS009	232-246	2	0.5	1.0	-0.333	0.625

The UPGMA dendrogram demonstrated the genetic relationships within genotypes at these SSR loci. Such that, the genetic distance among genotypes was around 78% (0.78) and 100% (1.00) (Figure1). According to the dendrogram there were 2 major clusters. While a group of genotypes namely S38Y (24) (long-type), S67 (55) (long-type), D35 (33) (bell pepper) formed a separate branch, the remaining genotypes and the reference cultivars were clustered in a different branch. Subsequently, group 2 was further divided into several subgroups containing the remaining genotypes and cultivars. In these subgroups, genotype named S31Y (67) that is a long type of pepper genotype formed a single branch and all other studied genotypes were clustered together with several subgroups. Within each subgroup genotypes that were identical at the 6 SSR loci analyzed were determined (Figure 1).

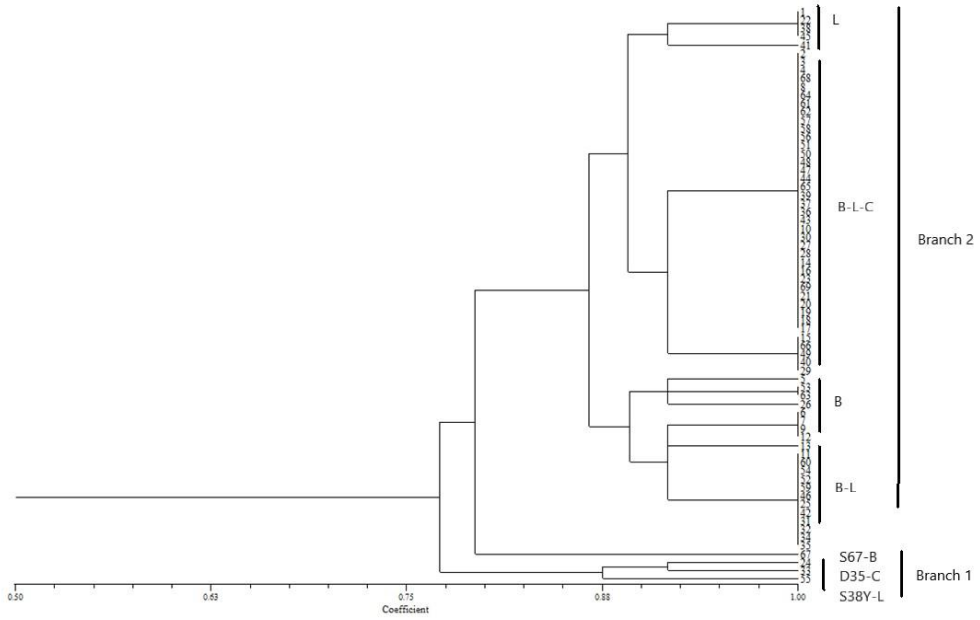

Figure1. UPGMA dendrogram of 69 pepper accessions with reference cultivars at six SSR loci (B: bell, L: long, C: capia).

AKYAVUZ *et al*. (2018) also worked on genetic variations and population structures of Turkish pepper based on peroxidase gene markers. They reported that all *C.annuum* varieties were separated from other *C.frutescens* and *C.chinense* varieties. Moreover, YILDIZ *et al*. (2020) reported that no perfect associations were found among clustering via iPBS markers and geographical origin or fruit characteristics of the varieties. However, at interspecies level the

clustering methods clearly identified *C.annuum*, *C.frutescens*, *C.chinense*. In another work, IBARRA-TORRES *et al*. (2015) used 24 SSRs and 36 ISSRs for addressing genetic diversity *C.pubescens* and *C.annuum*. Also, AKTAŞ *et al*. (2009) observed 56 AFLPs for explaining genetic diversity of Turkish *C. annuum* varieties. KRISHNAMURTY *et al.* (2015), reported 414 total bands using AFLPs between 59 *C. annuum* and *C. baccatum* varieties. Since both studies examined different polymorphic regions, the results were not similar to each other. However, all studies commonly supported that genetic associations among *C. annuum* varieties were change related with studied markers but enough to separate them.

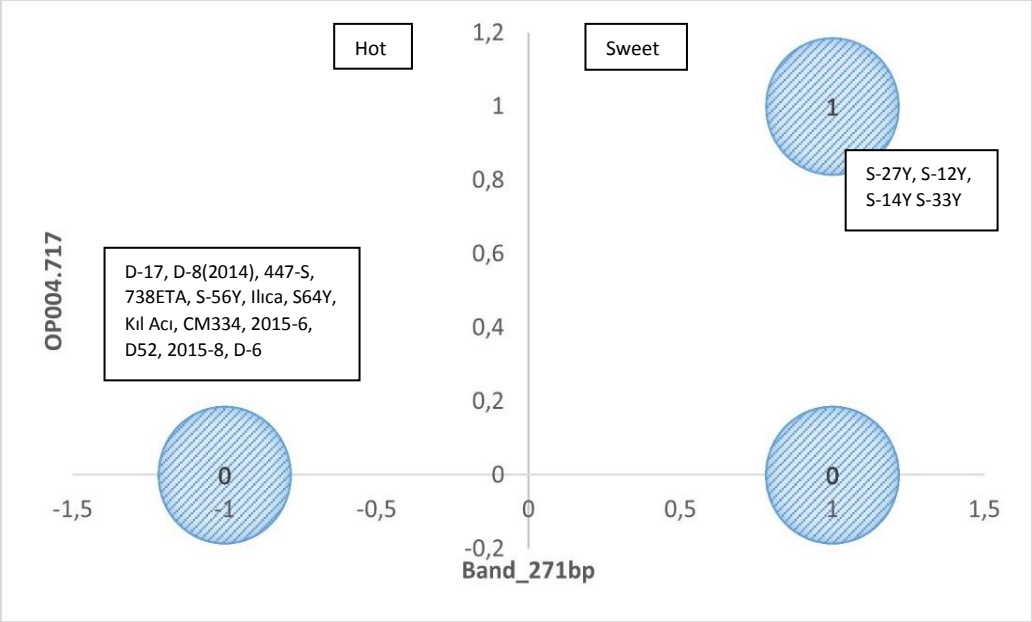

Figure 2. Graphical view of the segregation of genotypes based on the band profile of BF6-BF8 locus (absence -1, presence 1) and OP004.717 marker (presence 1, absence 0).

Pungency is an important trait in breeding studies aiming to develop varieties for the needs of consumers and industry. It is due to the presence of a group of compounds named capsaicinoids, found within the *Capsicum* genus. Marker-assisted selection can detect pungency even without fruit set in the early period and gives quick and easy results in large populations. SCAR markers (BF6 -BR8 and BF7 - BR9 primer pairs) linked to capsaicinoid synthesis (LEE *et al*., 2005) were used to identify pungency in pepper genotypes. According to LEE *et al*. (2005), the presence of a 2.8 kb band is associated with pungency whereas the existence of a band at 272 bp is associated with non-pungency when analyzed with BF6-BR8 SCAR marker. Similarly, the

presence of a band at 896 bp is linked to pungency when analyzed with the BF7-BR9 SCAR marker (LEE *et al*., 2005). This is explained as an indication of a deletion in the upper part of the CS locus in non-pungent peppers. In the present work, pungency was determined among genotypes using the SCAR marker BF6-BR8 (Figure2). However, the BF7-BR9 SCAR marker did not yield the 896 bp allele in pepper genotypes used in the current study.

Phytophtora capsici is a soil-borne fungal disease that causes severe damage to many species, including pepper. The SCAR marker OP004.717 linked to *Phytophtora capsici* (QUIRIN *et al*., 2005) was used to screen pepper genotypes based on the reference CM334 genotype. Previous work identified Phyto.5.2, which was an important QTL for resistance to *P. capsici* in pepper, suggesting that the QTL may account for different levels of resistance (ARNEDO-ANDRÉS *et al*., 2002). In the current study, 4 genotypes (S-27Y, S-12Y, S-14Y and S-33Y) were promising at the OP004.717 locus (Figure 2).

In the current study, to define the genetic relationships among local pepper genotypes of Central Anatolia at the molecular level, pepper genotypes with reference cultivars from the same province were analyzed with SCAR and SSR primers. All the data obtained were in accordance with the literature information. In the light of the molecular data, the genetic relationship of the genotypes according to the examined loci were revealed as shown in Figure 1. The genotypes were also evaluated in terms of SCAR markers linked to pungency and resistance to *Phytophthora capsica* (Figure2). Since the resistance to *Phytophtora capsici* is a complex trait, and multigenic in nature, the pathogenity tests will further confirm the efficiency of this marker within the *Capsicum annuum* genotypes.

ACKNOWLEDGMENTS

This project was funded by Kırşehir Ahi Evran University Project No PYO-ZRT.4001.14.006.

Received, December 10th, 2021

Accepted August 28th, 2022

REFERENCES

ADETULA, O.A. (2006): Genetic diversity of Capsicum using RAPD DNAs. Afr. J. Biotech., *5:*120-122.

AKTAŞ, H., K., ABAK, S., ŞENSOY (2009): Genetic diversity in some Turkish pepper (*Capsicum annuum* L.) revealed by AFLP analysis. Afr. J. Biotech., *8*:4378-86.

AKYAVUZ, R., B., TASKIN, M., KOÇAK, M., YILDIZ (2018): Exploring the genetic variations and population structure of Turkish pepper (*Capsicum annuum* L.) genotypes based on peroxidase gene markers. Biotech., *8*(8): 1-9.

ARNEDO-ANDRÉS, M., R., GIL-ORTEGA, M., LUIS-ARTEAGA, J., HORMAZA (2002): Development of RAPD and SCAR markers linked to the Pvr4 locus for resistance to PVY in pepper (*Capsicum annuum* L.). TAG, *105*(6-7): 1067-1074.

BECHER, S.A., K., STEINMETZ, K., WEISING, S., BOURY, D., PELTIER, *et al*. (2000): Microsatellites for cultivar identification inPelargonium. TAG, *101*, 643-651.

BOWCOCK, A.M., A., RUIZ-LINARES, J., TOMFOHRDE, E., MINCH (1994): High resolution of human evolutionary trees with polymorphic microsatellites. Nature, *368*: 455-457

GUERRERO-MORENO, A., J.A., LABORDE (1980): Current status of pepper breeding for resistance to *Phytophthora capsici* in Mexico. In Synopses of the IVth Eucarpia Meeting on Capsicum Wageningen (Netherlands) (pp. 52-56).

IBARRA-TORRES, P., E., VALADEZ-MOCTEZUMA, M., PÉREZ-GRAJALES, J., RODRÍGUEZ-CAMPOS, M.E., JARAMILLO-FLORES (2015): Interand intraspecific differentiation of *Capsicum annuum* and *Capsicum pubescens* using ISSR and SSR markers. Sci. Hortic., *181*(2):137–146.

JANG, I., J.H., MOON, J.B., YOON, J.H., YOO, T.J., YANG, Y.J., KIM, H.G., PARK (2004): Application of RAPD and SCAR Markers for Purity Testing of F 1 Hybrid Seed in Chili Pepper (*Capsicum annuum*). Molecules & Cells (Springer Science & Business Media BV), *18*(3).

KANG, B. C., J. W., YU, M.H., LEE, B.D. KIM (1997): Applicability of AFLP on hot pepper genetic analysis. J. Korean Soc. Hort. Sci., *38*, 698−703.

KANG, B.C., S.H., NAHM, J.H., HUH, H.S., YOO, J. W., YU, *et al*. (2001): An interspecific (*Capsicum annuum* × *C. chinese*) F2 linkage map in pepper using RFLP and AFLP markers. TAG, *102*: 531−539.

KRISHNAMURTHY, S.L., Y., PRASHANTH, A.M., RAO, K.M., REDDY, R., RAMACHANDRA (2015): Assessment of AFLP marker based genetic diversity in chilli (*Capsicum annuum* L. & *C. baccatum* L.). Indian Journal of Biotechnology, *14*: 49-54.

LEFEBVRE, V., A., PALLOIX, M., RIVES (1993): Nuclear RFLP between pepper cultivars (*Capsicum annuum* L.). Euphytica, *71*: 189–199.

LEFEBVRE, V., B., GOFFINET, J.C., CHAUVET, B., CAROMEL (2001): Evaluation of genetic distances between peppers inbred lines for cultivation protection purposes: comparison of AFLP, RAPD and phenotypic data. TAG, *102*: 741-750.

LEFEBVRE, V., S., PFLIEGER, A., THABUIS, C., CARANTA, A., BLATTES, J.C., CHAUVET, A.M., DAUBÈZE, A., PALLOIX (2002): Towards the saturation of the pepper linkage map by alignment of three intraspecific maps including known function genes. Genome, *45*: 839–854.

LEE, J.H., J.P., HONG, S.K., OH, S., LEE, D., CHOI, W., KIM (2004): The ethylene-responsive factor like protein 1 (CaERFLP1) of hot pepper (*Capsicum annuum* L.) interacts in vitro with both GCC and DRE/CRT sequences with different binding affinities: possible biological roles of CaERFLP1 in response to pathogen infection and high salinity conditions in transgenic tobacco plants. Plant molecular biology, *55*(1): 61-81.

LEE, C. J., E., YOO, J.H., SHIN, J., LEE, H.S., HWANG, B.D., KIM (2005): Non-pungent Capsicum contains a deletion in the capsaicinoid synthetase gene, which allows early detection of pungency with SCAR markers. Molecules and Cells, *19*(2): 262-267.

MCCOUCH, S.R., X.L., CHEN, O., PANAUD, S., TEMNYKH, Y.B., XU, *et al*. (1997): Microsatellite marker development, mapping and application in rice genetics and breeding. Plant Mol. Biol., *35*: 89-99.

MIMURA, Y., T., INOURE, Y., MINAMIYANA, N., KUBO (2012): An SSR-based genetic map of pepper (*Capsicum annuum* L.) serves as an anchor for the alignment of major pepper maps. Breeding Sci., *62*: 93–98.

MINAMIYAMA, Y., M., TSURO, M., HIRAI (2006): An SSR-based linkage map of *Capsicum annuum*. Molecular Breeding, *18*(2): 157-169.

MINAMIYAMA, Y., M., TSURO, T., KUBO, M., HIRAI (2007): QTL analysis for resistance to *Phytophthora capsici* in pepper using a high density SSR-based map. Breeding Sci., *57*(2): 129-134.

MINCH, E., A., RUIZ-LINARES, D.B., GOLDSTEIN, M., FELDMAN (1995): Microsat (Version 1.4d): a Computer Program for Calculating Various Statistics on Microsatellite Allele Data. Stanford University Medical Center, Stanford.

MOON, J. Y., S.I., YI, D.Y., PARK, I.H., SONG, H.Y., PARK, *et al*. (2003): Application of amplified fragment length polymorphism (AFLP) analysis for DUS test in pepper cultivars. Korean J. Breed., *35*: 306−312.

MORRIS, W. L., M. A., TAYLOR (2017): The Solanaceous Vegetable Crops: Potato, Tomato, Pepper, and Eggplant.

NICOLAÏ, M., C., PISANI, J.P., BOUCHET, M., VUYLSTEKE, A., PALLOIX (2012): Discovery of a large set of SNP and SSR genetic markers by high-throughput sequencing of pepper (*Capsicum annuum*). Genetics and Molecular Research, *11*(3): 2295-2300.

OELKE, L.M., P.W., BOSLAND, R., STEINER (2003): Differentiation of race specific resistance to Phytophthora root rot and foliar blight in *Capsicum annuum*. J. Am. Soc. Hortic. Sci., *128*:213–218.

ORTEGA, R.G., C., PALAZON-ESPANOL, J., CUARTERO-ZUECO (1991): Genetics of resistance to *Phytophthora capsici* in the pepper line 'SCM- 334'. Plant Breed., *107*:50–55.

PARAN, I., E., AFTERGOOT, C., SHIFRISS (1998): Variation in *Capsicum annuum* revealed by RAPD and AFLP markers. Euphytica, *99*: 167-173.

PARAN, I., R.W., MICHELMORE (1993): Development of reliable PCR-based markers linked to downy mildew resistance genes in lettuce. TAG, *85*(8): 985-993.

PAETKAU, D., W., CALVERT, I., STIRLING, C., STROBECK (1995): Microsatellite analysis of population structure in Canadian polar bears. Mol. Ecol., *4*: 347-354.

PFLIEGER, S., A., PALLOIX, C., CARANTA, A., BLATTES, V., LEFEBVRE (2001): Defense response genes co-localize with quantitative disease resistance loci in pepper. TAG, *103*:920–929.

PRINCE, J.P., E., POCHARD, S.D., TANKSLEY (1992): Restriction fragment length polymorphism and genetic distance among Mexican accessions of pepper. Genome, *36*: 404-417.

PORTIS, E., I., NAGY, Z., SASVARI, A., STAGEL, L., BARCHI, S., LANTERI (2007): The design of *Capsicum* spp. SSR assays via analysis of in silico DNA sequence, and their potential utility for genetic mapping. Plant Sci., *172*:640-48.

RAI, M.K., M., PHULWARIA, N.S., SHEKHAWAT (2013): Transferability of simple sequence repeat (SSR) markers developed in guava (*Psidium guajava* L.) to four Myrtaceae species. Molecular Biology Reports, *40*(8): 5067-5071.

ROHLF, F.J. (1988): NTSYS-PC Numerical Taxonomy and Multivariate Analysis System. Exeter Publishing, New York.

RETES-MANJARREZ, J. E., W. A., RUBIO-ARAGÓN, I., MÁRQUES-ZEQUERA, I., CRUZ-LACHICA, R.S., GARCÍA-ESTRADA, O., SY (2020): Novel Sources of Resistance to Phytophthora capsici on Pepper (*Capsicum* sp.) Landraces from Mexico. The Plant PathologyJ., *36*(6), 600–607.

QUIRIN, E.A., E.A., OGUNDIVIN, J.P., PRINCE, M., MAZOUREK, M.O., BRIGGS, T.S., CHLANDA, K.T., KIM, M., FALISE, B.C., KANG, M.M., JAHN (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. TAG, *110*:605-612.

STAGEL, A., I., GYURJAN, Z., SASVARI, S., LANTERI, M., GANAL, I., NAGY (2009): Patterns of molecular evolution of microsatellite loci in pepper (*Capsicum* spp.) revealed by allele sequencing. Plant Syst. Evol., *281*: 251-54.

THABUIS, A., A., PALLOIX, S., PFLIEGER, A.M., DAUBEZE, C., CARANTA, V., LEFEBVRE (2003): Comparative mapping of Phytophthora resistance loci in pepper germplasm: evidence for conserved resistance loci across *Solanceae* and for a large genetic diversity. TAG, *106*:1473–1485.

TRUONG, H.T.H., J.H., KIM, M.C., CHO, *et al*. (2013): Identification and development of molecular markers linked to Phytophthora root rot resistance in pepper (*Capsicum annuum* L.). Eur. J. Plant Pathol., *135*: 289–297.

UPOV-BMT, B.M.T. (2002): 36/10 Progress report of the 36th session of the technical committee, the technical working parties and working group on biochemical and molecular techniques and DNA-profiling in particular, Geneva, Switzerland.

WAGNER, H.W., K.M., SEFC (1999): Identity1.0. Centre for Applied Genetics. University of Agricultural Science, Vienna.

WALKER, S.J., P.W., BOSLAND (1999): Inheritance of Phytophthora root rot and foliar blight resistance in pepper. J. American Society for Horticultural Science, *124*(1): 14-18.

WU, F., N.T., EANNETTA, Y., XU, R., DURRETT, M., MAZOUREK, M.M., JAHN, S.D., TANKSLEY (2009): A COSII genetic map of the pepper genome provides a detailed picture of synteny with tomato and new insights into recent chromosome evolution in the genus Capsicum. TAG, *118*: 1279–1293.

YILDIZ, M., M., KOCAK, M. A., NADEEM, P., CAVAGNARO, K., BARBOZA, F.S., BALOCH, D., KELEŞ (2020): Genetic diversity analysis in the Turkish pepper germplasm using iPBS retrotransposonbased markers. Turkish Journal of Agriculture and Forestry, *44*(1): 1-14.

GENETIČKA KARAKTERIZACIJA GENOTIPOVA PAPRIKE (*Capsicum annuum* L.) IZ CENTRALNE ANADOLIJE SSR I SCAR MARKERIMA

Hakan BAŞAK[1]*, Gölge SARIKAMIŞ[2], Gamze ÇAKIRER[2], Mevlüde Alev ATEŞ[3]

[1]Odsek za hortikulturu, Poljoprivredni fakultet, Univerzitet Kirsehir Ahi Evran, 40200 Kiršehir, Turska

[2]Odsek za hortikulturu, Poljoprivredni fakultet, Univerzitet Ankara, 06110 Ankara, Turska

[3] Odsek za poljoprivrednu biotehnologiju, Poljoprivredni fakultet, Univerzitet Kirsehir Ahi Evran, 40200 Kiršehir, Turska

Izvod

Glavni cilj u programima oplemenjivanja paprike je stvaranje novih sorti visokog prinosa otpornih na štetočine i bolesti, tolerantnih na uslove abiotskog stresa sa poboljšanim osobinama kvaliteta ploda uključujući sadržaj kapsaicina. Kolekcije germplazme su važan izvor varijabilnosti za programe oplemenjivanja. Molekularni markeri su važni alati za procenu genetičkih odnosa među kolekcijama germplazme. Štaviše, markeri se koriste za odabir genotipova koji daju željene osobine putem selekcije uz pomoć markera (MAS) kao moćnog pristupa koji ubrzava programe oplemenjivanja. Uovom radu, 56 genotipova paprike odabranih između 313 genotipa paprike prikupljenih iz provincije Kiršehir u regionu Centralne Anadolije u Turskoj, prema njihovim agronomskim i morfološkim karakteristikama, korišćeno je za molekularne analize. Šest SSR markera od kojih su dva povezana sa morfologijom ploda, odabrano je za karakterizaciju genotipova paprike prema informacijama o polimorfizmu. Tri SCAR markera povezana sa sintezom kapsaicinoida i otpornošću na *Phitophtora capsici* (Phito.5.2) u paprici su korišćena za procenu oporosti i otpornosti među genotipovima. Prema rezultatima dobijenim sa SSR markerima, ukupan broj alela se kretao od 1 do 8. Najpolimorfniji SSR markeri su bili CaeMS015 i CAMS452 unutar populacije paprike. Genetska distanca između genotipova je određena u rasponu od 0,75-1,00. Segregacija SCAR markera BF6-BF8 vezanog za ljutinu kod paprike je određena u odnosu na opore i slatke referentne sorte. OP004.717 SCAR marker povezan sa *Phitophtora capsici* testiran je među genotipovima u odnosu na sortu paprike CM334, poznatu kao izvor otpornosti na *Phitophtora*.

Primljeno 10.XII.2021.
Odobreno 28. VIII. 2022.