



Development of molecular markers linked to quantitative trait loci and genes controlling endosperm-based hybridization barriers

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Abstract According to the theory of biological species, two species are considered different if there exist barriers to reproduction that prevent genes from flowing between them. Despite the undervaluation of hybrid seed lethality in the field of evolutionary biology, it represents a significant and promising area of research within the discipline of plant breeding. It is of the utmost importance to gain an understanding of and to overcome hybrid seed failure in order to facilitate the incorporation of desired traits into cultivated species. In this study, hybrid seed lethality between *Capsicum annuum* L. (21A) and *Capsicum frutescens* L. (PI 281420) cross was investigated. A genetic map was constructed using an F_2 population derived from a cross between *C. annuum* L. (21A) and *C. frutescens* L. (PI 281420). The quantitative trait loci (QTLs) for endosperm-based hybridization barriers were mapped using the F_2 population. A genetic map with a total length of 929.6 centimorgans (cM) and comprising 12 linkage groups was constructed using

61 markers, including 31 sequence-related amplified polymorphism markers, 19 simple sequence repeat (SSR) markers, and 11 random amplified polymorphic DNA markers. The 2 linked QTLs related with endosperm-based hybridization barriers character were mapped on linkage groups 11 and XX. This research contributes to our understanding of the genetic processes that underpin hybrid seed lethality, thereby providing insights for the enhancement of cultivated plant species through breeding.

Keywords DNA markers · Endosperm-based hybridization barriers · Genetic mapping

Introduction

Interspecific hybridisation is used to improve traits by transferring certain characteristics, such as resistance to biotic and abiotic stresses, from their wild relatives to cultivated plants. Interspecific hybrid populations also provide essential traits needed by pepper growers and consumers (Barchi et al. 2007). Based on interspecific hybridization, allopolyploids such as allotetraploids can also be used to double the chromosome number of F_1 hybrids. A successful allopolyploid generation may result in the formation of a new genome combination or the creation of a species that did not previously exist. However, considerable effort may be required to cross-breed cultivated species and wild species (Mavi 2020). It is reported that the first

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interspecific hybridization made by human hands was between *Dianthus caryophyllus* L. and *Dianthus barbatus* L. in 1717 (Stalker 1980). Thousands of interspecific hybridisation attempts have been made since then but the results have been limited. For example, it took plant breeders about a century to develop a new plant species, triticale through the process of hybridisation between wheat (*Triticum aestivum* L.) and rye (*Secale cereale* L.) (Zillinsky 2015). Interspecific hybridization has become an important goal for geneticists and plant breeders with its benefits and difficulties in success. There are two types of hybridisation barriers that prevent the development of interspecific hybrids: pre- and post-fertilisation barriers (Morgan et al. 2011). The formation of a fertilised is prevented by the presence of pre-fertilisation barriers. Pre-fertilisation barriers include asynchrony in flowering time, lack of viable pollen or stigma receptivity at pollination time, unnatural pollen tube growth in the recipient plant (Morgan et al. 2011; Kuligowska et al. 2015). It is also possible that post-fertilisation barriers prevent perfect hybrid formation after fertilisation. These factors encompass variations in polyploidy among species, chromosomal loss or rearrangements, apomixis, physiological seed dormancy, cytoplasmic incompatibilities, and hybrid breakdown caused by lethal endosperm defects or reduced plant vigor in the initial or subsequent generations. (Rieseberg and Carney 1998; Dwivedi et al. 2008). The cause of postzygotic failure of hybrid embryos in higher plants is usually incompatibility problems in the endosperm, not between the parental chromosomes (Lafon-Placette and Köhler 2016). Overcoming the interspecies hybridization barriers is of great importance for the *Solanaceae* group plants, which are very important for the world economy and nutrition.

Genus *Capsicum* includes both wild and cultivated peppers grown in a variety of geographical regions around the world. These peppers vary considerably in chemical composition, size, shape, colour and degree of spiciness (Chuah et al. 2008). Pepper (*Capsicum*) is a member of the *Solanaceae* family and is known to comprise approximately 30 species. However, only five of these (*C. annuum*, *C. chinense*, *C. frutescens*, *C. baccatum* and *C. pubescens*) are currently domesticated. The most widely cultivated of the five species is *C. annuum* (Bozokalfa and Eşiyok 2011). Utilizing genetic resources from wild *Capsicum* species, pepper cultivars can be improved in terms of

total productivity, nutritional quality, disease resistance, and adaptation to abiotic conditions (Kannan-gara et al. 2017). Most modern cultivars have been improved by using genetic diversity derived directly from a wild relative. The genetic proximity and cross-compatibility among the five domesticated species and their closely related wild species can be classified into three distinct genetic complexes (Shiragaki et al. 2020). The species *C. annuum* var. *annuum*, *C. chinense* and *C. frutescens* are classified in the *Annuum* clade (Liu et al. 2023). There have been some research of the ability of these species to interbreed under field conditions. It is clear that crosses between these three taxa have resulted in an increasing number of commercial cultivars.

There have been numerous investigations of interspecific hybridisation used to introduce insect and pest resistance traits into cultivars of many *Solanaceae* families. (Zewdie and Bosland 2000; Cho et al. 2016). Hybridisation of *C. annuum* with related species (*C. chinense*, *C. frutescens*, etc.) is currently one of the methods being considered to introduce resistance genes into cultivars. (Manzur et al. 2015). However, successful large-scale hybridisation attempts to introduce disease resistance traits into *C. annuum* have been few and far between (Yoon et al. 2004; Eggink et al. 2014). Post-zygotic barriers that either prevent fertilization because of pollen-pistil incompatibility or result in embryo/endosperm abortion, hybrid weakening, or sterility are the main causes of cross-compatibility difficulties among these species (Aniel Kumar et al. 1987; Jae et al. 2006).

Growth of the embryo is supported by the endosperm, which is a nourishing tissue. In hybridisations between closely related plant species or between species with different ploidies, failure of endosperm development frequently leads to seed lethality and embryo arrest. The nature of the endosperm and the direction of hybridization influence the formation of hybridization barriers, which can result in the development of non-reciprocal seed defects. It can be concluded that this type of hybridisation barrier is caused by genes specific to the parents of origin, which implies that the parental genomes are not equivalent (Lafon-Placette and Köhler 2016).

Early 20th-century hybridization research, most of which were intended to develop crop hybrids, showed that angiosperms are susceptible to seed lethality as a result of interploidy and interspecific hybridization

(Sansome et al. 1942; Cooper and Brink 1945; Williams et al. 1976; Gill and Waines 1978; Johnston and Hanneman 1979; Sukno et al. 1999). A similar conclusion has been reached by the majority of investigations that have examined hybrid seed lethality using histological observations and embryo rescue experiments in vitro. These investigations have demonstrated that the primary cause of the inviability of hybrid seeds is endosperm deficiency (Sansome et al. 1942; Cooper and Brink 1945; Williams et al. 1976; Gill and Waines 1978). To overcome this problem, an alternative approach is to use a genetic bridge between phylogenetically closer species and rescue embryos (Hossain et al. 2013; Manzur et al. 2015). Cross-compatibility between pepper species has not been systematically studied and the nature of hybridisation barriers is unclear. Finding information about the number of chromosomes in the genus is also limited. The aims of this study were the identification of the nature of hybridizing barriers occurring in crosses between selected pepper species, and the identification and mapping of genes or quantitative trait loci (QTLs) that control endosperm-based hybridizing barriers.

Materials and methods

Plant materials

The parents *C. annuum* L. (21A) and *C. frutescens* L. (PI 281420) were crossed to create the mapping population. In order to create the F₂ population (127 plants), the F₁ plants were selfed. The leaves of the F₂ plants were sampled at a time when normal fertilization was occurring and the plants were growing healthily in order to obtain DNA from them. Out of the F₂ plants, 127 plants were chosen for mapping.

In vitro embryo rescue

Through experimental vitro culture, hybrid embryos derived from seeds produced by crossings between *C. frutescens* (PI 281420) and *C. annuum* (21A) were saved between 39 and 56 days after pollination (DAP). After a brief incubation period in 70% (vol/vol) ethanol, the embryos were carefully removed from the siliques using needles that were hypodermic and placed in hormone-free Murashige and

Skoog (MS) medium supplemented with 3% (wt/vol) sucrose. Plates were incubated in a light chamber. Surviving seedlings were transferred to soil after 15 day.

Development of F₂ population

In vitro embryo rescue was successfully used to produce 10 healthy F₁ plants. These robust plants were grown in ideal climatic circumstances within a plastic greenhouse, along with the required upkeep and attention. After the first flowering, all F₁ plants were selfed in a controlled manner and the F₂ population was obtained. The seeds of F₂ plants were germinated and 200 of them were grown in the greenhouse. From these plants, 127 healthy growing F₂ plants were selected and 3 fruits were taken from each F₂ plant (127 F₂) during the red harvest period and their seeds were harvested. The percentages of blacking and non-blackening seeds were determined by using a setup in which fluorescent light was reflected from the bottom.

Germination assays

Following the standard methodology, the seeds were surface sterilized one month after harvesting and subsequently plated on MS2 medium devoid of sucrose. In order to break seed dormancy, the plates were maintained at 4 °C for a period of 6 weeks, after which they were transferred to normal growth conditions for a month. The germination rates were evaluated by enumerating the seeds that had ruptured their seed coats and exhibited protruding radicles.

Extracting DNA, analysis of markers, cluster analysis and constructing genetic map

Genomic DNA was extracted from young leaves of 127 F₂ pepper plants, as well as the parent plants, using a modified CTAB method as outlined by Shams et al. (2020). To determine the quantity and quality of the isolated pepper DNAs, electrophoresis was performed in a 1% agarose gel in TAE [(1XTAE; 4.84 g Tris, 1.142 mL acetic acid, 2 mL 0.5 molar (M) EDTA (tris-acetate-EDTA, pH:8)] solution at 50 mA electric current and imaging was performed under ultraviolet (UV) light. Furthermore, the concentrations of DNA and the purities of isolation were determined with a NanoDrop spectrophotometer

Table 1 The primer combinations and the number of polymorphic bands obtained in sequence-related amplified polymorphism (SRAP) primer research

No	Primer combination	Polymorphic band numbers	No	Primer combination	Polymorphic band numbers
1	Em11 Me11	6	15	EM1 Me2	4
2	Em6 Me13	2	17	Em10 Me16	4
3	Em16 Me3	2	18	Em13 Me5	4
4	Em14 Me1	2	19	Em4 Me9	1
5	Em6 Me1	2	20	Em5 Me12	3
6	Em15 Me3	1	21	Em11 Me12	3
7	Em10 Me5	3	22	Em4 Me13	2
8	Em13 Me5	1	23	Em10 Me11	2
9	Em7 Me6	3	24	Em5 Me11	6
10	EM10 Me6	3	25	Em5 Me13	5
11	EM3 Me6	1	26	EM16 Me4	3
12	EM3 Me10	4	27	EM9 Me1	1
13	EM5 Me8	3	28	EM16 Me13	1
14	EM6 Me6	3	29	EM11 Me8	2
16	EM8 ME7	4		Total	80

Table 2 The primers and polymorphic band numbers obtained in random amplified polymorphic DNA (RAPD) primer research

No	Primers	Polymorphic band numbers
1	OP108	6
2	OPAC10	4
3	OPAH2	4
4	OPB01	4
5	OPB20	5
6	OPM19	4
7	OPM10	5
8	OPM11	7
9	OPL04	5
10	OPI03	4
11	TIB17	5
12	TIBMBB04	7
	Total	61

2000 (Thermo Scientific) and the DNA samples were diluted to 50 ng/μl.

Cluster analysis

In this study, 29 SRAP primer combinations (Table 1) and 12 RAPD primers (Table 2) were

Table 3 X^2 test results for seed blacking of 127 F_2 plants

Model	Observed (O)	Expected (E)	O-E	X^2 Values
12:3:1	76 (0–20%)	75	1	0.013
	18.48 (21–40%)	18.75	0.27	0.073
	5.89 (41–70%)	6	0.11	<u>0.012</u>
			Total	0.098
				<i>p</i> -value:0.051

The mode of segregation indicated that dominant epistasis with 12:3:1 ratio explains the trait in two independent tests using F_2 population ($X^2=0.098$, $P=0.051$, $N=127$). 0.012: show that the model fits very well

used. Polymerase chain reaction (PCR) products were subjected to electrophoresis in 1X TAE buffer and 2% agarose gel, stained with ethidium bromide, and imaged in a gel imaging unit (KODAK). Subsequent to the gel electrophoresis and imaging, the images were scored as follows: (1) for existing bands, (0) for the absence of bands and (9) for non-amplified ones. Data were gathered with the aid of NTSYS software. A similarity matrix was constructed using the Dice method, and a dendrogram of F_2 pepper plants was generated using the UPGMA method (Table 3).

Simple sequence repeat (SSR) analysis

An integrated map of *C. frutescens* × *C. annuum* was constructed using a list of co-dominant markers, from which the SSR markers exhibiting the highest degrees of polymorphism were first chosen. All 127 F₂ individuals were genotyped using markers linked to 12 chromosomes. 24 markers with the highest polymorphism were found throughout this approach and mapped using SRAP and RAPD methods. Sixty primer pairs were first evaluated on the parent plants using sequencing data and mapping information from the Sol Genomics Network database (Solgenomics.net) (Wu et al. 2009). Out of them, the 24 primers (Online Appendix 1a) with the largest polymorphism were chosen, and the 127 F₂ plants were evaluated using these primers.

Sequence-related amplified polymorphism (SRAP) analysis

In the SRAP analysis, 208 SRAP primer combinations were utilized to identify parental polymorphism, from which 31 primer combinations (Online Appendix 1b) were selected. Both parents and the F₂ population were genotyped using these 31 SRAP primer combinations, which were recorded as dominant markers. PCR amplification was conducted in accordance with the methodology delineated by Li and Quiros (2001).

Random amplified polymorphic DNA (RAPD) analysis

RAPD analysis was conducted using 12 distinct 10-mer RAPD primers (Supplementary Fig. 1) with the PCR amplification conditions outlined by Williams et al. (1990). A total of 150 RAPD primers were employed to identify polymorphisms between the parents with 12 primers exhibiting the highest polymorphism selected. These polymorphic primers were then used for genotyping the parents and the F₂ population; they were categorized as dominant markers.

Electrophoresis and gel imaging

The PCR products were mixed with a 3 µl loading buffer containing 40% glycerol (20 ml), sterile

water (30 ml), and bromophenol blue (0.05 g). The mixtures were then loaded onto a 2% agarose gel for SRAP and RAPD analysis and electrophoresed at 115 V for 3.5 h. For SSR marker analysis, the PCR products were resolved on a 3% high-resolution agarose gel.

Map construction and QTL detection

SRAP and RAPD bands obtained after filtering were further considered for genetic analysis. A chi-square (χ^2) test was applied on polymorphic SRAP and RAPD markers with a null hypothesis that two alleles from both parents of F₂ population (127 plants) at a particular locus segregate in a 1:1 ratio. The markers showing high segregation distortion (χ^2 -test, $P < 0.001$) were filtered out and not considered for the linkage map construction. The genetic map was constructed using JoinMap (version 4.0) (Ooijen et al. 2006) with logarithm of odds (LOD) threshold ranging from 4 to 10.0. The significant LOD scores for detection of the QTL were calculated based on 1000 permutations at $P < 0.05$ and 0.01 grouping and ordering of the markers were performed using the regression mapping algorithm. Linkage groups were constructed using JoinMap 4.1 software (Ooijen 2011) and the QTL analysis was carried out by the rank sum test of Kruskal–Wallis mapping and Interval Mapping using MapQTL 6 software, including ‘Kruskal–Wallis (KW)’, ‘interval mapping (IM)’, MQM, and ‘composite interval mapping (rMQM)’, was conducted (Ooijen et al. 2000) depending on trait or trait transformed distribution. The point detected with a maximum log-of-odds (LOD) score was determined as the most likely position of the QTL on the map for Interval Mapping and the confidence interval of a QTL location was calculated based on one-LOD support intervals.

Results and discussion

Although fruit set was achieved through the hybridization of *C. frutescens* and *C. annuum*, endosperm blackening was observed in the seeds harvested during the red ripening period, and these seeds did not germinate as expected. To address this issue, embryos were rescued at 3-day intervals starting from the 24th day after crossing under in vitro conditions. Successful embryo

rescue was performed between the 39th and 56th days, resulting in viable plants. The obtained F_1 plants were then self-pollinated to generate the F_2 population.

In higher plants, postzygotic failure of hybrid embryos is typically attributed to incompatibility issues in the endosperm, rather than to incompatibility between the parental chromosomes. In such cases, embryos must be rescued from fruits developed after interspecific hybridization at an early stage; otherwise, the failure of the embryo and/or the inability to obtain seeds capable of germination will result due to endosperm degeneration. Embryos may be utilised for the generation of new plants, even in instances where they are in an immature or endosperm-deficient state. Embryo rescue technique used in integration with tissue culture is an important technique that will enable further advances in interspecies hybridization (Mavi 2020). The rate of transformation of hybrid embryos into plants remains very low in interspecific hybridisations within the *Capsicum* genus, due to the presence of postzygotic incompatibility barriers (Hossain et al. 2003). This technique has received very limited attention for pepper, but recent research has shown that it has significant potential for interspecific hybridization (Hossain et al. 2003; Debbarama 2013; Sui and Hu 2015; Manzur et al. 2015; Cremona et al. 2018).

Cluster analysis of F_2 mapping population (*C. annuum* × *C. frutescens*)

In this study, genetic variation between the F_2 population and the parent plants was assessed using 29 SRAP marker combinations and 12 RAPD primers. A total of 80 polymorphic bands were obtained with the SRAP primer combinations (Table 1) and 61 bands were obtained with the RAPD markers (Table 2). The genetic similarity, as depicted in the dendrogram derived from the 141 total bands, ranged from 0.55 to 0.89. The similarity values for all genotypes within the F_2 population were found to be between those of the female parent (*C. annuum*) and the male parent (*C. frutescens*) (Fig. 1).

Endosperm-based hybridization barriers trait evaluation

In the study, 127 healthy growing F_2 plants were grown and harvested at red fruit harvest period and

the percentages of blackening of seeds in the fruits were determined. The seed blackening rates for the F_2 population are presented in Fig. 2. The results revealed considerable variation in seed blackening among the F_2 plants produced from the *C. frutescens* × *C. annuum* hybrid. Seed blackening ranged from a maximum of 66.7% to a minimum of 0%.

Map construction and QTL detection

The JoinMap 4.1 program was used to create 12 linkage groups (LGs) using 163 polymorphic markers. The LGs were obtained from 31 SRAP, 19 SSR, and 11 RAPD markers. The generated map had a total length of 929.6 cM and included 61 polymorphic bands dispersed over 12 LGs. Table 4 shows the lengths of each LG, with an average marker distance of 14.99 cm. The LGs were assigned using SSR markers, as published by Wu et al. (2009) on Solgenomics.net, for the *C. annuum* × *C. frutescens* population.

QTL mapping

The LGs were built with JoinMap 4.1, and then QTLs were mapped using MapQTL 6. ‘Kruskal–Wallis’ and ‘Interval Mapping’ analyses were used to map the quantitative data related to endosperm-based hybridization barriers that were collected from F_2 families onto the LGs. Table 5 lists the QTLs linked to endosperm-based hybridization barriers along with the related chromosomal locations.

Endosperm-based hybridization barriers ratio (%)

The ratio (%) of Endosperm-Based Hybridization Barriers produced from 127 F_2 populations was recorded at the conclusion of seed harvest and evaluated with MapQTL. Two QTLs were identified on LGs 11 and XX. The important markers for this feature were EM5ME11.220 and EM1ME2.350, which had LOD values of 4.5 and 3.1, respectively (Table 1, Fig. 3). These QTLs explained 15.4% of the phenotypic variation in endosperm-based hybridization barriers.

The most commonly used plant materials to genetically study and map a trait are populations of expansions obtained by crossing two genotypes that are expansions for a trait. The most suitable populations

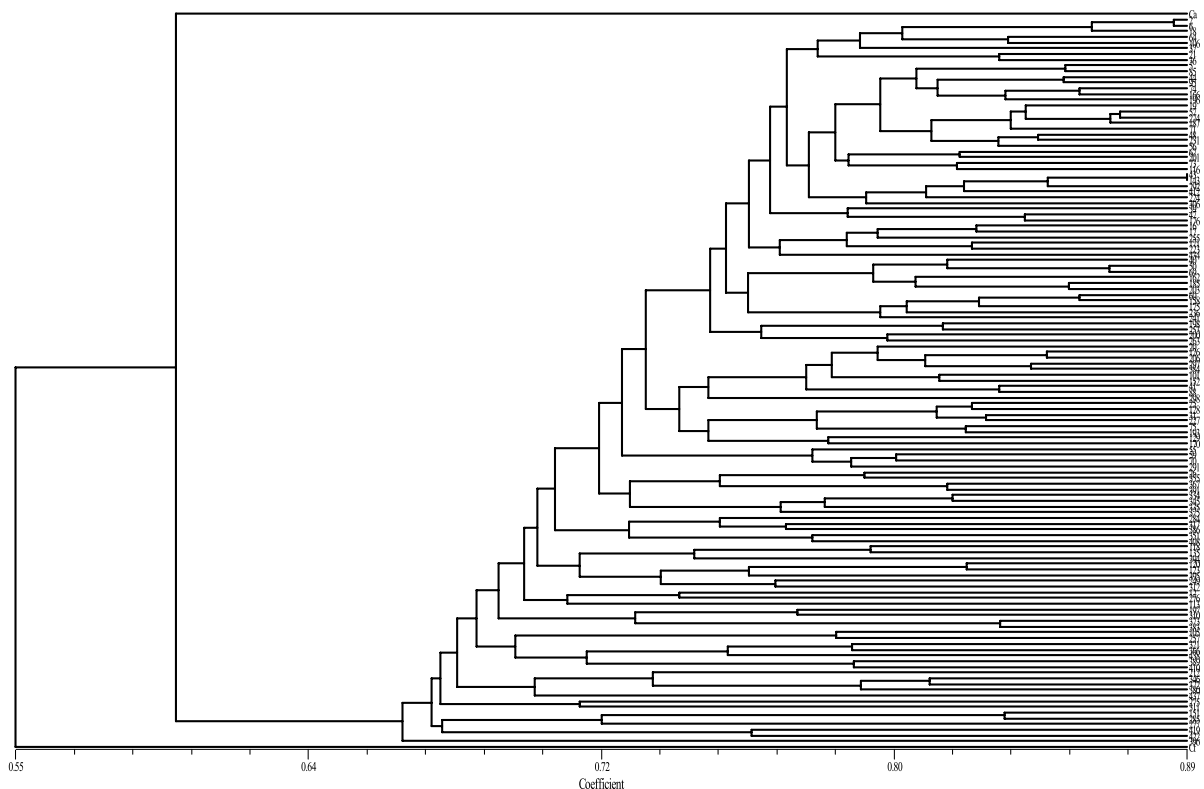


Fig. 1 Cluster analysis of 127 F_2 population and their parents using sequence-related amplified polymorphism (SRAP) and random amplified polymorphic DNA (RAPD) markers

for mapping are F_2 populations. Polymorphic molecular markers are used for genotyping a population in the development of linkage maps. Statistically, the relationships between different marker alleles obtained from previous research and the marker alleles showing expansion are evaluated. When marker locations in the genome are known, linkage groups on chromosomes can be determined. If the genomes of plant species are sufficiently saturated with markers, the number of linkage groups that will meet the haploid chromosome number in the genomes can be obtained. Although constructing a linkage map is necessary to identify genes controlling quantitative traits, it is not necessary to identify genes and associate markers with them when the target trait is controlled by major genes (Franco et al. 2001). Effective mapping research depends on two types of data. These are field or greenhouse data of the trait showing expansion and laboratory data obtained from marker expansion. The quality of phenotypic data is critical to the success of gene/QTL analyses. Because laboratory data

of marker expansion within a population must be parallel with field and greenhouse data to determine QTLs. Therefore, trials should be planned carefully to reduce phenotypic assessment errors (Franco et al. 2001). Several traditional approaches have been used to overcome hybridization barriers among species within the genus *Capsicum*. For this purpose, the use of genetic bridges (Kannangara et al. 2017), growth regulators (Jae et al. 2006), pollen irradiation (Onus and Pickersgill 2004) and in vitro pollen tube development (Zijlstra et al. 2019) investigations were performed. It has been suggested that biotechnological techniques such as somatic hybridization is another strategy for getting around these interspecific barriers in the genus *Capsicum* (Morrison et al. 1986). However, these techniques are not sufficient to eliminate all the obstacles to interspecific hybridization in pepper species.

The primary issue underlying the incompatibility between the two species is post-zygotic incompatibility, particularly in the form of embryo/endosperm

Fig. 2 Frequency distribution for the endosperm-based hybridization barriers ratio (%) traits analyzed in the pepper (F_2) population

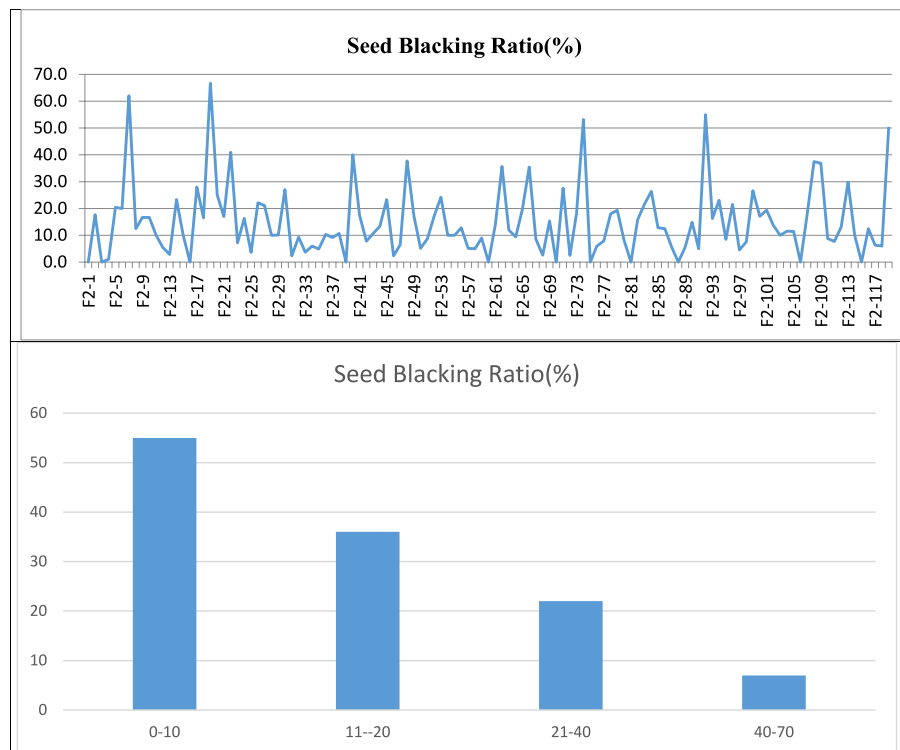


Table 4 Marker distribution across the linkage groups developed from the *Capsicum annuum* x *C. frutescens* F_2 population (Pinar et al. 2021)

Chromosome (Chr)	Chr1	Chr2	Chr3	Chr5	Chr6	Chr8	Chr9	Chr10	Chr11	ChrX	ChrXX	ChrXXX	Total
Total	5	3	3	7	5	8	7	7	5	4	5	3	31
SSR	2	2	1	1	1	5	3	2	2	—	—	—	7
SRAP	3	—	2	5	3	3	3	4	1	3	4	1	15
RAPD	—	1	—	1	1	—	1	1	2	1	1	2	8
Length (cM)	95.4	2.1	83.7	113.5	59.3	95.5	114.4	104.9	94.3	88.5	39.3	19.7	461.1
Average interval (cM)	19.1	7.03	27.6	16.2	11.9	11.9	16.3	14.9	18.9	22.1	7.86	6.6	14.99

Table 5 The list of quantitative trait loci (QTLs) and corresponding markers found in pepper that indicate endosperm-based hybridization barriers

Trait number	Trait name	QTL symbol	Chr	Marker ^a	QTL position (cM)	R^2 ^b	Lod score	Direction ^a
1	Endosperm-Based Hybridization Barriers	<i>ebhb11</i> <i>ebhbxx</i>	11 XX	EM5ME11.220 EM1ME2.350	21.305 0.00	4.476 3.242	4.5 3.1	PI 281420 21A

^aIndicates the parent that contributes to the rise in the numerical value of the trait

abortion and hybrid sterility (Kotcherlakota 1992). The first barrier in many plant species is caused by faulty zygote cell division or delayed endosperm development, which makes the plant incompatible

with the growth of embryos (Nowack et al. 2010). The second factor is the result of a number of different processes, including the divergence of genes, changes to the karyotype, the transposition or loss of genes,

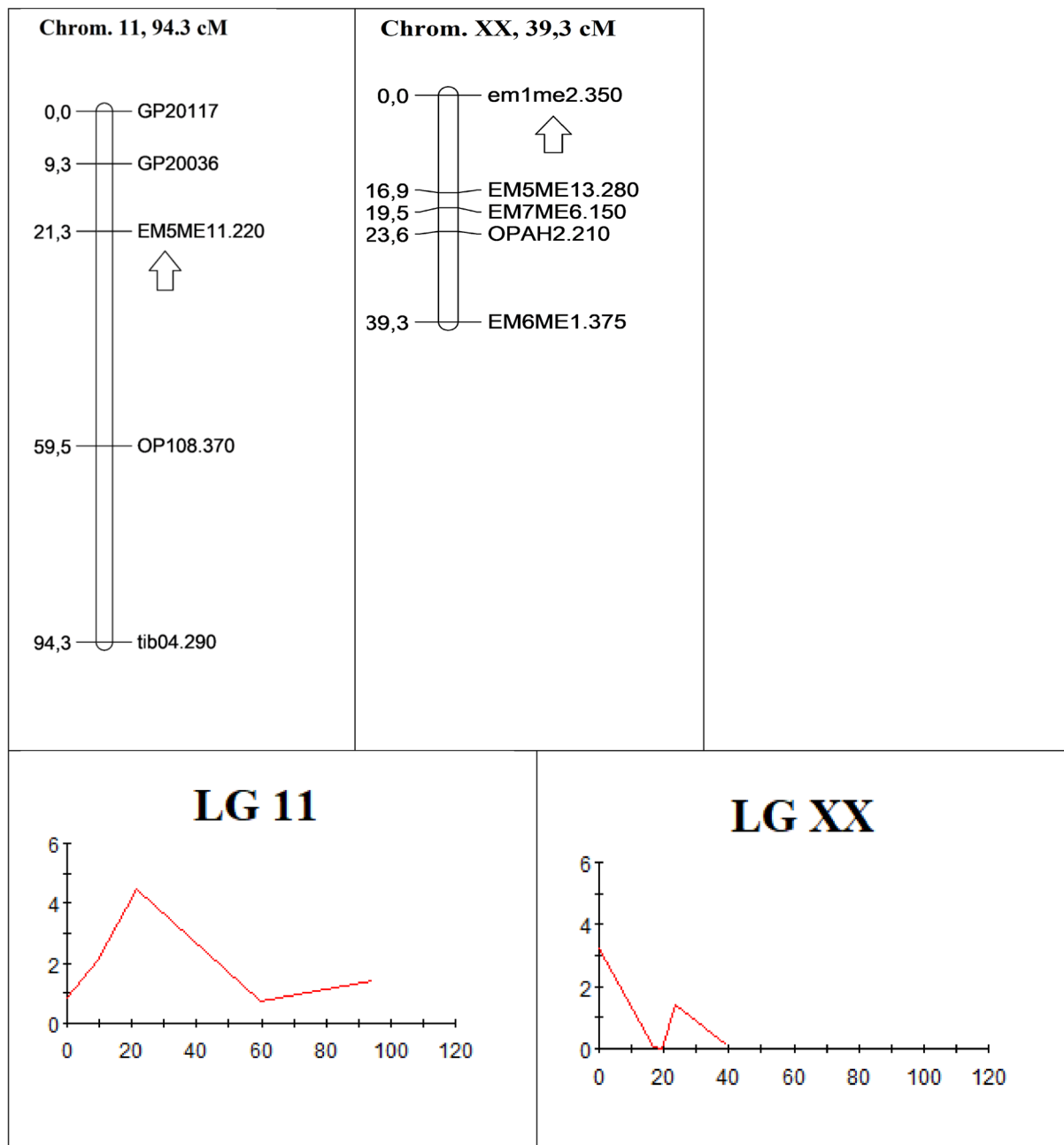


Fig. 3 Distribution of simple sequence repeat (SSR), sequence-related amplified polymorphism (SRAP) and random amplified polymorphic DNA (RAPD) markers and endosperm-

based hybridization barriers ratio (%) related quantitative trait loci (QTLs) on 11 and XX linkage groups of pepper

sequence divergence and dosage imbalance, among others (Maheshwari and Barbash 2011). Learning about the molecular processes behind hybrid incompatibility is a major objective in evolutionary biology. This knowledge is also important for the breeding

of plants since it may help create new hybrid types. Using *C. annuum* L. (21A) and *C. frutescens* L. (PI 281420) as parents, two closely related pepper species, we investigate the hybrid incompatibility between them in this work. Interspecific hybridization

is the main technique used to transfer biotic and abiotic tolerance/resistance genes from *C. annuum* to *C. frutescens*. However, as noted by Jae et al. (2006), there are major obstacles due to genetic barriers across species. According to our research, the primary cause of the interspecific incompatibility between *C. annuum* and *C. frutescens* is embryo abortion. This abortion happened about 39 days after pollination, before the globular embryo stage. Within the Solanaceae family, particularly in tomatoes, these genetic barriers have been well characterized. Two steps were taken to address the problem of early embryo abortion. This work is the first to report on crossing *C. annuum* with *C. frutescens* to produce an interspecific F_2 population. Investigations on tomatoes have shown similar outcomes. Epigenetic alterations, including as DNA methylation and histone modifications, control genomic imprinting at the molecular level. This is the first research that presents the generation of molecular markers associated to QTLs/genes that influence endosperm-based hybridization barriers in an interspecific F_2 population produced from *C. annuum* and *C. frutescens*. Two quantitative trait loci (QTLs) were identified on linkage groups (LG) 11 and XX in the study. Additionally, the mode of segregation indicated that dominant epistasis with a 12:3:1 ratio is responsible for the trait in the F_2 population. These results may provide insights into interspecific hybridization and genetic research in pepper.

Conclusion

Interspecific crosses are used in many plant species to generate new genetic diversity. However, endosperm-induced hybrid mortality occurs as a result of interspecific hybridization. This situation creates a barrier to breeding and prevents the increase of genetic diversity. Currently, there are very few investigations on the genetic-based mechanism of hybrid mortality, or the research results do not point to the genes involved. This study contributes to our understanding of the genetic processes underlying hybrid seed lethality and thus provides insights for the improvement of crop species through breeding. The QTLs identified in this study will provide time and financial advantages for future breeding research.

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Author contribution NM, HP proposed the concept. All authors conceived the manuscript. HA and VD wrote the manuscript. CŞ and SD prepared the figures. All authors reviewed and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Conflict of interest The authors declare no competing interests.

Consent to participate The authors consent to participate.

Consent for publication The authors consent for publication.

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