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Unlocking genetic potential in Capsicum species through interspecific hybridisation

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Abstract

Capsicum chinense, Jacq, commonly called habanero-type pepper and *Capsicum frutescens* L., commonly called wild pepper, have higher capsaicin content and better adaptability to changing climatic scenarios. These two species could be a potential source of variation to improve *Capsicum annuum* L, the cultivated pepper. Introgression of beneficial genes from wild relatives to cultivated crops through interspecific hybridisation has always been advantageous. The present study explored the possibility of interspecific crosses using three different *Capsicum* species viz., *C. annuum* (four genotypes), *C. frutescens* (two genotypes) and *C. chinense* (nine genotypes), to develop F_1 hybrids in chilli for high capsaicin content. The genotypes of *C. chinense* and *C. frutescens* having high capsaicin content were chosen to transfer genes to the cultivated *C. annuum* genotypes to improve their commercial value. The percent of fruit set was better in *C. annuum* × *C. chinense and C. fruitescens* × *C. chinense.* Seeds were more in *C. annum* × *C. chinense* (20 numbers of seeds) than in *C. fruitescens* × *C. chinense with* 8number of seeds. Irrespective of the parents involved, there was variation in seed germination in different cross combinations. Percent of germination and seedling survival was less in the crossed fruits. This experiment offers scope for further studies on pollen viability and the introgression pattern can be confirmed using molecular markers.

Keywords

chilli; F_1 hybrids; gene introgression; interspecific hybridisation; wild relatives

Introduction

Chilli is a high-value horticultural crop, with its fruits consumed both as vegetables and spice in fresh and dried forms. It is a native of Central and South America and is one of the oldest cultivated crops (1). There are five domesticated species of chilli peppers *viz*., *C. annuum*, *C. baccatum*, *C. chinense*, *C. frutescens* and *C. pubescens* (1). Of the five domesticated species, *C. annuum* (2n= 2 x= 24), a native species in Mexico, is one of the economically important vegetable crops (2). This species is also the most common and extensively cultivated in tropical and subtropical regions worldwide (3). However, the genetic base of cultivated pepper (*C. annuum*) is narrowing due to domestication, limiting opportunities for crop improvement. On the contrary, the wild relatives of chilli are characterised by a relatively larger genetic variation. The genus *Capsicum* comprises over 20 wild species, which have been utilized in breeding programs for several decades. These wild relatives are useful for broadening the narrow genetic base of cultivated pepper (4).

Interspecific hybridization has immense potential to create wide variability and to develop newer varieties and hybrids by diversifying the genetic base. Interspecific hybridization allows the introgression of desirable genes governing important traits such as yield, disease resistance and bioactive compounds, allowing breeders to develop genetically superior varieties and hybrids (5). However, for the success of such a transfer, the species must be genetically close, minimizing incompatibility problems and thus enabling hybridization. Incompatibility barriers in interspecific crosses may be due to pre-fertilization and post-fertilization barriers (6). Also, some interspecific crosses are unidirectional, wherein the reciprocal crosses are unsuccessful such that in a cross (A x B) when species A is the female parent and species B is the male parent, successful pollination and seed set is observed, but in the reciprocal cross when species B is the female parent and species A is the male parent (B x A) pollination and seed set is not successful. In chilli, not much success has been obtained in interspecific hybridisation due to cross incompatibility. An interspecific hybrid has been obtained in a cross combination between *C. chinense* × *C. frutescens* (7). In another study, interspecific hybridisation between *C. chinense* and *C. annuum* resulted in fruits in which the seeds were viable (8). A total of nine hybrids were obtained from the combination of *C. annuum* × *C. baccatum*; however, out of nine hybrids, only seven seedlings reached maturity.

Considering this, 45 crosses were made to assess the success of interspecific hybridization between different species of chilli, including their reciprocals.

Materials and Methods

The study was conducted at the College Orchard, Department of Vegetable Science, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. The location is at an altitude of 426.6 m above the mean sea level and at 11°N latitude and 77°E longitude. Three species of chilli, *Capsicum annuum, C. chinense* and *C. fruitescens,* were used in this study.

Molecular characterisation of different species of chilli

The different species of chilli, *C. annuum*, *C. chinense* and *C. fruitescens* were subjected to molecular characterisation using 30 SSR markers because they are highly polymorphic, co-dominant and readily transferable. This makes them a powerful tool for identifying genetic diversity within chilli populations. This is crucial for selecting desirable traits in breeding programs and accurately distinguishing different chilli varieties to estimate their genetic diversity (Table 1).

Nursery preparation

Chilli seedlings were raised under a shade net with 50% shade, covered with 40 mesh insect-resistant nylon nets that ran along the surrounding walls. The seeds were treated with the bio-control agent *Trichoderma viride* @ 4g/kg of seeds overnight the day before sowing to prevent them from pathogenic infection. The pretreated seeds were sown in 98 cells protrays filled with completely decomposed coco peat supplemented with *Azospirillum*. Two to three seeds were sown in each planting cell and later thinned to a single seedling per cell. The protrays were watered every day using a rose-can and drenched with 19:19:19 of N.P and K @ 5g/l to promote quick germination and good seedling growth 18 days after sowing.

Main field preparation

The experimental plot was prepared under a shade net with 50% shade. The soil was ploughed 2-3 times to break the soil clods after each ploughing to achieve fine tilth. Farmyard manure (FYM) @ 25 t/ha was added to the soil at the time of the last ploughing. Ridges and furrows were made at 90 × 60 cm spacing and the ridges were irrigated with drip irrigation before transplanting.

Transplanting

Forty-five days old chilli seedlings of 15 cm height were transplanted with the ball of the earth at one seedling per hill on either side of the ridges with 60 cm spacing between the plants. For each genotype, about 20 seedlings per replication were transplanted to the main field. All the recommended Standard Horticultural practices for chilli cultivation (Crop production guide of the Government of Tamil Nadu) were followed throughout the cropping period.

Production of interspecific F_1 hybrid

The crossing block was raised with different species of Chilli viz., *Capsicum annuum, C. chinense and C. fruitescens* in Line x Tester (L×T) mating design. The mature balloon-shaped buds of the female parents were emasculated the previous day at anthesis to avoid self-pollination and covered with butter-paper bags. Pollination was made in the morning using freshly mature pollen from flowers collected from the male parent. The pollinated flowers were tagged with genotypes of the parents involved in the cross and the date at which it was finished. The rest of the buds and the naturally pollinated ones were cut off. Crossing was attempted in all possible combinations.

Identification of interspecific hybrid F_1

Different species of chilli *viz*., *C. annuum*, *C. fruitescens* and *C. chinense* were raised in crossing the block in Line x Tester mating design. Crossing was attempted in all possible combinations. Observations were recorded for the percent fruit set, number of seeds per fruit, seed germination percentage and

Table 1. List of SSR markers used in the study and their detailed information

Primer	Sequence	Expected base pair	T_a	Reference
AVRDC PP-208	**F:** TATTCCTTCTTCACCCCTCC **R:** GAAAGAGGCGCTAACTGGAC	153-310	55.5 °C	(Dhaliwal *et al.*, 2014)
AVRDC PP 3	**F:** GCTAGGCTTGATCCTTCACC **R:** CGCTTGAAATCATGCTCACT	83-113	55 °C	(Dhaliwal *et al*., 2014)
CaBR76	**F**: gCTAgTCACgTCAATCTgTT **R**: CATTCTCTTCTTCTCAAACg	116-140	56 °C	G.S.C. Buso *et al*.2016

seedling survival in *Capsicum* species and their interspecific crosses.

Results

Molecular characterization was done using 30 SSR molecular markers to observe diversity. The results showed that out of thirty molecular markers used, three markers, viz., AVRDCPP 208, AVRDCPP 3 and CaBR76, exhibited polymorphism effectively differentiating the three *Capsicum* species (Fig. 1, Fig. 2 and Fig. 3).

The study observed that *C. fruitescens* had a higher percentage of fruit set (92.1%) than *C. annuum* (80.0%) and *C. chinense* (66.6%). When comparing the number of seeds per fruit, *C. annuum* registered a higher number (40), followed by *C. chinense* (31) and *C. fruitescens* (20) (Table 2).

A successful fruit set was found in *C. annuum* × *C. chinense, C. chinense* × *C. annuum, C. fruitescens* × *C. chinense and C. fruitescens* × *C.annuum* showing that these combinations were compatible (Table 3). It was observed from the study that *C. annuum* was crossable with *C. chinense* in both direct and reciprocal. Similarly, *C. fruitescens* was cross-compatible with *C. chinense* and *C. annuum.* Among the intercrosses, the fruit set percent was higher (20%) in *C. annuum* × *C. chinense* followed by *C. fruitescens* × *C. chinense* with 15%. Likewise, the number of seeds per fruit was higher in *C. annuum* × *C. chinense* (20) and a lesser number (8) was recorded in *C. fruitescens* × *C. chinense.*

Significant differences existed in the percent of seed germination and seedling survival in *Capsicum* species and their interspecific crosses (Table 4). Of the different species used, *C. fruitescens* recorded the highest germination percent (38.0%), followed by *C. annuum* (25%), whereas among the intercrosses, *C. annuum* × *C. chinense* showed the highest percentage (23.6%). The seedling survival percent was the highest in *C. fruitescens* (92.1%) followed by *C. annuum* (80%). Among the intercrosses, *C. annuum* × *C. chinense* exhibited a higher percent of seedling survival (18.1%) and a lower level (8%) was noticed in the intercross, *C. fruitescens* × *C. annuum*.

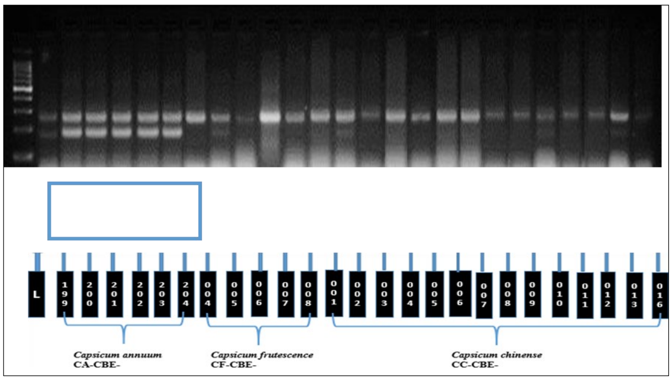

Fig. 1. DNA Profiling of chilli species using AVRDCPP208 marker.

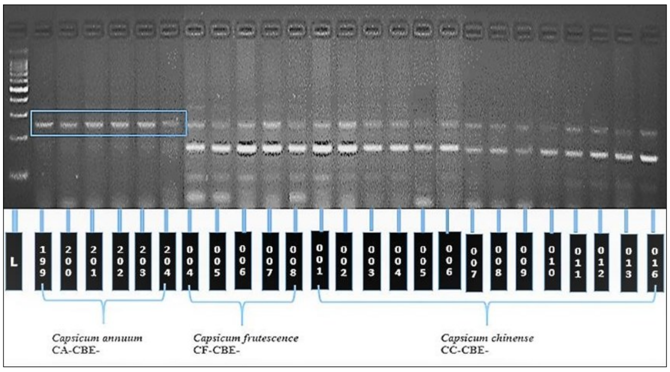

Fig. 2. DNA Profiling of chilli species using AVRDCPP3 marker.

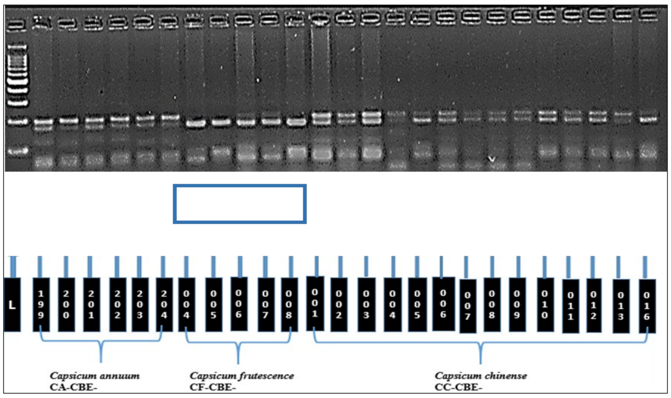

Fig. 3. DNA Profiling of chilli species using CaBR76 marker.

Discussion

The observed polymorphism in the three *Capsicum* species aligns with findings from previous studies (9, 10)

Significant differences were evident between and within the species for the various characters studied. The variation amongst different species of *Capsicum* observed in the present study has been studied and established through previous studies in *Capsicum*. The classification of the accessions of *C. annuum, C. baccatum* and *C. chinense* into three main clusters using cluster analysis based on

Table 2. Percent fruit set and number of seeds per fruit in *Capsicum* species at F_0 level

Species	Number of selfed flowers	Number of fruits	Fruit set (%)	Number of seeds per fruit
C. annuum	25	20	80.0	40
C. chinense	15	10	66.6	31
C. fruitescens	38	35	92.1	20

Table 3. Percent fruit set and number of seeds per fruit in interspecific crosses at F_0 level

Crosses	Number of crossed flowers	Number of fruits	Fruit set (%)	Number of seeds per fruit
C. annuum × *C. chinense*	20	4	20.0	20
C. chinense × *C. annuum*	21	3	14.3	13
C. fruitescens × *C. chinense*	33	5	15.0	8
C. fruitescens × *C. annuum*	35	4	11.0	9

Table 4. Percent of seed germination and seedling survival in *Capsicum* species and their interspecific crosses at the F_1 level

Species/crosses	Seed germination (%)	Seedling survival (%)
C. annuum	25.0	80.0
C. chinense	15.0	66.6
C. fruitescens	38.0	92.1
C. annuum × *C. chinense*	23.6	18.1
C. chinense × *C. annuum*	19.5	10.3
C. fruitescens × *C. chinense*	15.9	12.2
C. fruitescens × *C. annuum*	14.2	8.0

characters recommended by the IPGRI descriptor (1995) has been reported (11).

Evaluating the compatibility of crosses between *C. chinense* and *C. annuum* on the seed germination showed that interspecific crosses produced fruit set rates between 8.9% and 40.0%. Germination ranged from 0.0% to 45.3%, concluding that it is possible to obtain fruit and viable seeds in interspecific crosses of *C. chinense* with *C. annuum* (12). Germination rates of interspecific hybrids between *C. chinense* and *C. annuum* ranged from 0% to 97% (13). In another study, germination rates ranging from 0% to 6.6% were obtained in crosses using *C. chinense* as the female parent and *C. annuum* as the male parent and between 0% and 47% when *C. chinense* was used as the male parent (14).

Chromosomal disharmony may have affected the initial seedling development in hybrids, leading to development arrest or mortality. During this critical phase of cell differentiation, many genes responsible for tissue and chlorophyll formation are activated simultaneously and any disruption in protein synthesis can adversely affect plant growth.

Conclusion

From the study, it can be concluded that the percent fruit set was better in *C. annuum* × *C. chinense and C. fruitescens* × *C. chinense*. Seeds were more in *C. annum* × *C. chinense* (20 numbers) than in *C. fruitescens* × *C. chinense* (8 seeds). Irrespective of the parents involved, there was variation in seed germination in different cross combinations. Percent of germination and seedling survival was less in the crossed fruits. The seed germination can be enhanced using hydration treatments like seed priming and seed encapsulation like seed pelleting, which significantly impacts seedling emergence and establishment. Alternatively, seed abortion can also be prevented by biotechnological approaches. The study signifies that interspecific hybridization is possible in chilli despite pre- and post-fertilization barriers, such as unilateral incompatibility and genotype specificity and development of abnormal/empty F_1 seeds, lack of vigour and hybrid sterility of the F_1 plants. This offers ample scope to broaden the genetic base of chilli and to develop novel cultivars through transferring desired characteristics like high capsaicin content from *C. chinense* and a higher number of fruit set in *C. fruitescens.*

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Authors' contributions

UNDH conceptualised and performed the experiment and manuscript preparation PL verified the analytical methods, revision of the manuscript All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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