Mapping of fruit length related QTLs in interspecific cross (Capsicum annuum L. × Capsicum galapagoense Hunz.) of chilli

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Fruit length in chilli is quantitatively inherited trait and selection based on phenotypic performance is tedious and time consuming. To detect QTLs determining fruit length in Capsicum spp., an interspecific $F_2$ mapping population was developed from the cross of $C.\ annuum$ L. cv. ‘FL 201’ with $C.\ galapagoense$ Hunz. accession ‘TC 07245’. Fruit length in this cross showed a quantitative inheritance with the population depicting a symmetric distribution in histogram. To map quantitative trait loci (QTLs) for fruit length 400 SSR markers were surveyed on the parental genotypes but only 28 markers were observed to be polymorphic indicating less genetic diversity between the two Capsicum species. Polymorphic markers were then analyzed in $F_2$ population consisting of 210 plants and 24 of these markers were mapped on to three linkage groups (LGs): LG 1, LG 2 and LG 3. Two fruit length determining QTLs designated as paufl2.1 and paufl2.2 were identified and both the QTLs were mapped on to LG 2. The two QTLs together explained 21.78 per cent of the phenotypic variation. Apart from the two QTLs, positive alleles were detected in the small fruited parent ‘TC 07245’ which might be of potential use in chilli breeding programs.

Key Words: chilli pepper, fruit length, molecular markers, paufl2.1, paufl2.2, QTL mapping.

Introduction

Chilli or hot pepper belongs to the genus Capsicum five species of which namely $C.\ annuum$ L., $C.\ chinense$ Jacq., $C.\ frutescens$ L., $C.\ baccatum$ L. Ruiz. & Pavon, and $C.\ pubescens$ Ruiz. & Pavon are domesticated. $C.\ annuum$ L. is the widely cultivated species worldwide and includes both, the chilli pepper and the bell pepper (Bosland 1992). Improving yield related traits have remained a major goal for chilli breeding (Barchi et al. 2009, Zygier et al. 2005). Fruit length in chilli determines consumer acceptability and contributes directly to the yield. This is a polygenic trait with narrow sense heritability of 0.68–0.76 (Ben Chaim et al. 2001). The conventional biometrical approaches, although descriptive of inheritance, but do not explain the effects of individual quantitative trait loci (QTL) affecting a trait. Therefore, breeders grow large populations over locations and years to reliably make selection on phenotypic basis. This makes the field evaluation for fruit length and yield improvement cumbersome and time consuming with low genetic gains.

In recent years, progress in molecular marker technology has permitted breeders to identify individual QTLs and estimate their effects on phenotypic performance (Barchi et al. 2009, Lu et al. 2012). Introgression of QTLs from the related species to the commercial types has been accelerated through Marker-Assisted Breeding (MAB). Among the molecular markers, Simple Sequence Repeats (SSRs) have been extensively used in mapping programs due to their suitability for automation, high throughput and good genome coverage (Hearne et al. 1992, Mimura et al. 2012, Powell et al. 1996, Sugita et al. 2013). Despite the advances made in marker technology, progress in mapping QTLs in chilli has been rather slow when compared with other Solanaceous crops such as tomato ($Solanum\ lycopersicum$ L.). The probable reasons could be that chilli has larger genome size, approx. $3\times$ that of tomato (Park et al. 2011) and exhibit low levels of polymorphism (Dhaliwal et al. 2014, Dwivedi et al. 2013). To facilitate MAB in chilli, Barchi et al. (2009), Ben Chaim et al. (2001), Dwivedi et al. (2013), and Han et al. (2016) detected QTLs for fruit length, fruit diameter, fruit shape, fruit weight and other yield related traits in populations originating from intra- and inter-specific crosses.

The objective of this study was to identify and map QTLs for fruit length in $F_2$ population derived from an...
inter-specific cross of long fruited *C. annuum* cv. ‘FL 201’ × short fruited *C. galapagoense* accession ‘TC 07245’. The identified QTLs would facilitate their introgression to the commercial types through MAB and might serve as potential target regions for cloning of the candidate genes through fine mapping.

**Materials and Methods**

**Plant material**

The plant material was developed from the inter-specific cross involving long fruited *C. annuum* cv. ‘FL 201’ and short fruited *C. galapagoense* accession ‘TC 07245’. Plants of ‘FL 201’ are medium tall (50–55 cm) with light green foliage, pale green anthers and erect fruit bearing habit. Plants of ‘TC 07245’ are tall (80–85 cm) with dark green foliage, purplish anthers and pendent fruit bearing habit. The parental lines were maintained by continuous selfing for several generations. The F1 seed was generated by controlled pollination in 2013 using ‘FL 201’ as a maternal parent, ‘TC 07245’ as a paternal parent, confirmed hybridity of the F1 seed was raised in 2014 in an insect-proof cage to generate the F2 seed. The F2 seed were sown in October 2014 and the mapping population comprising of 210 F2 plants along with the two parents was transplanted to the field in February 2015.

**Trait evaluation**

Individual F2 plants and the two parents were phenotyped for fruit length at the second harvest. Five red ripe fruits were taken randomly from different plant positions and fruit length, the distance from the pedicel attachment to its apex, was measured in cm. Means were used for classification of fruit length following the scale described in Table 1.

Broad sense heritability 

\[ h^2 = \frac{\text{VF}_2}{\text{VF}_2 - \frac{1}{2}(\text{VP}_1 \times \text{VP}_2)} \]

\( \text{VF}_2 \) = phenotypic variance of F2, \( \text{VP}_1 \) = phenotypic variance of P1, \( \text{VP}_2 \) = phenotypic variance of P2, and \( \text{VF}_1 \) = phenotypic variance of F1.

**Table 1.** Scale used for classification of the fruit length in F2 population derived from the cross *C. annuum* ‘FL 201’ × *C. galapagoense* ‘TC 07245’ chilli

| S.No. | Fruit length (cm) | Classification       |
|-------|------------------|----------------------|
| 1     | 3.80 to 4.79     | Very small fruit     |
| 2     | 4.80 to 6.79     | Small fruit          |
| 3     | 6.80 to 7.79     | Medium long fruit    |
| 4     | 7.80 to 8.79     | Long fruit           |
| 5     | 8.80 to 10.79    | Very long fruit      |
| 6     | 10.80 and above  | Extra-long fruit     |

1 S.No. = Serial Number.

**SSR amplification and polymorphism**

The genomic DNA was isolated from fresh leaves following the method described by Singh et al. (2009). DNA was quantified using spectrophotometer (Nanodrop 1000, Thermo scientific Inc., Waltham, MA USA) and adjusted to 50 ng/μl by adding TE. The PCR was performed in a reaction mixture (25 μl) containing 2.0 μl (50 ng) genomic DNA, 1.5 μl (5 μM) of each forward and reverse primers, 0.5 μl (10 mM) dNTP mix, 1.5 μl (25 mM) MgCl2, 5 μl (5×) green flexi PCR buffer, 0.12 μl (5 U/μl) Taq polymerase and 12.8 μl of sterile distilled water. The PCR reagents were procured from Promega, Madison, WI, USA.

SSR marker analysis was performed to determine the polymorphism between the two parents, ‘FL 201’ and ‘TC 07245’. In total, 400 SSR markers including 112 genic (gene based SSR markers with a known location on a chromosome) and 288 genomic markers (developed from SSR enriched genomic libraries) covering the whole *Capsicum* genome were screened. The genic markers were selected from genetic maps developed by Ince et al. (2010), Lee et al. (2004), Minamiyama et al. (2006) and Yi et al. (2006). Primer sequences for genomic SSR primer pairs were received from Dr. Roland Schafleitner, the World Vegetable Center, Taiwan. List of 400 primer pairs used for parental polymorphism survey is given in Supplemental Table 1.

The DNA amplifications were performed in Master Cycler 5331-Eppendorf version 2.30.31-09, Germany. A touchdown PCR program was followed to amplify the DNA fragments, that is initial denaturation at 94°C for 3 min followed by 10 cycles of denaturation at 94°C for 30 s, annealing at 60°C (the annealing temperature for each cycle being reduced by 1°C per cycle) for 1 min and extension at 72°C for 1 min, and subsequently, 30 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 1 min, extension at 72°C for 1 min and final extension at 72°C for 5 min. The PCR products of SSR markers were first fractioned at 4.0 per cent agarose gel. The markers which did not show any detectable parental polymorphism on agarose gels were subjected to 6.0% polyacrylamide gels (PAGE) using 0.5× Tris-borate-EDTA (TBE) buffer containing ethidium bromide (HiMedia Labs. Pvt. Ltd., Mumbai India) and visualized under UV light on an Alpha Imager® HP imaging system (Fisher Scientific Ltd., Loughborough UK). The results were then confirmed through high resolution MCE®-202 multiNAMicro-chip automated electrophoresis system.

**QTL analysis and genetic mapping**

Means of individual F2 plants were used for QTL analysis. MAPMARKER/EXP v3.0b program (Lander and Botstein 1989) was used to create a framework linkage map for QTL detection. The map was generated at a Logarithm of Odds (LOD) score of 3.0 and maximum recombination fraction of 30 centi Morgan (cM). Linkage distances in terms of cM were calculated by means of the Kosambi’s mapping function. The commands ‘Order’ and ‘Rip’ were used to assign the order of markers on the map. Mapping was...
performed using the QTL Cartographer v2.5 (Wang et al. 2010). Percentage of phenotypic variation accounted for individual QTL was estimated by the coefficient of determination ($R^2$). Additive (a) and dominance (d) effects were determined by single-marker analysis with the highest F-value within a given QTL region. The detected QTLs were designated as per the International Rules of Genetic Nomenclature. In the assigned nomenclature, ‘pau’ indicated name of the institution ‘Punjab Agricultural University’, ‘fl’ indicated the trait ‘fruit length’, the first number indicated the pepper linkage group (LG) involved, and the second number indicated the position within the LG to which the QTL is mapped.

**Results**

**Phenotypic evaluation of F$_2$ population**

The phenotypic diversity in fruit length between the parental lines ‘FL 201’ and ‘TC 07245’, and a wider range within the F$_2$ population is depicted in Figs. 1, 2, respectively. The fruit length data of the 210 F$_2$ plants were classified into six categories: very small, small, medium long, long, very long and extra-long fruit length. Variation in the F$_2$ population is represented graphically through the frequency distribution in Fig. 3. The maximum number of segregants (29.52%) was grouped under the ‘medium long’ category. The frequency distribution formed nearly the symmetric histogram indicating that fruit length in chilli is quantitatively inherited trait with additive gene effects. Some segregants with mean values on either side of the parental range were also observed. Broad sense heritability ($h^2_{bs}$) of fruit length in F$_2$ population of chilli was 0.75.

**Marker analysis and QTL mapping**

Of the 400 SSR markers used in parental polymorphism survey, only 28 markers were polymorphic. The list of polymorphic SSR markers is given in Table 2. For genotyping, banding pattern obtained across 210 F$_2$ population was scored and marker segregation pattern was checked for their

fitness to the expected 1:2:1 (AA:AB:BB) genetic ratio. The observed $\chi^2$ values for all the polymorphic markers are less than the tabulated $\chi^2$ value 5.99 at 5 per cent level of significance and 2 degree of freedom. Thus, genotypic segregation data fitted well and do not deviate from the expected genotypic ratio. Segregation pattern of selected SSR markers resolved on agarose and PAGE are shown in Fig. 4a, 4b, respectively.

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**Fig. 1.** Phenotypic variability for fruit length between parental lines ‘FL 201’ and ‘TC 07245’.

**Fig. 2.** Phenotypic variation for fruit length in F$_2$ population derived from the cross *C. annuum* ‘FL 201’ × *C. galapagoense* ‘TC 07245’ chilli.
as shown in Fig. 5. Linkage analysis mapped 12 markers on to LG-1, 8 markers on to LG-2, and 4 markers on to LG-3. The remaining 4 markers could not be linked to any of the Linkage groups. The genetic map covered a total map length of 399 cM with an average marker interval of 16.62 cM between two markers. Based on the BLAST analysis, position of the unlinked markers AVRDC-PP161, AVRDC*MD911, CAMS 684 and Hmps 2-9 was located on the chromosome 12, 1, 6 and 3, respectively.

Marker analysis following composite interval mapping (CIM) of the F2 population detected two QTLs determining fruit length. The QTLs designated as paufl2.1 and paufl2.2 were conditioned by the marker pairs AVRDC-PP236-AVRDC*MD 705 and GPMS 100-AVRDC*MD 782 (Fig. 5). Both the QTLs were mapped on to the LG-2. The QTL likelihood plots of LG-2 showing the two LOD peaks for fruit length in F2 population are shown in Fig. 6. The QTL paufl2.1 was found to be the major QTL explaining 13.38 per cent of the phenotypic variation with LOD score of 5.53. The minor QTL paufl2.2 explained 8.40 per cent of the phenotypic variation with LOD score of 5.26. The identified QTLs showed positive additive effects and were derived from the long fruited ‘FL 201’. The F2 population mean for the four markers viz., AVRDC-PP236, AVRDC*MD705, GPMS 100 and AVRDC*MD782 spanning QTLs Paufl2.1 and Paufl2.2 respectively were analyzed. At marker locus,

### Table 2. Test of significance for segregation of polymorphic SSR markers in the F2 population derived from the cross FL 201 × TC 07245

| SSR primer          | Plant population scored | Observed frequency values* | Expected frequency values | \( \chi^2 \) (1:2:1) | P-value at P ≤ 0.01 |
|---------------------|-------------------------|-----------------------------|---------------------------|------------------------|--------------------|
|                     | P1 allele   | H allele  | P2 allele | Not amp. | P1 allele | H allele | P2 allele | Ratio |                         |
| AF244121            | 210         | 48        | 110       | 52       | 52.5      | 105      | 52.5      | 1:2:1 | 0.628                    | 0.730 |
| AF130118            | 210         | 47        | 110       | 53       | 52.5      | 105      | 52.5      | 1:2:1 | 0.819                    | 0.664 |
| AVRDC*MD711        | 210         | 54        | 113       | 43       | 52.5      | 105      | 52.5      | 1:2:1 | 2.372                    | 0.305 |
| AVRDC-PP231        | 210         | 47        | 112       | 51       | 52.5      | 105      | 52.5      | 1:2:1 | 1.086                    | 0.581 |
| AVRDC*MD678        | 210         | 52        | 110       | 48       | 52.5      | 105      | 52.5      | 1:2:1 | 0.874                    | 0.646 |
| AVRDC-PP124        | 210         | 54        | 112       | 44       | 52.5      | 105      | 52.5      | 1:2:1 | 1.774                    | 0.412 |
| CAMS 844           | 210         | 48        | 109       | 53       | 52.5      | 105      | 52.5      | 1:2:1 | 0.543                    | 0.762 |
| AVRDC-PP171        | 210         | 51        | 111       | 58       | 52.5      | 105      | 52.5      | 1:2:1 | 1.306                    | 0.520 |
| AVRDC-PP102        | 210         | 47        | 113       | 50       | 52.5      | 105      | 52.5      | 1:2:1 | 1.300                    | 0.520 |
| HpmsE063           | 210         | 49        | 111       | 50       | 52.5      | 105      | 52.5      | 1:2:1 | 1.600                    | 0.740 |
| HpmsE028           | 210         | 52        | 108       | 50       | 52.5      | 105      | 52.5      | 1:2:1 | 2.101                    | 0.900 |
| HpmsE062           | 210         | 46        | 110       | 54       | 52.5      | 105      | 52.5      | 1:2:1 | 1.115                    | 0.573 |
| CAMS 888           | 210         | 48        | 111       | 51       | 52.5      | 105      | 52.5      | 1:2:1 | 0.773                    | 0.679 |
| AVRDC-PP250        | 210         | 48        | 113       | 49       | 52.5      | 105      | 52.5      | 1:2:1 | 1.230                    | 0.541 |
| AVRDC-PP236        | 210         | 50        | 112       | 48       | 52.5      | 105      | 52.5      | 1:2:1 | 0.973                    | 0.615 |
| AVRDC*MD705        | 210         | 48        | 109       | 53       | 52.5      | 105      | 52.5      | 1:2:1 | 0.542                    | 0.763 |
| GPMS 100           | 210         | 54        | 110       | 46       | 52.5      | 105      | 52.5      | 1:2:1 | 0.967                    | 0.671 |
| AVRDC*MD860        | 210         | 43        | 112       | 53       | 52.5      | 105      | 52.5      | 1:2:1 | 2.250                    | 0.325 |
| AVRDC*MD782        | 210         | 51        | 110       | 49       | 52.5      | 105      | 52.5      | 1:2:1 | 0.514                    | 0.773 |
| CAMS 808           | 210         | 49        | 109       | 52       | 52.5      | 105      | 52.5      | 1:2:1 | 0.391                    | 0.822 |
| AVRDC-PP711        | 210         | 45        | 114       | 51       | 52.5      | 105      | 52.5      | 1:2:1 | 1.885                    | 0.390 |
| AVRDC-PP116        | 210         | 50        | 112       | 48       | 52.5      | 105      | 52.5      | 1:2:1 | 0.972                    | 0.615 |
| HmpsE119           | 210         | 53        | 113       | 44       | 52.5      | 105      | 52.5      | 1:2:1 | 1.643                    | 0.440 |
| HmpsE010           | 210         | 52        | 109       | 49       | 52.5      | 105      | 52.5      | 1:2:1 | 0.390                    | 0.823 |
| Hmps 2-9           | 210         | 48        | 111       | 51       | 52.5      | 105      | 52.5      | 1:2:1 | 0.772                    | 0.680 |
| AVRDC-PP161        | 210         | 49        | 114       | 47       | 52.5      | 105      | 52.5      | 1:2:1 | 1.580                    | 0.454 |
| AVRDC*MD911        | 210         | 53        | 112       | 45       | 52.5      | 105      | 52.5      | 1:2:1 | 1.543                    | 0.462 |
| CAMS 684           | 210         | 46        | 113       | 51       | 52.5      | 105      | 52.5      | 1:2:1 | 1.456                    | 0.483 |

* Where: P1, allele from FL 201; H, Heterozygous allele; P2, allele from TC 07245.
Mapping of fruit length related QTLs in chilli

This work was undertaken to identify fruit length determining QTLs so as to facilitate their introgression in elite chilli breeding lines through MAB.

The frequency distribution for fruit length in the F2 mapping population showed nearly normal distribution, which indicated that the trait is quantitatively inherited with additive effects. However, some transgressive segregants on either side of the parental means were observed. This suggested that some positive alleles were also contributed by the short fruited parent ‘TC 07245’. Utilizing such alleles from the wild germplasm, Tanksley et al. (1996) increased fruit size in cultivated tomato lines by the introduction of genes from the small fruited *S. pimpinellifolium*. Similarly, red fruit color of a processing tomato line was enhanced by the introgressions from the green fruited *S. hirsutum* (Tanksley and McCouch 1997). The positive alleles detected in ‘TC 07245’ might be of potential use to the chilli breeders.

**Discussion**

An important development during the last few decades has been the ability of crop breeders to tag genomic regions called QTLs responsible for variation in quantitative traits. The linked markers have facilitated genetic enhancement in major field crops through pyramiding of QTLs and breaking of undesirable linkages (Ordon et al. 1998, Ribaut and Hoisington 1998). However, the progress in molecular breeding in chilli has been rather slow, especially with respect to the quantitative traits (Barchi et al. 2009). This work was undertaken to identify fruit length determining QTLs so as to facilitate their introgression in elite chilli breeding lines through MAB. The frequency distribution for fruit length in the F2 mapping population showed nearly normal distribution, which indicated that the trait is quantitatively inherited with additive effects. However, some transgressive segregants on either side of the parental means were observed. This suggested that some positive alleles were also contributed by the short fruited parent ‘TC 07245’. Utilizing such alleles from the wild germplasm, Tanksley et al. (1996) increased fruit size in cultivated tomato lines by the introduction of genes from the small fruited *S. pimpinellifolium*. Similarly, red fruit color of a processing tomato line was enhanced by the introgressions from the green fruited *S. hirsutum* (Tanksley and McCouch 1997). The positive alleles detected in ‘TC 07245’ might be of potential use to the chilli breeders. Therefore, it is imperative to identify such genes in ‘TC
Though the parental species were phenotypically diverse, yet the low level of polymorphism could be attributed to their genetic relatedness as both the species have been grouped under Annuum complex (McLeod et al. 1983). Secondly, genic SSR markers have been reported to be less polymorphic compared with genomic SSR markers (Cho et al. 2000). However, the low level of SSR polymorphism may be compensated for by their potential of inter-specific transferability (Thiel et al. 2003).

Most of the QTLs identified by Ben Chaim et al. (2001) for fruit and growth characteristics had relatively small effects, controlling less than 20% of the total phenotypic variation, and were clustered in a few chromosomal regions. We identified two fruit length determining QTLs designated as paufl2.1 and paufl2.2 clustered together on LG 2. These QTLs together explained 21.8% of the phenotypic variation.

Our results are consistent with the previous inheritance studies, in that additive gene effects for fruit length are of primary importance (Barchi et al. 2009, Ben Chaim et al. 2001, Dwivedi et al. 2013, Lee et al. 2011, Mimura et al. 2012) with moderate estimates (0.6–0.8) of $h^2_{bs}$ (Ben Chaim et al. 2001, Santos et al. 2014). We found 13.7 % polymorphism in our mapping population. The rate of polymorphism is less than the earlier reported by Lee et al. (2011), Mimamiyama et al. (2006) and Moulin et al. (2015) but is comparable with those reported by Dhaliwal et al. (2014) and Dwivedi et al. (2013). Considering that we used high-end resolution MultiNAMicro-chip electrophoresis technique, there are least chances of detecting further polymorphism between the two parents.

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QTL regions were not reported in the previous studies by Ben Chaim et al. (2001), Dwivedi et al. (2013), Han et al. (2016) and Rao et al. (2003). The two QTLs together contributed 21.78% to the phenotypic variation. QTLs for fruit traits were previously detected in colinear positions on LG 2, LG 3, LG 4 and LG 10 in intra- as well as inter-specific crosses (Barchi et al. 2009, Ben Chaim et al. 2001, Zygier et al. 2005). It is likely that additional QTLs that control this trait might exist that could not be detected in the current study due to low level of parental polymorphisms.

In Capsicum, few mapping studies resulting from interspecific crosses have been conducted. Inter-specific linkage maps developed have originated from the crosses involving C. annuum × C. frutescens (Ben Chaim et al. 2006, Blum et al. 2003, Rao et al. 2003, Wu et al. 2009); C. annuum × C. chinense (Kang et al. 2001, Lee et al. 2004, 2009, 2011, Tanksley et al. 1988) and C. annuum × C. baccatum (Eggink et al. 2014, Moulin et al. 2015). Ours is the first report of the inter-specific partial parental genetic map developed from the cross C. annuum × C. galapagoense. The linkage analysis mapped 12 markers on to LG 1, 8 markers on to LG 2, and 4 markers on to LG 3. The genetic map with 24 linked SSR markers containing 3 Linkage groups covers a total distance of 399.0 cm. The genetic map constructed can be considered as one of the precise maps, since it involved the high throughput marker technique and none of the marker showed segregation distortion. The newly mapped markers would enrich the already available Capsicum genetic maps and provide wider option to the breeders in selection of the markers, especially with respect to the Linkage groups 1, 2 and 3. However, additional markers are required to generate whole genome linkage map in this cross.

Since SSR markers are transferable between the species, this genetic map will have wider application across the populations. The linked markers will facilitate introgression of the two QTLs identified in C. annuum accession ‘FL 201’ into the diverse genetic background of C. chinense, C. frutescens, C. baccatum and C. pubescens. Since fruit length is quantitatively inherited trait with moderate level of heritability, MAB would improve efficiency of selection in the segregating generations. The identified QTLs might also serve as potential target regions for identifying candidate genes through fine mapping. The positive alleles for fruit length present in the short fruited parent ‘TC 07245’ might be of potential use to the breeders. Therefore, those genes are required to be identified and mapped on to the Capsicum genome. Advanced QTL backcross analysis would be useful to simultaneously discover and transfer the positive alleles from the unadapted parent C. galapagoense accession ‘TC 07245’ into elite chilli breeding lines. Secondly, with availability of advanced genotyping techniques, Genomic selection may also help to identify favourable combination of alleles from both the parents based on estimation of genomic assisted breeding values.

Acknowledgements

The authors are thankful to Dr. K.V Ravishankar, Principal Scientist, Division of Biotechnology, IIHR Bangalore, India for providing lab facilities; Dr. Parveen Chhuneja and Dr. Dharminder Bhatia, PAU Ludhiana, for technical inputs; and Dr. Roland Schafleitner, Molecular Geneticist, World Vegetable Center, Taiwan for providing information about one set of SSR primers.

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