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Transferability of Simple Sequence Repeat Markers from other Members of Family Fabaceae to Chickpea (*Cicer arietinum* L.)

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ABSTRACT

Background: Limited genetic variation exists within chickpea (*Cicer arietinum* L.) owing to its narrow genetic base. Consequently, the DNA-based markers *i.e.* simple sequence repeat (SSR) markers that show considerable polymorphism in other crops reveal limited polymorphism in chickpea. Development of saturated linkage maps, marker assisted selection and gene cloning needs larger number of polymorphic markers which necessitates development of additional SSR markers for chickpea. Microsatellite marker development is costly, requires a great research expertise and effort. The cross-genera transferability of pre-developed SSR markers is a good alternative to new SSR marker development.

Methods: To generate additional SSR markers for chickpea, a total of 292 SSR markers from horsegram (*Macrotyloma uniflorum*, 94 SSRs), lentil (*Lens culinaris*, 66 SSRs) and pea (*Pisum sativum*, 132 SSRs) were evaluated for cross-transferability to chickpea using a panel of four chickpea genotypes-GPF2, ICC16349, ICC10685 and ICC15614.

Result: Lentil SSR markers had highest transferability to chickpea (36.36%) followed by pea (18.18%) and horsegram (14.89%). Limited polymorphism was detected in chickpea; 10.61% by lentil markers, 4.25% by horsegram markers and 3.79% by pea markers. Overall, 62 new SSR markers were added to repository of chickpea SSRs.

Key words: *Cicer arietinum*, Fabaceae, Marker-transferability, Microsatellites, Simple sequence repeats.

INTRODUCTION

Chickpea (*C. arietinum* L.) is the third most important food legume in the world. With advent of marker technologies, DNA-based markers are being used frequently in crop improvement (Reddy *et al.*, 2021) wherein marker linked tightly to gene of interest is being used to select the progeny plants rather than phenotype, a technique called marker assisted selection. The SSR markers are the markers of choice in chickpea for gene mapping (Barmukh *et al.*, 2021) and marker assisted selection (Kosgei *et al.*, 2022) owing to co-dominant nature and high reproducibility (Gupta and Gopalakrishna, 2010). The marker assisted selection in this crop is, however, limited due to paucity of polymorphism (Sharma and Muehlbauer, 2007; Gaur *et al.*, 2012). Following sequencing of genome of chickpea (Varshney *et al.*, 2014, Jain *et al.*, 2013), several new microsatellite markers were generated. The majority of SSR markers developed so far (70%) are however, monomorphic (Jhanwar *et al.*, 2012) and narrow genetic base of chickpea is the reason for limited SSR polymorphism. Low polymorphism limits the use of SSR markers in chickpea genotyping (Amina *et al.*, 2020; Nayak *et al.*, 2010; Gaur *et al.*, 2012; Gujaria *et al.*, 2011; Choudhary *et al.*, 2012; Suman *et al.*, 2018) due to which scientific community resorted to single nucleotide polymorphism (SNP) as an alternative to SSRs (Gaur *et al.*, 2020). Since, SSR marker technology is within the reach of ordinary laboratories, it is highly desirable to develop new SSR markers for chickpea, so that genetic mapping of genes including quantitative trait loci (Jha *et al.*, 2021) and marker assisted selection (Henkrar and Udupa, 2020) can become a routine procedure as is the case in crops like rice (Courtois *et al.*, 2000).

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Development of SSR markers is, however, expensive and time consuming process which limits the development of new markers (Gutierrez *et al.*, 2005). A cheaper alternative to SSR marker development is cross-transferability from closely related genera and species usually termed as cross-genera and cross-species transferability (Scott *et al.*, 2000; Zhang *et al.*, 2005).SSR marker's transferability within taxa of family Fabaceae is well documented (Raghu *et al.*, 2021; Hou *et al.*, 2012; Gupta and Gopalakrishna, 2010; Bakir, 2019; Choudhary *et al.*, 2009).

The aim of the present study is to add new SSR markers to repository of already existing markers in chickpea by cross-transferability from other legume genera. In the present study, we report transferability of SSR markers from three genera of family Fabaceae *viz.*, horsegram (*Macrotyloma uniflorum*), lentil (*Lens culinaris*) and pea (*Pisum sativum*) to chickpea (*Cicer arietinum*). The study

added new SSR markers in chickpea that can be used in molecular breeding, germplasm characterization, mapping and comparative genomics in chickpea.

MATERIALS AND METHODS

The present investigation was carried out at the Department of Agricultural Biotechnology, CSKHPKV, Palampur during the year 2018-20.

Plant material

Cross-transferability of SSR markers from lentil, pea and horsegram was estimated on a panel of four *Cicer arietinum* genotypes *viz.*, GPF2, ICC16349, ICC10685 and ICC15614. Of these four genotypes, GPF2 is cold sensitive, ICC16349 is cold tolerant, ICC10685 is heat sensitive and ICC15614 is heat tolerant. The seeds were sown in 10″ diameter pots filled with standard potting mixture (Soil: Sand: FYM:: 1: 1: 1). At 3-4 leaf stage, a small amount of leaf tissue from each genotype was harvested and transported immediately in ice to lab for DNA extraction.

DNA isolation

Genomic DNA was extracted using the CTAB method (Murray and Thompson, 1980).

Primers

A total of 292 SSR primers were used. Out of these, 94 were from horsegram (*Macrotyloma uniflorum*, Kaldate *et al.*, 2017), 66 from lentil (*Lens culinaris*, Saha *et al.*, 2010) and 132 were from pea (*Pisum sativum*, Loridon *et al.*, 2005).

PCR amplification

The primers from horsegram, lentil and pea were used to amplify genomic DNA of four genotypes of chickpea *i.e.* GPF2, ICC16349, ICC10685 and ICC15614. For polymerase chain reaction (PCR) assay, 10 µl PCR reaction mixture *i.e.* 6.7 µl PCR water, 1 µl 10X *Taq* buffer, 1.2 µl DNA (20 ng/µl), 0.3 µl dNTPs (2 mM), 0.2 µl DNA polymerase (1 U/µl), 0.3 µl of each forward and reverse primer was prepared. PCR profile was followed with initial denaturation of 5 min at 95°C; 35 cycles with denaturation at 94°C for 30 seconds, annealing temperature depending on primer used for 30 seconds, followed by extension at 72°C for 1 min; and a final extension at 72°C for 8 mins. The amplification products were resolved on 3% agarose gel (0.5X TAE Buffer) using gel electrophoresis system at 100V for 2 hours and amplified products were stained with ethidium bromide (0.5 µg/ml). Gel documentation system (Biorad, USA) was used to visualize the amplified products. The size of the amplicons was estimated by 100 bp ladder (Sigma-Aldrich). The primers which showed clear amplicons were identified. Transferability percentage and percentage polymorphism were calculated as:

$$\text{Transferability (\%)} = \frac{\text{No. of amplified markers}}{\text{Total no. of markers}} \times 100$$

$$\text{Polymorphism (\%)} = \frac{\text{No. of polymorphic marker}}{\text{Total no. of amplified markers}} \times 100$$

RESULTS AND DISCUSSION

Transferability of horsegram SSRs to chickpea

Out of 94 horse gram primers used in the present study, 14 amplified genomic DNA of chickpea with percent transferability of 14.89% (Table 1). Of these, only four were polymorphic (percentage polymorphism 4.25%). Transferable SSR markers were: MUGR601, MUGR608, MUGR613, MUGR614, MUGR621, MUGR622, MUGR623, MUGR624, MUGR625, MUGR630, MUGR635, MUGR645, MUGR646 and MGR-23. Among polymorphic markers, MUGR601 showed amplicons in all the genotypes and polymorphism was due to variable length of amplification product (Fig 1a). In other three polymorphic markers, *viz.*, MUGR613, MUGR630 and MGR-23, polymorphism was due to presence or absence of bands in genotypes suggesting differences in SSR flanking sites in chickpea and horsegram. Among the three, MUGR613 showed amplification only at a single locus in GPF2 and ICC16349, MUGR630 in GPF2, ICC16349 and ICC15614 whereas, MUG-23 showed amplification only in GPF2 (Fig 1a). While transferability of horsegram SSR markers to chickpea was lower in present study *i.e.* 14.89% (Raghu *et al.*, 2021, Sharma *et al.*, 2015), the transferability of chickpea SSR markers to horsegram was also reported to be relatively higher (Jingade *et al.*, 2014, Kaldate *et al.*, 2017).

Transferability of lentil SSRs to chickpea

Of 66 lentil SSR primers tested for their transferability to chickpea, 24 (36.36%) displayed amplification and seven (10.61%) showed polymorphism. The transferable primers were: L-48-4, L-48-6, L-48-7, L-48-9, L-48-13, L-48-14, L-48-18, L-48-22, L-48-24, L-48-25, L-48-26, L-48-27, L-48-28, L-48-31, L-48-32, L-48-33, L-48-34, L-48-35, L-48-36, L-48-37, L-48-39, L-48-41, L-48-42 and L-48-45 (Table 2). Polymorphism by three markers *i.e.* L-48-27, L-48-28 and L-48-22 was due to amplicon size variation and in remaining four, *viz.*, L-48-7, L-48-13, L-48-24 and L-48-25, it was due to presence or absence of amplification in some genotypes. Primer L-48-7 showed amplification only at a single locus in genotypes GPF2, ICC16349 and ICC10685, L-48-13 showed amplification in ICC16349 and ICC10685, L-48-24 showed amplification in ICC16349, ICC10685 and ICC15614, L-48-25 showed amplification in GPF2 and ICC15614 (Fig 1b). Transferability of lentil SSRs to chickpea was highest (36.36%) among the three genera tested. Higher transferability rates of lentil SSR markers to chickpea (Bakir, 2019) and vice-versa (Agarwal *et al.*, 2008; Rana *et al.*, 2004; Choudhary *et al.*, 2009) were also reported suggesting that lentil genomic resources can be exploited effectively to add new SSR markers to chickpea genome.

Transferability of pea SSRs to chickpea

Twenty four pea SSR primers out of one hundred thirty two were transferable to chickpea (Table 3) with per cent transferability rate of 18.18%. Transferable markers were: P-18391, P-16758, P-16534, P-16452, AC-17, AD81, AF109922, PSU81287, CHPSCPA1, P-16208, PS1AA6D,

Table 1: Horsegram (*Macrotyloma uniflorum*) simple sequence repeat markers that showed transferability to chickpea (*Cicer arietinum*).

Primer name	Forward (5′-3′)	Reverse (3′-5′)	Tm (°C)
MUGR601*	GGTGGGAACACCTTTTAGCA	TGATAGGTGGTTTGTTGGCA	60
MUGR608	GGGAAACTGGACAGGGATTT	AAATGTTGGGGGTTCCTTTC	60
MUGR613*	CGGTTAGGGGAAAATCAGTG	TGCAAATTACATGCGTCGAT	55
MUGR614	TTGGGAACAGAGGAAGCAAG	CTCACCACGAAAATTGACCA	60
MUGR621	ACAAGATGCCCCTTCCTTTC	TCACTCGCAGTTCCTTCTGA	60
MUGR622	CCACACATCCTTACACGGAA	GGGCACTCGTTTAAGACCAA	60
MUGR623	CTCAAAGTAACGGAGACGGC	ATCTCCTCTCGATTTTCGGG	60
MUGR624	TTCTTTGGCCCATCTCATTC	TAAGCCCACTGAAAACCCAG	60
MUGR630*	TTGAGCAATAGAAGTAACCCTACA	CGTTTGGCTGAATTCTAAGGTG	60
MUGR635	TACAACCCCCTCTCTCCACA	GCAAACGATGCCATATTCCT	60
MUGR645	ACCGTCACCATCTTCTCCAC	AATTGCTTGATGTTGGAGGG	60
MUGR646	ACCGTCACCATCTTCTCCAC	AATTGCTTGATGTTGGAGGG	60
MGR-23*	CATCTCATCCAAGGAGATCCA	TCAAGCACCTAGCCACCTCT	55
MUGR625	TCCTCTATTCCCAAACACGC	TGGATTTGGTTGTTCTGGTG	60

*Polymorphic markers.

Table 2: Lentil (*Lens culinaris*) simple sequence repeat markers that showed transferability to chickpea (*Cicer arietinum*)

Primer Name	Forward (5′-3′)	Reverse (3′-5′)	Tm (°C)
L-48-4	TCGACAGAGTCGAATACAACAT	TGTTGTGTTACAGTTTGCCATT	58
L-48-6	GGGTGAAAAGAAACTACGCTGA	AAAACAACAACACTGACGAATCA	60
L-48-7*	GCTTCAAAAAGCTTTATCACAAAAG	TGGGAGATTTCCAAGACTGG	60
L-48-9	GCACACATCACAGCAAATCC	CCGATTCATATATTTTGCCTGA	66
L-48-13*	AAATGCTTCTAGAAAAGCTGTAATCA	TGGAACAAGTGGTATATTTGGAGA	54
L-48-14	TCCTTGCTGAATTGCATGTT	GAGCCACTGGTCCATTCATT	59
L-48-18	CCTTTAATAACATTCTCATTTGTGG	ACTTTCAAAGCCACCTTCAA	58
L-48-22*	CAAATTGAAGAAAGAAAGTGACGA	TTGCTTCAAAAAGCTATATCACC	59
L-48-24*	AACCCAATGTCTCTGTTGTTTTT	AGATCTTAGTAAACATGAGTTTTGAGG	58
L-48-25*	CACAAGTTAAGGGCAATGACA	AGTGCTCTTGTGGCTTGTGA	59
L-48-26	GCCCTAGTCTCTCAAGGAACAA	TCAAGAAAGAGAAAAACAACACAC	59
L-48-27*	AAGGGATGTGTTGGGTGAAA	GGCAATGATGAAAATGATGG	60
L-48-28*	TGGTTCACCAAAATGTATAATGC	CCTTCAATCTTAGGTAACTGCTACG	59
L-48-31	TCCTCCATAGGAGGTGTTGA	TTCACGTCACCTGCAAAAAG	59
L-48-32	AATCGTTCTCATAGGGAAAAGTTC	CGACTTATACTTAGAAATGAAGGGAGT	59
L-48-33	TCAACGGCTCCTTTGTTAGAA	TGCTTCAAAAAGATGTATCACAAAC	59
L-48-34	GGGAAAAGTTCCCTAATCATTTT	ACATTTAGAAATGAAGGGAGCTAA	58
L-48-35	CATATGAGTCGTATTTGCAGTGG	GCTCCTTGGCCAAACTATCA	60
L-48-36	GCGCAAGATTGGACATAAAGAT	TCTTTCTCTTGAGTATGAACGTG	60
L-48-37	ATCCTGACTAAGCCCCATCTCT	TGGGAGTTTTCCAAGACAGAAT	60
L-48-39	CGGAGGAGCTTAATCCATAGAA	: TTAACAGCTTTTCCAATCTCAGC	59
L-48-41	GAGAGTCTGATTTGGTTTGTCTT	CCAAAAGGAAAGTGATGTTCGT	60
L-48-42	CTGACTCACAACCATGGTCACT	CCACTCTAGCCTTTTCACGACT	60
L-48-45	CCATGCTGCTAGCCTACTACAA	TGTAATGTGATTATGGGGGAGA	59

*Polymorphic markers.

P-16697, P-17056, P-17181, P-17560, P-17122, P-17526, P-18341, P-18542, P-18938, P-18781, P-18702, P-17684 and AB53. Among transferable markers, five (PS1AA6D, AF109922, P-17560, P-17526, P-18781) were polymorphic (3.79% polymorphism). Of these five, PS1AA6D produced amplicons of variable sizes in the panel of four genotypes. Rest four polymorphic markers showed dominant polymorphism *i.e.* presence or absence of amplified products. Primer AF109922 showed amplification in genotypes ICC10685 and ICC15614. P-17560 showed amplification only in one genotype *i.e.* ICC16349, P-17526 showed amplification in ICC16349, ICC10685 and ICC15614, P-18781 showed amplification in genotypes ICC16349 and ICC10685 (Fig 1c). SSR marker transferability rates from pea to chickpea were in conformity with an earlier study (Pandian *et al.,* 2000) whereas higher

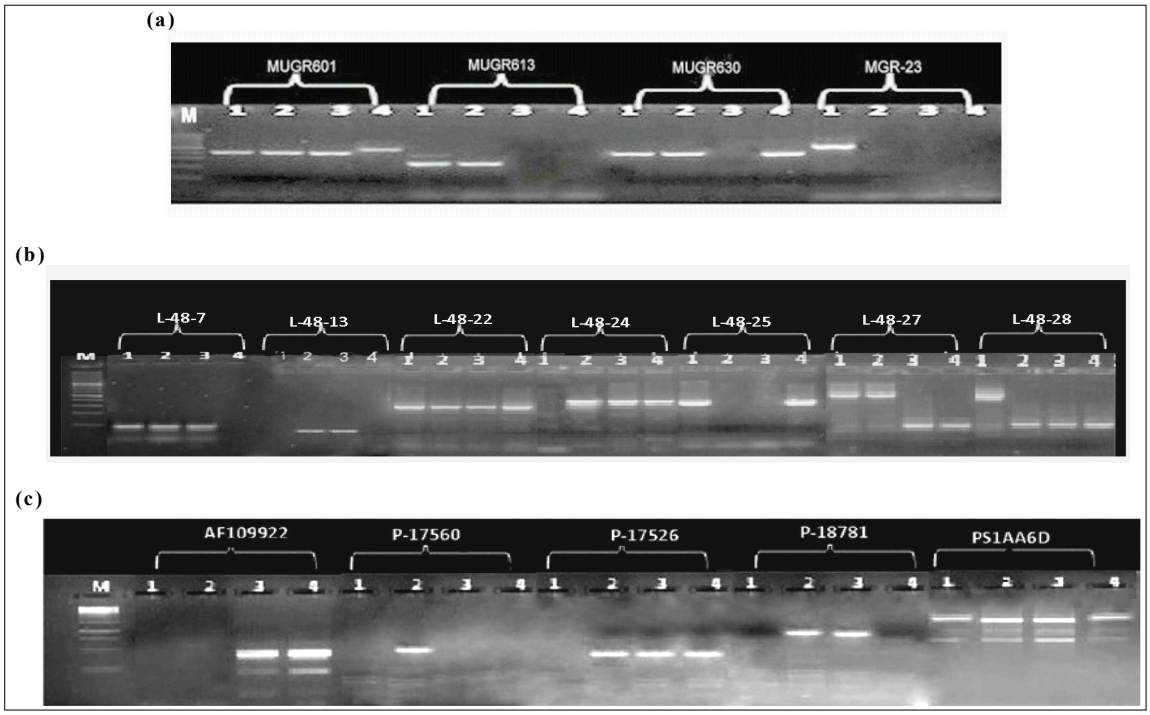

Fig 1: Amplification of genomic DNA of four genotypes of chickpea (GPF2, ICC16349, ICC10685 and ICC15614) using simple sequence repeat (SSR) primers from three genera of family Fabaceae that generated polymorphism in chickpea. (a) Horsegram SSRs, (b) lentil SSRs, (c) pea SSRs. Lanes are - M: 100 bp ladder, 1: GPF, 2: ICC16349, 3: ICC-10685, 4: ICC15614.

Table 3: Pea (*Pisum sativum*) simple sequence repeat markers that showed transferability to chickpea (*Cicer arietinum*)

Primer Name	Forward (5′-3′)	Reverse (3′-5′)	Tm (°C)
PSI AA6D*	TGGTGATTGGATGCTGGTT	TGGTGATTGGATGCTGGTT	60
AF109922*	CCTCGACAAAAAAGTCCGTCCCC	CCTTGGCCTGCCCCTGAAGTGC	54
P-17560*	TCACTGACGTTCCCACTCTG	CGTCGCTGTCAGTTCCAGTA	52
P-17526*	CAAGCTTCCATGCTCAACCT	AGCCAGGCTTGGAATGACTA	52
P-18781*	CAGTCACACCCTCAAAAGTGAA	AGCCTTCTCCAGCAAAGACA	52
P-18391	CCATCCTCCACGTGTCTCTT	TCGCATATCCAAATGCAAAC	52
P-16758	CCCTTCAACAAAGCCTAACG	AGGGTGCGAAGGAGGTTAGT	52
P-16534	TTGCAAATATACCAATTCCAAAA	ATTGGAGCCTGGTGAAGACC	52
P-16452	CGATGGTTGCTGTTGTGAGA	ACCCCAAACAAACACCAATG	52
AC17	ACAGAGTGATATGATAAAGCAA	ATGACAATATCACCAAAGTAAG	55
AD81	GGTTTCATAAGTGGCTCTGATACC	GCAGGAGCATTTGAGAAGTTGT	61
PSU81287	AGAGACACCGGAAGATCGAG	CATCCCCATAGCCACCAC	58
CHPSCPA1	GGGTTGTTGATTTGGCTGAC	GCACAATGAGGAGCAAGAG	60
P-16208	GGGTATTTGAGGGGAGAGTCA	CACGTGCCCTTTTGAGTTTT	52
P-16697	GTTCTGGGTGGAGGAAAGGT	GAAGGACGTGAGGAGACCAG	52
P-17056	AGAAACCAGCCTCCACCAG	TGTTTGTTTTCCGACGATGA	52
P-17181	AGTCCTCACAACGCCATCTT	GCCCACTGTCTCAACCTTGT	52
P-17122	ATTGTTGAGGCGAAACATGA	TGGTGAGGCTTATGATAACTGC	52
P-18341	AACCAAACGTAAGCCTCAAG	TGGCCTTTTAGTGACGGTCT	52
P-18542	TGAAAATCAGGGTTTCTTCTTTG	TCTCCTCCAAACGACCAGAT	52
P-18938	GACCTGCAGGACAAGGCTAC	TCTCTTCAATTGGGAGGTCTT	52
P-18702	TGAATTTACCACTACAATATCCAATCA	GCGTACGAGGGAGAGAAAGA	52
AB53	CGTCGTTGTTGCCGGTAG	AAACACGTCATCTCGACCTGC	51
P-17684	TGATTGAAGCTGATGGTGCT	CAATTGCAGACCGCAGTTTA	52

*Polymorphic markers.

Table 4: Transferability of SSR markers of related legumes to chickpea.

SSR markers	Total markers used	Transferable to chickpea	Polymorphic markers	Per cent transferability	Per cent polymorphism
Horsegram	94	14	4	14.89%	4.25%
Lentil	66	24	7	36.36%	10.61%
Pea	132	24	5	18.18%	3.79%

rates were also observed (Mishra *et al.*, 2012; Gangadhar *et al.*, 2016).

Findings of the present study are summarized in Table 4. Lentil SSR markers showed highest transferability (36.36%) to chickpea which is comparable to an earlier study on legumes (Raghu *et al.*, 2021). SSR markers of lentil also showed maximum polymorphism in chickpea as compared to horsegram and pea SSRs. This high polymorphism in chickpea may again be attributed to higher evolutionary similarity between lentil and chickpea (Pandian *et al.*, 2000). Limited polymorphism in chickpea by the SSR markers transferred from lentil as observed by us was also reported earlier (Bakir, 2019; Amina *et al.*, 2020). Lack of variability in chickpea was further proved by the fact that even the intra-specific SSR markers showed limited polymorphism in chickpea (Nayak *et al.,*2010; Hiremath *et al.,*2011; Gaur *et al,.* 2012; Gujaria *et al.,*2011; Choudhary *et al.,*2012). Hence, the polymorphism revealed in chickpea by lentil SSR markers, though, appears to be low, can be considered adequate in the context of chickpea.

CONCLUSION

The study added 62 new SSR markers to the existing chickpea genomic resources from three legumes lentil, horsegram and pea. Presence of few polymorphic markers in chickpea suggests that lower polymorphism remains a major bottleneck for marker assisted breeding in chickpea owing to narrow genetic base. The study further revealed higher SSR marker transferability from lentil to chickpea. Hence, emphasis should be on the exploitation of lentil genomic resources and all lentil SSR markers must be screened in chickpea to add more markers to the repository of existing chickpea markers. The new SSR markers are expected to contribute in molecular breeding, gene cloning, germplasm characterization and comparative genomics in chickpea.

Conflict of interest: None.

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