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Cross transferability of Chickpea genic SSR markers developed from *Fusarium* wilt resistance loci to orphan legumes

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ABSTRACT

The availability of genomic resources in orphan legumes is limited for molecular research because of the high cost involved in development of SSR marker pool. In this study, 162 genic SSR markers derived from *Fusarium* wilt resistant genomic loci of chickpea were tested for cross-genera transferability across nine other legumes *viz*., cowpea, horse gram, black gram, pigeon pea, dolichos, rice bean, winged bean, soya bean and moth bean. Nineteen markers of chickpea amplified the genomic DNA of all other legumes indicating the sequence conserved across the genera. However, the markers exhibiting the cross-genera transferability is ranging from 24.40 to 32.14%. The observed cross-genera transferability was highest in the cowpea (32.14%), reflected in the closer relationship with chickpea followed by rice bean (31.55%). The least transferability was reported in moth bean (24.40%). These transferable genic SSR markers will serve as a valuable tool in genomic research of orphan legumes.

Key words: Chickpea, *Fusarium* wilt, Genic SSR, Transferability.

INTRODUCTION

Chickpea (*Cicer arietinum* L.), a self-pollinated diploid (2n=16) annual crop species with genome size ~738 Mb (Varshney *et al*., 2013; Jain *et al*., 2013) belong to the family *Leguminosae* and subfamily *Faboideae.* It ranks second position in pulse cultivation after common-bean (*Phaseolus vulgaris*) and ranks third in production after field pea (*Pisum sativum*) in the world. Advances in high throughput next generation sequencing (NGS) technology provide a rapid means of discovery of genes/markers based on genome/ transcriptome sequences. Among DNA markers, simple sequence repeat (SSR) markers are gaining importance in recent days due to their highly reproducible nature, characterization of co-dominant loci, multi allelic, high abundance, even distribution throughout the genome, highly polymorphic and easily amenable for high throughput methods and these features have made SSR markers, a valuable tool in genotyping, genetic mapping and marker-assisted selection (MAS) in plant breeding programs (He *et al*., 2006; Miah *et al*., 2013).

Chickpea genome sequence projects had generated massive amounts of genomic sequence information and are currently available in public database (Varshney *et al*., 2013). With the availability of sophisticated bioinformatics tools, the genome sequences information is easily available for *in silico* analysis for the development of gene based marker (*i.e.,* genic molecular markers or functional markers), fine mapping and identification of candidate genes (Ramu *et al*., 2010 and Varshney, 2010). The markers developed from genes or expressed sequence tags (ESTs), whose putative gene function is known, are referred as genic molecular markers (GMMs) or also called as functional markers (Andersen and Lubberstedt 2003; Varshney 2010). Many researchers have developed genic SSR or EST-SSR markers based on *in silico* analysis in chickpea (Choudhary *et al*., 2009; Varshney *et al*., 2009; Gujaria *et al*., 2011; Jain *et al*., 2015; Raghu and Ravikmar, 2016), *Oryza* (Cho *et al*. 2000), *Hordeum* (Thiel *et al*. 2003), *Triticum* (Gupta *et al*. 2003), *Coffea* (Poncet *et al*. 2006; Aggarwal *et al*. 2007), *Medicago* (Gupta and Prasad, 2009), barley (Zhang *et al*., 2014), mulberry (Thumilan *et al*., 2016) and proso millet (Hou *et al*., 2017). Genic molecular markers are useful for molecular breeding program such as genetic diversity, genetic mapping, MAS and cross-transferability across related species and genera (Varshney *et al*. 2005). But the limitation of using SSR markers in molecular breeding is the cost and efforts required for designing specific primers for locus specific SSRs, which requires sequence information of targeted region in genome (Squirrell *et al*. 2003). An alternative to overcome this issue is cross-transferability of SSR markers to closely related species or genera with limited or no genome sequence information (Peakall *et al*. 1998). Several studies have been reported the feasibility of transferring SSR markers across species or genera of chickpea (Choudhary *et al*., 2009; Jingade *et al*., 2014),

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soyabean (Kuleung *et al*., 2004), medicago (Jafari *et al*., 2013), sorghum (Disasa *et al*., 2016), cotton (Satya *et al*., 2016), mulberry (Thumilan *et al*., 2016), melilotus (Yan *et al*., 2017) and so on. The objective of present study was to evaluate the cross-transferability of novel chickpea genic SSR markers to orphan legumes, which adds valuable genomic resources in these orphan legumes.

MATERIALS AND METHODS

Plant material and DNA extraction

One genotype each from cowpea (*Vigna unguiculata*), horse gram (*Vigna biflorus*), blackgram (*Vigna mungo*), pigeonpea (*Cajanus cajan*), dolichous (*Lablab purpureus*), rice bean (*Vigna umbellata*), winged bean (*Psophocarpus tetragonolobus*), soyabean (*Glycine max*) and moth bean (*Vigna aconitifolia*) were selected for assessing the transferability of chickpea genic SSR markers (Table 1).

The DNA was isolated from three chickpea genotypes (JG62, K850 and WR315) and nine genotypes from other legumes. For DNA extraction 0.1 g of fresh growing buds were excised and immediately DNA was isolated using CTAB extraction method (mini preparation) with little modifications (Doyle and Doyle, 1987). The quantification of DNA was done by nanodrop (Eppendorf Biospectrometer) and stored at -20°C.

Genic markers and PCR amplification

A total of 168 chickpea genic SSR primers (Raghu and Ravikumar, 2016) specifically designed from selected *Fusarium* wilt resistance genomic loci were chosen for transferability studies (Table 2). PCR amplification was carried out in 15 µl reaction mixture with ~35-55 ng (1 µl) of plant genomic DNA as template, 0.4 µl of 1 U/µl 3B DNA polymerase (3B BlackBio Biotech India Ltd.), 2 µl *Taq* buffer (10X), 0.5 µl dNTPs (2 mM), 0.5 µl of forward and reverse primer each (10 p mole) and 10.1 µl of PCR water. Polymerase chain reaction (PCR) was carried using Eppendorf mastercycler nexus gradient. PCR conditions for amplification of SSR markers were as follows, initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 50-55°C for 45 seconds and extension at 72°C for 1 minute, followed by final extension at 72°C for 8 minutes and final hold at 4°C. PCR products were analyzed in 3-4 per cent agarose gel with ethidium bromide staining and analyzed using the gel documentation system Alpha innotech, FluorChem® FC2 Imaging System. Fragment size of each SSR markers were evaluated using standard size markers.

Table 1: List of other legumes and their varieties used for assessing transferability of novel chickpea genic SSR markers.

Selected legumes	Varieties
Cowpea (*Vigna unguiculata*)	NBC-40
Horse gram (*Vigna biflorus*)	IC-47132
Black gram (*Vigna mungo*)	TU-1
Pigeon pea (*Cajanus cajan*)	BRG-5
Dolichos (*Lablab purpureus*)	HA4
Rice bean (*Vigna umbellata*)	KBR-1
Winged bean (*Psophocarpus tetragonolobus*)	KBWB-1
Soya bean (*Glycine max*)	RKS-1
Moth bean (*Vigna aconitifolia*)	MB-16

Transferability

A total of 168 novel genic SSR primers of chickpea tested for the transferability in other legume crops. The results were recorded based on presence or absence of amplicon on agarose gel. Based on band intensity the amplified products were classified into four classes, a) strong signal easy to score, b) weak signal able to score, c) very weak signal unable to score, and d) no signal (Kuleung *et al*., 2004). To determine transferability of SSR markers across other legumes, only two classes of bands *i.e.,* a and b were considered as amplified and the rest of the classes (c and d) were considered as no amplification. The SSR markers which successfully amplified DNA of other pulses were considered as transferable SSR markers. The percent transferability was calculated based on number of SSR markers amplified to the total number of markers selected for transferability studies.

RESULTS AND DISCUSSION

The study was carried out to determine the cross-genera transferability of 168 chickpea genic SSR markers to other legumes. PCR amplification of 168 chickpea genic SSR markers against DNA templates of three chickpea genotypes (JG62, K850 and WR315) yielded scorable profile in 162 (96.42%) markers. Out of 162 chickpea markers, 73 (45.03%) were amplified in atleast one of the other legumes whereas, only 19 (UASBC-2, 35, 51, 53, 55, 57, 59, 61, 70, 71, 80, 84, 86, 88, 89, 94, 100, 114 and 144) markers (11.72%) were showed amplification in all the legumes tested indicating 100% transferability (Fig 1). Amplification of 162 primer pairs in other legumes including chickpea revealed a minimum of one allele and maximum of six alleles (UASBC94) with a total of 211 alleles at 162 loci yielding on average of 1.30 alleles per locus. The intensity of SSR bands amplified in other legumes was weaker than the amplification in donor chickpea genotypes. Kuleung *et al*., (2004) also reported the weaker bands amplified in related species than in donor species.

The transferability study revealed highest transferability in cowpea (32.14%) followed by rice bean (31.55%), black gram (30.95%), pigeon pea (30.36%), winged bean (29.17%), horse gram (28.57%), dolichous (28.57%), soyabean (26.79%) and the least transferability was observed is 24.40% in moth bean with an average transferability of 29.16% (Table 3). SSR markers amplified from donor genome resulted in similar banding pattern in other legumes however; the probable reason may be that they were derived from the same loci and allelic region of the conserved primer binding sites (Erayman *et al*. 2014).

The lack of availability of genomic resources particularly in orphan legumes hinder the genomic studies such as linkage studies, QTL mapping and fine mapping of genes for particular trait. To overcome these limitations it is better to use SSR markers readily available in other related

Table 2: Genic SSR markers developed through *in silico* physical mapping of *Fusarium* wilt resistance genomic regions in chickpea.

Marker name	GenBank ID	Chromosome /scaffold	Markers flanking genomic regions	Forward/reverse primers (5′-3′)	SSR motifs	Expected product size (bp)
UASBC1	LOC101489302	Ca4	A07C-TS72	GTCTCACTTTTGTGCCTTCTAACA/ GTTGCTAAGAGTATCCAAACCCA	(TTTTG)6	378
UASBC2	LOC101491241	Ca4	A07C-TS72	GCTGCTGGGTTACATAAAATGA/ CGAAAGTAGCGGAGAGTGGTAT	(AT)6	393
UASBC3	LOC101493838	Ca4	A07C-TS72	GAACACATACATTTGACCGTGTG/ AAGGCAACCCTTACTTTTGTGA	(AAT)7	146
UASBC4	LOC101495550	Ca4	A07C-TS72	GAAACCTTAACGATCCTCATGC/ ACCTGATATGCTTCGCCTAAAA	(TTTA)10	195
UASBC5	LOC101507081	Ca4	A07C-TS72	TCAGGAAAGAGGTTGGAACTTG/ ATCAAACTGGGAATGCACTTG	(TA)9	260
UASBC6	LOC101508554	Ca4	A07C-TS72	GCTCTCTGTGTTGCTTAGGGAT/ GACTGACTCCTCAAGTGGAACC	(TAA)25	242
UASBC7	LOC101506964	Ca4	A07C-TS72	TAGCCTGTTCTCTATTGGGGTG/ TATCACATGGTTGTTTGGCAGT	(TG)6	230
UASBC8	LOC101509945	Ca4	A07C-TS72	AACCTTCCTTCCAACCACTAAAC/ GAAAACCACGCCAAAACTATGT	(AAT)20	168
UASBC9	LOC101509615	Ca4	A07C-TS72	GCCTAATGGAAGGATGGGTAA/ GCACTTTCGTTTCACTTTGCTT	(TAAAT)6	381
UASBC10	LOC101509301	Ca4	A07C-TS72	CGTGACATTGGGGAATAGTTGT/ GCACAATTTAGCTGAGGTTGCTA	(AAT)6	347
UASBC11	LOC101507600	Ca4	A07C-TS72	TGCTTTAGGGATTGTCTCATCA/ CTTGATAATGTAGTTGGCATACAG	(TC)8	396
UASBC12	LOC101506964	Ca4	A07C-TS72	TAGCCTGTTCTCTATTGGGGTG/ TATCACATGGTTGTTTGGCAGT	(TG)6	230
UASBC13	LOC101504059	Ca4	A07C-TS72	ACGGGAGAAGAGAAGAAGTTGA/ GTTAGGCCATGTTTGATGGTTA	(TA)16	389
UASBC14	LOC101489033	Ca2	TA110-H3A12	TTCTCTCCTTCCTCTCCTTCCT/ GAATATGGGCAAGTGTTGTTGA	(CA)12	334
UASBC15	LOC101498460	Ca2	TA110-H3A12	GTCCCTTCCTTCTCACAACAAC/ AGGGTGCTCTGTCTTTGTCTTC	(CT)8	181
UASBC16	LOC101491810	Ca2	TA110-H3A12	CTCTTTCTCCGCGCTAACC/ CGTATGGATCTTCCTGAGCTTC	(ACG)6	143
UASBC17	LOC101495484	Ca6	CaM1402-CaM1101	ACCCTCTAATGCAACATCATCA/ TATAAATCCCACTTGACCCACC	(TAA)7	348
UASBC18	LOC101515041	Ca6	CaM1402-CaM1101	CAACTGGTCATATCTCTGGTGC/ CCCCAAGAATACACGTAACAAA	(AT)13	374

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UASBC19	LOC101498533	Ca6	CaM1402-CaM1101	CTATGACTTGGCAGCATTGAAG/ GATAGGTTCTCCATCTCTTTGAGC	(AT)7	253
UASBC20	LOC101501211	Ca6	CaM1402-CaM1101	CACTTTTACCCCTCTCCATTTG/ AGAGTAGCACTGGGGATTCAAG	(TC)20	356
UASBC21	LOC101511520	Scaffold174	-	CATCTATGTATGGTTGTGTGGTCA/ TTTGGCTTTAGCTTCCTACCAA	(AT)8	303
UASBC22	LOC101489480	Ca2	TA110-H3A12	CTAATTGTCCGAAGTTGACGTG/ GGAATGCAAGAAGGTGATAAGTG	(AT)15	386
UASBC23	LOC101512685	Ca2	TA110-H3A12	CACGGACAGTTTTACGATCTCA/ TCGAATCCAACATCACACAATC	(TATT)7	303
UASBC24	LOC101513982	Ca2	TA110-H3A12	TCAATGGGTGTATTAAGCACAG/ GTCAGTGTTTCTGGGTGTTTTC	(TAA)18	389
UASBC25	LOC101501439	Ca2	TA110-H3A12	TCGATTTGCTCTAATACTGCTGAC/ CCTCTGCCTTGAATACTCCTTG	(AT)11, (TA)9	344
UASBC26	LOC101502078	Ca2	TA110-H3A12	TACTCCTTCAACGTACCAACCC/ TGGCGAGACAAATACACAACTC	(TTTTA)6	393
UASBC27	LOC101490665	Ca2	TA110-H3A12	TGCCCCAACCTTAGAAGATAAA/ CTTTGCTCACAACACAACCATT	(AT)12, (A)11, (A)12	355
UASBC28	LOC101503797	Ca2	TA110-H3A12	TTGTTGCTCTTTATGGTCCTCA/ GTGCTTGCATTAGTCTTTCTTCC	(GATTAT)6	364
UASBC29	LOC101488825	Ca2	TA110-H3A12	ACCCTGCTCTTCTTCCTTTACC/ ACAACCCCAAATGCAATGTT	(GA)7	400
UASBC30	LOC101488825	Ca2	TA110-H3A12	GTGTGGTGTCTGTTGGGCTAT/ ACCTCATTAGAGAAGGTTTACATGC	(TTTA)6	378
UASBC31	LOC101510540	Ca2	TA110-H3A12	TGTGGGTTACAAAGCAAGAGAA/ TTCAAAACAGGAAGTGGGTCTT	(TA)12	399
UASBC32	LOC101505498	Ca2	TA110-H3A12	GGATGATGCTACCGTTGATTTC/ TTGGAGAAGGTCCGATAAGAGA	(AT)21	202
UASBC33	LOC101506689	Ca2	TA110-H3A12	CATCCAAATGCAGTCCTCAGT/ CCGTTTCATATAGTTGTGTCGG	(TAAT)6	377
UASBC34	LOC101494099	Ca6	CaM1402-CaM1101	AGGGAACAACACCCAGATAGTC/ TTAAGAAGGGTTGGGCTCAC	(AT)21	398
UASBC35	LOC101513415	Ca6	CaM1402-CaM1101	GGTCAAGCCCAATAAATCACTC/ CGTTGCACATCCACATTCTACT	(TTAT)11	195
UASBC36	LOC101498314	Ca6	CaM1402-CaM1101	GCTCCAAAAGATTCACAAAAGG/ AGCTGCACTTAATGGAAATGGT	(AAT)12	387
UASBC37	LOC101507117	Ca6	CaM1402-CaM1101	AACAATCAAGACGCTAATTGGG/ GGATCGGGGTAGCATAACAAC	(ATA)9, (AAC)9	380

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UASBC38	LOC101508263	Ca6	CaM1402-CaM1101	CATACAAATATGGTGGGTGGC/ CAATGCAAGGACAGGAACATAA	(TA)21	395
UASBC39	LOC101499413	Ca6	CaM1402-CaM1101	CTTTGAGGACTTGGTCAGATTG/ CATGTTTCGTTATAGGAGGAGC	(ATA)17	395
UASBC40	LOC101497778	Ca6	CaM1402-CaM1101	GTTGGAATGTTGCGAAGATGTA/ GTTTTGTCCTTTGCGTCTCTCT	(ATT)13	365
UASBC41	LOC101514741	Ca2	TA200-TA37	GTCAAGAATGCCGATGTACG/ ATATTAGACCATTTCCTTGGGC	(AT)16	376
UASBC42	LOC101488826	Ca2	TA200-TA37	GGCAGGCAAACAAACTACATACT/ ATCACTCCACAAGCCGTTTAAT	(AAT)11	359
UASBC43	LOC101488826	Ca2	TA200-TA37	GCTTGTGGAGTGATGGTTTTAT/ AGAGTTGAATGAGAGAGAACGG	(TA)6, (A)10, (AT)7	335
UASBC44	LOC101492140	Ca2	TA200-TA37	TTCTGCTGGTAAGAAATGGGAT/ CAAAGATCAAGAGCAATGAGGA	(TGG)8	191
UASBC45	LOC101494422	Ca2	TA200-TA37	AACCCAGAGAAGTCAGATCCAA/ TACCATCCCAACGTGTCATCTA	(ATATT)14	338
UASBC46	LOC101494422	Ca2	TA200-TA37	CCAGCAATCAAAGAATGAACAC/ GCTAGTCTCACCAACGGGTC	(TA)13	230
UASBC47	LOC101512465	Ca2	TA200-TA37	GAAGTTGTAAGACCCGCATTTT/ TAGAAGTGGGGAACGTGGTATT	(TAA)18	306
UASBC48	LOC101507653	Ca2	TA110-H3A12	TCCTTTTCTCTTGAGCTTGACTG/ ACGATGGGATAACTGAGGAAGA	(AC)24	160
UASBC49	LOC101514850	Ca2	TA110-H3A12	GTACTCCCTCCCTGTCGC/ GCACCACACTTCATTCCTATGT	(AT)7, (TA)7	364
UASBC50	LOC101492815	Ca2	TA110-H3A12	ATCATGTGTTCCCCTATTTGTG/ AACTCTAATGCACACCAACCAA	(TAT)23	282
UASBC51	LOC101500751	Ca4	A07C-TS72	CAAGCTCTGGTAAGCATAAGCA/ CTTTACTTCTACACTCAACGGATCA	(TA)10	369
UASBC52	LOC101508554	Ca4	A07C-TS72	ACGCTCTTGTCTTTTGTCACTT/ AGTAGTCATCCTCCGGTAATCA	(T)11	389
UASBC53	LOC101510924	Ca4	A07C-TS72	TATTTTGTACGGCATAGCCAGA/ GAAAACAATGAGACCCAACACA	(TA)22	313
UASBC54	LOC101494165	Ca4	A07C-TS72	ACTTCAGCAACAGAATCAACCC/ CCCATCATATACTCCAAGTCCAA	(A)17	399
UASBC55	LOC101512465	Ca2	TA27-TA59	GAAGTTGTAAGACCCGCATTTT/ TAGAAGTGGGGAACGTGGTATT	(TAA)18	306
UASBC56	LOC101490121	Ca2	TA27-TA59	CCAACAATTCAACTTCCTCCAT/ TGAGAAACAGATAAACGATACACCC	(AT)20	359

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Table 2: Continue...

UASBC57	LOC105851616	Ca2	TA27-TA59	GACAAGGGAAAGAAGACATTGC/ AGTTTTGGGACTGCATGAGC	(ATA)21, (AT)10	252
UASBC58	LOC101498462	Ca2	TA27-TA59	TCCTGTGTGACCATTTCTATGC/ CCCTGTCTAGTAAGATCGTGGG	(AG)16	367
UASBC59	LOC101503022	Ca2	TA27-TA59	TCAGCCACACCATTCTAATCAC/ TAAAGTCACCAACCAAAACCCT	(TC)18	355
UASBC60	LOC101500167	Ca2	TA27-TA59	TTACTTTTACGAGGCGACTTCC/ ACAATTCGAGCACACAAATCAC	(GA)16	384
UASBC61	LOC101502609	Ca2	TA27-TA59	ATCAATATCGTGTGCCCATACA/ GTGTCAACAAACAAATCGGGTA	(AT)23	272
UASBC62	LOC101502926	Ca2	TA27-TA59	GGGAAATCGTTATTAGCCACAA/ CAAAGCAAAATCCACAACTCTG	(TTAAT)7	314
UASBC63	LOC101498007	Ca2	TA27-TA59	CGAGTAACAATAAGGAAAGGGC/ GTTTGGGTTCTCATGTAGCAAA	(TA)17	279
UASBC64	LOC101515069	Ca2	TA27-TA59	AGTATTCTTCACGCTGCAATCA/ CTCTTACAAACCACCAAAACACC	(TA)26	347
UASBC65	LOC101505338	Ca4	TR20-TS82	GCATTGGTTGTTTGCTCTGTAT/ TCACTTGGAGTCATTTCGATGT	(TTA)14	191
UASBC66	LOC101510592	Ca4	TR20-TS82	AGGCTGAAGGGATAATGACAAA/ TCTAGTGGAAAGGTCGAATGCT	(TA)23	397
UASBC67	LOC105851931	Ca4	TR20-TS82	CAAAGCAGAATGTCCATCAA/ CGTTTGTAGCGCAGAATGT	(AGA)18	382
UASBC68	LOC101499060	Ca4	TR20-TS82	TCCAGTGACAAATACAATTCCG/ ACAATAGCCCCTGAAACAGAGA	(TCAT)8	171
UASBC69	LOC101488417	Ca4	TR20-TS82	GAAAGAAGAAAGGAAGGGGAAG/ GTTAGTGATGTGATGAGTGTGATGA	(AT)19	340
UASBC70	LOC101507596	Ca4	TR20-TS82	CCAAACTCTCCTCCCTACCTCT/ AGACATTCCATCTCCCAAGAAA	(TCA)10	386
UASBC71	LOC101508452	Ca4	TR20-TS82	CCACACACAACACAAATCACAA/ AAGCACCAAAGGTAGTGAGGAA	(A)19	313
UASBC72	LOC101491183	Ca2	TA110-H3A12	CAAAGCGTTTCTGTGTAACCAA/ TTTGGATGAAGATGTGATGTCC	(ATT)8	285
UASBC73	LOC101515723	Ca2	TA110-H3A12	TCTTCCTCACTTCAGATCCCTC/ GAAAACTTCGCTCATCTTTGCT	(TTTC)8	192
UASBC74	LOC101493792	Ca2	TA110-H3A12	TGCTTTCGTTCTTGTTGTTG/ CCAGCACATCATAAATACATCC	(AAT)9	287
UASBC76	LOC101498314	Ca6	CaM1402-CaM1101	GCTCCAAAAGATTCACAAAAGG/ AGCTGCACTTAATGGAAATGGT	(AAT)12	387

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UASBC77	LOC101497987	Ca6	CaM1402-CaM1101	AAGTCCTTAATGTATCTCGCGG/ CTTCCTTGTTTTGCAGCTTTCT	(GAT)10	278
UASBC78	LOC101499300	Ca6	CaM1402-CaM1101	AAACTCAGCATTTTAGGCCG/ GAAGGCTACCACTACGCAATCT	(TTAT)7	398
UASBC79	LOC101504726	Ca6	CaM1402-CaM1101	TTTTCTGAGGCAACATGCTCTT/ CCGAATGACAAGATTCCACACT	(TA)19	305
UASBC80	LOC101491914	Ca6	CaM1402-CaM1101	CTGGAATCTCCCATTCGGT/ GAGAACTTAGAGAAGGTCAATGCAA	(AT)6	396
UASBC81	LOC101514179	Ca6	CaM1402-CaM1101	CACAATTTCCCTTTCTCTGCTT/ TCTTACCCTGACATTTTCCTTGTAG	(T)53	238
UASBC82	LOC101510511	Ca6	CaM1402-CaM1101	TGCTGGTTACACCTTGCTTATG/ TCTAAAGCCAAAGGAATGGAAG	(ATT)26	271
UASBC83	LOC101492347	Ca6	CaM1402-CaM1101	ATGTGTGTTGTATCCATTGCGT/ GATTGATGCCTAAAATGCAGGT	(ATCT)22	355
UASBC84	LOC101510625	Ca6	CaM1402-CaM1101	TGCAACGTAGAGCAATTTTGAC/ TCATTTCCATGACTTTCTGAGC	(TAT)20	278
UASBC85	LOC101509650	Ca6	CaM1402-CaM1101	ATTAGAGGCAAACAAGAACCGA/ ATGTTGAAGTTGGGAAAACGAC	(TAT)22	391
UASBC86	LOC101509650	Ca6	CaM1402-CaM1101	TTTCAAGTTTTCAACCTAGCGG/ ATTCTCGTGCCAAACATTACCT	(TTA)14	239
UASBC87	LOC101507324	Ca6	CaM1402-CaM1101	AATGGAAAGGAAGAGAGCAACA/ TATTGTCCCCTAGCAAGCATTT	(TCA)21	314
UASBC88	LOC101502054	Ca6	CaM1402-CaM1101	CTTTCAGAGGAAAACGAACGAT/ GGAAGGGAGGCTATAAAATGCT	(TA)8	201
UASBC89	LOC101502054	Ca6	CaM1402-CaM1101	GAGCGCGAGGAGATTCAG/ CTGAGGTGGGAGAGTTAAGGAG	(CT)7	400
UASBC90	LOC101502054	Ca6	CaM1402-CaM1101	GTGTCATTGGCTTTGAGATTGT/ AACAACAGCAGGTTCGTCCTAT	(TC)7	264
UASBC91	LOC101489008	Ca6	CaM1402-CaM1101	ACTGGATGCTGGTTGGAGAT/ GTTGGCTTGTTCCCATCTTTT	(TA)18	395
UASBC92	LOC101500886	Ca6	CaM1402-CaM1101	GGACCTAGTTTTCCCGTCTTCT/ TGACAAGGACTATTTTGAGCGA	(AT)20	317
UASBC93	LOC101506664	Ca6	CaM1402-CaM1101	AGTGCAGGTGTAAATCATGTGG/ CCTTCGTACTCATCCTCTAAGGC	(AT)8	233
UASBC94	LOC101500576	Ca6	CaM1402-CaM1101	ACCAAGTGTATGGGACACCTTT/ ACATCTCCATTGCTTCCATCTT	(TA)8	174
UASBC95	LOC101489545	Ca6	CaM1402-CaM1101	GCGGTTCAAAAGAGTGAAGAAT/ CGTCATACTCATCAAGCGGTTA	(GAA)14	335

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UASBC96	LOC101488897	Ca6	CaM1402-CaM1101	TGAAAGCACCACTATGGCTAAA/ TGTCAACTTTATGTCTCCGATAGC	(CT)23	391
UASBC97	LOC101506340	Ca6	CaM1402-CaM1101	TCTTCACTTCCCAGGTTCCTTA/ TGAGTTGCTTGTCTCACCAGTT	(T)44	368
UASBC98	LOC101499296	Ca6	CaM1402-CaM1101	GCGGCCACACACCTTATT/ AAAATAGGAGGGCTAATTCCGT	(AT)23	226
UASBC99	LOC101505364	Ca6	CaM1402-CaM1101	TGCAGAAGATATGGGGAGTTTT/ AAAGCTAGGAAACATAGGCACG	(TC)19	205
UASBC100	LOC101512664	Ca6	CaM1402-CaM1101	AGTGGAAGTAGAGGGATTAGACGA/ TGTAATGTGATTGGTAGACCGC	(CTATTA)33	368
UASBC101	LOC101514938	Ca6	CaM1125-H4E09	TTGAAAAGTCACGATGGATCAG/ ACCCTGCAACCAAAGAATGA	(TG)6	342
UASBC102	LOC101514938	Ca6	CaM1125-H4E09	AACAAACACGACCCTGTCACTA/ ATCAGTTGCTGCTTCCTCATCT	(T)16	350
UASBC103	LOC101507318	Ca6	CaM1125-H4E09	CATATCTCCAAACACGTCATGC/ AACCATATCACAACTGGGTCATC	(ATT)6	321
UASBC104	LOC101509330	Ca6	CaM1125-H4E09	TATCACACTCGATACACACCCC/ TAAATTCGCTCTTTCCTGCAAC	(T)65	241
UASBC105	LOC101508375	Ca6	CaM1125-H4E09	TCTATGGAAACCCAGTGAGCTA/ CTATGCACAAATTCATTCACCC	(TTA)18	332
UASBC106	LOC101494922	Ca6	CaM1125-H4E09	AGTCGAAATGAAGAACTCCACC/ TATATCTCATCCTGCACCCAAA	(AAT)17	365
UASBC107	LOC101494922	Ca6	CaM1125-H4E09	AGTCGAAATGAAGAACTCCACC/ TATATCTCATCCTGCACCCAAA	(TAT)20.33	365
UASBC108	LOC101490890	Scaffold 96	-	ATAGCACGGGGTCATATAATGG/ TCCCCATCAGAACCTTCGTA	(TA)18	333
UASBC109	LOC101490890	Scaffold 96	-	ATTTGTCATTTGAGTTGCGG/ ACACAAGGCTATGGAGGAAAAT	(TA)16	267
UASBC110	LOC101499861	Scaffold 766	-	TTGAGATGTGAGTGAAGTGATGTG/ AAAATGAAGACGACTGTTTCGG	(TA)13	396
UASBC111	LOC101500195	Scaffold 766	-	TCTTCGCACTCTTCAGGTATTG/ CCGCAGTCTATGTTGTTAGTTTG	(TA)14	303
UASBC112	LOC101488843	Scaffold 766	-	AGTTTCCTTTGTGAGAGGGTTG/ CCCATCATCTTTTACATGCTCA	(ATG)8	361
UASBC113	LOC101500363	Ca6	H4E09-LOC101504400	CACGCAGTTTTATAGTCGGTGT/ TTGACGAAGGATGACACTTGAT	(TA)17	374
UASBC114	LOC101500675	Ca6	H4E09-LOC101504400	ATTCGGATAGGTTGGTTTGAGT/ CTTTGTTTCATTTAGTCCTCGG	(TA)17	234

Table 2: Continue...

Table 2: Continue...

UASBC115	LOC101489737	Ca4	TR20-TS82	ATTGGCATCAATGTGTGAGAAG/ CTCAATGTCCCCTAAACCAAAA	(TGA)8	366
UASBC116	LOC101500679	Ca6	CaM1402-CaM1101	GATTCACCGCAAATTCAGACA/ AATGACCCCACACACCACTAAT	(TTATT)6	287
UASBC117	LOC101514293	Ca6	CaM1402-CaM1101	CTGTTGACACATGGCTTATTGC/ TCGTATCCAGGCCAACTAAAAT	(ATT)9	377
UASBC118	LOC101496017	Ca6	CaM1402-CaM1101	CAATGTGGGTTTGGTATGTTCA/ GGTGGAGTGTGCAAAGGTATTT	(TA)18	315
UASBC119	LOC101511722	Ca2	TA110-H3A12	CATGATTTTCGGTGTTCGACT/ TGGTGTGCGAGTTTTAACAAAG	(AAC)7	399
UASBC120	LOC101494641	Ca2	TA110-H3A12	TATGGGTGGTGATCTAGGGTTC/ ATTCATGTCTCAGTGTTGGTGC	(TTG)6	325
UASBC121	LOC101505729	Ca2	TA110-H3A12	AAGAATAAAAGAAGCGCGAGTG/ GGCGGTTGAGAGTAAGAGAGAG	(CAT)7	200
UASBC122	LOC101507653	Ca2	TA110-H3A12	TTGGATTCTCAGGGTTCATTCT/ CCATTTTCAAGCAAGTGCAA	(TTTA)6	390
UASBC123	LOC101507145	Ca2	TA110-H3A12	GTCATGTCAAATTAGCACGCTC/ AGAGACCAATCCTGAAAGCAAG	(TTG)7	363
UASBC124	LOC101490870	Ca2	TA110-H3A12	CTGGGTATATGCTATTCTGGGG/ TCGGGTACTTCTCCATCTTCAT	(CAT)7	262
UASBC125	LOC101498006	Ca2	TA27-TA59	GTGCTGAGGAGAAGTAGAAGCAA/ AATCAAACCACCATTCACACCT	(GGT)7	327
UASBC126	LOC101502926	Ca2	TA27-TA59	GGGAAATCGTTATTAGCCACAA/ CAAAGCAAAATCCACAACTCTG	(TTAAT)7	314
UASBC127	LOC101496486	Ca2	TA27-TA59	ATTTTGAATCTATGCAGGCACC/ TCCACCACCACAATCTTCATTA	(CAA)7	386
UASBC128	LOC105851616	Ca2	TA27-TA59	GGGTCATATAGGGTCACACAGC/ TCCCAGAGTAGAGGAGATGAGG	(TAAA)6	365
UASBC129	LOC101502968	Ca4	TR20-TS82	CAAGTTTTGCCTCCATCTATGA/ TCACCAAAGCAATCTTATCCG	(TTA)9	369
UASBC131	LOC101510183	Ca6	CaM1402-CaM1101	CTCTTTTGCATGAATTGGAGC/ GCCACACACAGAACTCAACTTT	(ATA)8	297
UASBC132	LOC101497650	Ca6	CaM1402-CaM1101	GGACCCTATTGCCTAAATCACA/ CTCCCTCCGTTTCTAAATACAAGA	(TAAT)6	283
UASBC133	LOC101493243	Ca6	CaM1402-CaM1101	TGTAAATGTGTGAACCTCGCTC/ GACGAAAACGAGAACGAAGAAG	(CTT)7	332
UASBC134	LOC101488343	Ca6	CaM1402-CaM1101	AGACGGAGGAGAATGACTGAAA/ AGGCAAGGTGAAGAAGCATAAC	(TTA)7	305

Table 2: Continue...

Table 2: Continue...

UASBC135	LOC101492117	Ca6	CaM1402-CaM1101	CACTTATTTCACACACACACTCTCTC/ TTGTCCCAAAAGGATCAACTCT	(ACA)7	162
UASBC136	LOC101492791	Ca6	CaM1402-CaM1101	TTCCTCTCTCTCTCCTCCTTTTC/ AAGGGTGTAATGGGTTGTTCTC	(CTT)8	114
UASBC137	LOC101503235	Ca6	CaM1402-CaM1101	ATTCAACTCGGGACCTTACTG/ AAAGGGTACATAACACAATGCC	(AT)20	397
UASBC138	LOC101498648	Ca6	CaM1402-CaM1101	CTTCTTCCCTCTTCTTCCAACA/ ACCTGATCCAAAACAACACACA	(TC)18	397
UASBC 139	LOC101495609	Ca2	TA110-H3A12	CCTTTTGTTGTTTGGGAAGCTA/ TATTTGAATGTGTAGGGCATGG	(AT)14	337
UASBC 140	LOC101500284	Ca2	TA110-H3A12	CCCACCACACAGAAGAAAGAA/ AGAAGGCAAGAGAGAAGAGGGT	(TC)13	113
UASBC 141	LOC101505729	Ca2	TA110-H3A12	AAGAATAAAAGAAGCGCGAGTG/ GGCGGTTGAGAGTAAGAGAGAG	(CAT)7	200
UASBC 142	LOC101506051	Ca2	TA110-H3A12	GTAATGAGCCCAAAACGAAGTC/ CTGATATGCGATTGTGTGTGTG	(TC)11	255
UASBC 143	LOC101506576	Ca2	TA110-H3A12	TGGTTATGAGTGGTTGACTGTG/ TATCTTGAATGACCCTGACTGC	(AT)12	398
UASBC 144	LOC101489481	Ca2	TA110-H3A12	CCTCTAGGGAAACACCTCAAAA/ CAATGCTTACTTCACTTGGTGC	(GAA)7	207
UASBC 145	LOC101508291	Ca2	TA110-H3A12	AAGGTAGGAAGGCTGTAGAGGG/ TCCATTTCTTCACTTTGGGACT	(AT)13	310
UASBC 146	LOC101497475	Ca2	TA110-H3A12	AGAGATAAGCAAAACTTGGCGA/ ATCAATGGGTCAAGAAACCTGT	(AT)10	182
UASBC 147	LOC105851616	Ca2	TA27-TA59	TCATTAAGAGATTCCCCGTTCT/ GGTGTATTTTGGATTTTGGCTC	(TA)16	345
UABCS 148	LOC101503575	Ca2	TA27-TA59	GGCTTGAATCTACTTCGACTCTTG/ TGCTCCCAAAACACGATTTTAC	(TA)11	316
UASBC 149	LOC101511725	Ca2	TA27-TA59	AGAGGCAATAATGGGTGTGTTT/ TGGCCTGACCTAGAAGCATATT	(AT)11	250
UASBC 150	LOC101511725	Ca2	TA27-TA59	GCCAAATACCGATGGAAAATG/ TCTCACCTGCAATAAGCATCAA	(TA)14	343
UASBC 151	LOC101495940	Ca2	TA27-TA59	AGTTTCTATGAATTTGGCGCTC/ TGTGTGTCTTCCCTTTATGACC	(CT)13	199
UASBC 152	LOC101496486	Ca2	TA27-TA59	CGTCCTCTTCTGCTGGTCTACT/ CTTATGATCGTGGTGCAAATGT	(T)34	330
UASBC 153	LOC101506851	Ca4	TR20-TS82	TACAGGTCAGAAGCAAAGGACA/ TGTAACCGAAAGTTCATCGCTA	(TA)11	368

Table 2: Continue...

Table 2: Continue...

UASBC 154	LOC101508663	Ca4	TR20-TS82	GCTCACAGTTGCCACAATTTAG/ ATTTATGGTCTCATGCAATCCC	(AT)13	398
UASBC 155	LOC101507596	Ca4	TR20-TS82	TTCAGGGAGATAGACGACCATT/ TGGTAACTTGCATTCACCCAT	(TA)13	364
UASBC 156	LOC101511284	Ca2	TA200-TA37	GGTCTTTTGAGGGCTTATTTCC/ CGTGGGCATAGTTCATATCTCA	(TC)12	275
UASBC 157	LOC101498314	Ca6	CaM1402-CaM1101	ATGATGCGTGGAGAAGGAAA/ CACACCACCACTACCATAAATCA	(TAA)7	363
UASBC 158	LOC101501846	Ca6	CaM1402-CaM1101	GCGCTGTAAAAGACCTCCTTAT/ TGACCTGCCATAACTGATTTTC	(TA)11	348
UASBC 159	LOC101512758	Ca6	CaM1402-CaM1101	GTGTCATGTTCGGTGATGTCTT/ TCTCTCTCAAGCCAAACCTTTC	(GT)11	202
UASBC 160	LOC101498313	Ca6	CaM1402-CaM1101	GACAAACCTTGAAAGCACATGA/ AGGAGGGGTGTTGACATATAGC	(AT)13	377
UASBC 161	LOC101497651	Ca6	CaM1402-CaM1101	GAGCATCTTTTCAATCGACCTT/ AGGTGGTCTTCCAGAACTTTGA	(CAA)7	342
UASBC 162	LOC101489660	Ca6	CaM1402-CaM1101	TTCCTATTCTCCCAATGCAA/ GTACTCATCTTGCGGGTTTAAT	(AT)13	293
UASBC 163	LOC101502686	Ca6	CaM1402-CaM1101	ATTAACCATCGCGCTCTCTCT/ TGGATCAGAAATGACGAATCAC	(CT)14	209
UASBC 164	LOC101502383	Ca6	CaM1402-CaM1101	TTATCCCACGCGGTGTTATT/ TGTGGAGTGCTTTTGTCTCACT	(TA)15	242
UASBC 165	LOC101497985	Ca6	CaM1402-CaM1101	TATGGTTGAGTTTTGGGTAGGG/ ACGTGGGTTACGTGAAATCAG	(TA)12	226
UASBC166	LOC101500194	Ca3	TR24-TC14801	AACGCAAGTGAAGAAAGAAACC/ CGAACCAGTGTAATGCAATTCT	(CT)10	343
UASBC167	LOC101501469	Ca3	TR24-TC14801	AACCAAGTGTACCTGCTGTTTT/ GCATGACGACTACAAAATTACG	(TA)12	264
UASBC168	LOC101510893	Ca3	TR24-TC14801	GTTGATGGATTGGAATGTGTTG/ TATATGCAAAGCCGCCGT	(CT)73	380
UASBC169	LOC101503824	Ca3	TR24-TC14801	TTACTCTTTCTCTGCAACTGCG/ CAATTTAGCATCGGTTGGTTTC	(TTA)16	275
UASBC170	LOC101509709	Ca3	TR24-TC14801	ACCGTCCAATAAAGAATGTGCT/ GAAGTGAAAGCCGAATCAAAAG	(AT)16	397

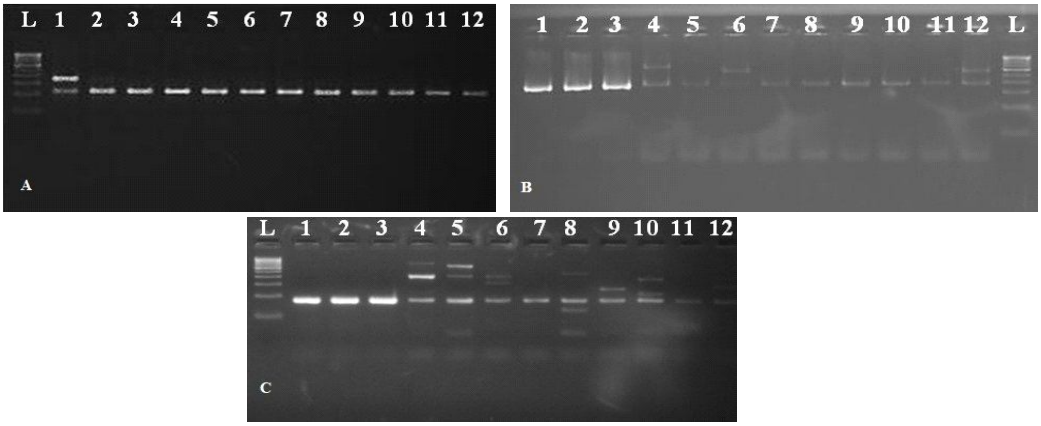

Fig 1: PCR profile of Primers UASBC51 (A), UASBC71 (B) and UASBC94 (C), L-100bp. Ladder 1, 2 and 3- Chickpea, 4- Cowpea, 5- Horse gram, 6- Black gram, 7- Pigeon pea, 8- Dolichos, 9- Rice bean, 10- Winged bean, 11- Soya bean and 12- Moth bean.

Table 3: Cross-transferability of Chickpea Genic SSR markers to other legumes.

Other legumes	No. of SSR markers amplified	Transferability (%)
Cowpea (NBC-40)	54	32.14
Rice bean (KBR-1)	53	31.55
Black gram (TU-1)	52	30.95
Pigeon pea (BRG-5)	51	30.36
Winged bean (KBWB-1)	49	29.17
Horse gram (IC-47132)	48	28.57
Dolichos (HA4)	48	28.57
Soya bean (RKS-1)	45	26.79
Moth bean (MB-16)	41	24.40

legumes. However, the rate of transferability is influenced by percentage of conserved primer binding sites between two closely related species (Jingade *et al*., 2014 and Fan *et al*., 2013). In general, in legumes transferability of SSR markers may varied from 100% Azuki bean to blackgram (Gupta and Gopalkrishna 2010), 90% from Azuki bean to *Vigna umbellate* (Chaitieng *et al*. 2006), 61.7 % from Chickpea to *Medicago truncatula* (Choudhary *et al.,* 2009), 43.32% from *Medicago truncatula* to chickpea (Jafari *et al*., 2013) and in some reports no transferability from pigeon pea and chickpea to black gram (Cannon *et al*., 2011). However, in present study high proportion of cross-genera transferability of chickpea genic SSR markers to cowpea (32.14%) was observed. But, this result was lower than the transferability of chickpea EST-SSR markers to *Medicago truncatula* (61.7%) (Choudhary *et al.,* 2009) and to black gram (64.5 %) (Jingadhe *et al*., 2014). The most probable reason is that, genic or EST-SSR markers should have high rate of transferability than genomic SSR markers as they are derived from the coding region of DNA (Fan *et al*., 2013). Whereas, the low rate of transferability in the present study may be due to genic SSR markers derived particularly from the chromosomal segments representing *Fusarium* wilt resistance genomic region (Raghu and Ravikumar, 2016).

CONCLUSION

However, highest rate of genic SSR markers transferability in cowpea (32.14%) to lowest rate of transferability observed in moth bean (24.40%) were less than 35% but, it may also been considered for building an SSR pool for molecular breeding research program in orphan legumes where development of sequence based SSR markers may not be possible due to lack of availability of genome sequence information.

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