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Research Note

Transferability of heterologous SSR Markers to cotton genotypes

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Abstract
The use of transferable cross species/ genera SSR markers is considered as cost effective strategy to ensure availability of markers in genomic resources of cotton. Forty one of 88 SSR primer pairs constitutes 46.59 % [24 from safflower *i.e* 27.27% and 17 from pulses *i.e* 19.32%] were found transferable as they amplified SSR priming genomic regions of both the genotypes. Out of these 88 markers, 27 (30.68%) were shown monomorphism and 14 (15.91%) were shown polymorphism. Among amplified markers, 45.83% and 17.65% polymorphism was observed by these heterolgous markers of safflower and pulses respectively.

Key words
Cotton, SSR markers, RGA, heterologous primers, polymorphism

Cotton is an important fibre crop of global importance. Cotton known as the "king of fibre" and in recent times called as "white gold", is the most vital crop of commerce to many countries including India. Cultivated cotton (*Gossypium* spp.) is the world's leading natural fibre crop and it is the cornerstone of textile industries worldwide. Inspite of several competitions from synthetic fibres, cotton continues to enjoy a place of prime importance in textile industry. In India, cotton provides means of livelihood to millions of farmers and workers and sustains cotton textile industry which annually produces cloth of the value exceeding a thousand crore rupees. Cotton seed had also gained the additional economic importance as a major contributor to edible oil, protein and other by products. The valuable biomass from cotton stalks has become very useful raw material for manufacture of particle boards, paper and other stationaries. In total, cotton has become a highly agro-industrial crop producing 90 *per cent* raw material to textile industry and contributes 60 *per cent* of oil requirements.

The importance of molecular markers used in the development of disease resistant lines over a conventional breeding is recognized, as, it saves the time from substitution of complex field trials (that need to be conducted at a particular times of a year or at specific locations, or technically complicated) with molecular tests and selection of genotypes at seedling stage, gene pyramiding or combining multiple genes can be done simultaneously. Estimation of unreliable phenotypic evaluations associated with field trials due to environmental effects. Avoid the transfer of undesirable or deleterious genes (linkage drag) and selecting for trials with low heritability. Testing for specific traits where phenotypic evaluation is not feasible and also assessment of cultivar, genetic diversity and parental selection and also used to exploit heterosis.

The number of amplified bands varies among RGA (resistant gene analogue) primer pairs, there was no obvious relationship berween the group of primers used and the level of polymorphism detected. A significant advantage of RGAs over arbitrary DNA markers is that RGAs represent potentially functional genes. Although not all amplified products are functional disease resistant genes, they all contain conserved sequences representing LRR, Kinase, and (or) NBS domines (Chen *et al.,* 1998). RGA markers offer a number of advantages for evaluating resistant germplsm compared with existing molecular markers. The RGA markers serve as both candidate genes for cellular recognition and informative markers for inferring genetic relationships between germplasm accessions (Naik *et al.,* 2006).

DNA markers which are crop stage non specific, environmental neutral, easily assayable and simply inherited are increasingly being used as surrogates to augment the pace and efficiency of breeding crop plants. Of several markers systems, those based on simple sequence repeats(SSR) are the primery choice of crop breeders owing to hyper variability, higher reproducibility, mono-locus, multi-allelic and co-dominant inhreritance, possibility of multiplexing and amenability for automation (Powell *et al.*,1996). *De novo* development of SSR markers is expensive laborious and labour intensive (Powell *et al.,* 1996). Nevertheless, the use of cross species/genera SSR markers (Transferable or heterologous markers) in crop where are not available is an alternative strategy to ensure availability of markers in genomic resources of cotton. The present study was carried out with an objective of exploiring and assessing transferability of heterologous SSR markers to cotton genotypes.

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The Two cotton genotypes (DS-28 and SBYF-425), 37 SSR primers from safflower, 51 resistant gene analogues (RGAs) from pulses (mungbean and urdbean) were used in this study. DNA was extracted from both the genotypes using CTAB method with few modifications.

PCR amplification and electrophoresis: A set of 88 SSR primers (37 from safflower and 51 from pulses RGA) were used for the amplification of genomic DNA of parents (DS-28 and SBYF-425). The SSR reaction mixture consisted of 20 ng of template DNA, 0.5µl of Forward and Reverse primer each, 2.0 µl of dNTPs, 3 unit of *Taq* polymerase (Bangalore Genei, India), 10X PCR buffer (100mM Tris pH 9.0, 500 mM KCl, 15mM $MgCl_2$ and 0.1% Gelatin) in a volume of 20µl. Thermal cycler gradient was used for cyclic amplification of DNA (Thermocycler). PCR duration of both safflower and pulses (mungbean and urdbean) was different and the annealing temperatures for each of the primers were determined by a gradient PCR and were standardized. The initial denaturation was at 94°C for 5 minutes followed by 38 cycles of 30 seconds at 94°C, 45 seconds at 56°C, 45 seconds at 72°C and final 10 minutes extension at 72°C. For pulses, the initial denaturation was at 94°C for 5 minutes followed by 40 cycles of 30 seconds at 94°C, 35 seconds at 53°C, 45 seconds at 72°C and final 10 minutes extension at 72°C. The horizontal electrophoresis of PCR amplified products was carried out on agarose gel of 3 % in 1X TAE at 70V for 3 hours. 2µl of tracking dye was added to 20 µl of PCR products and mixed well before loading into the wells. Lambda DNA double digest was used as DNA molecular weight marker. The electronic images of ethidium bromide (0.5 µg/ml) stained gels were captured and documented using UV Tran illuminator and Alpha Innotech.

DNA quantity and quality estimation: The concentration of DNA was assessed spectrophotometrically and also by gel electrophpresis using 0.8% agarose with known concentration of uncut DNA. In spectrophotometric analysis, 5µl of DNA sample diluted with TE buffer and volume made up to 3000µl was subjected to spectrophotometer readings at absorbance of 230 nm, 260 nm. A good DNA preparation generally exhibits the following spectral properties.

$A_{230}<0.10$, $A_{230}<0.45$, $A_{280}/A_{260}<0.55$, or $A_{260}/A_{280}>1.80$

Concentration of DNA (µl/ml) = O.D at 260 x 50. To test the quality of DNA, samples were run on 0.80 % agarose gel 1x TAE (Tris Acetic Acid EDTA) buffer and stained with ethidium bromide and checked for contamination by RNA (which usually runs a head) and the DNA was evaluated by comparing it with a standard undigested DNA sample.

Data scoring: The products of PCR were scored visually based on presence or absence of amplicon in agarose gel matrix. Based on variation in the size of amplification product, the loci or trait were classified as monomorphic or polymorphic.

Cross-species transferability is a quick and economic method to enrich SSR database, particularly for minor crops where little genomic information is available. However, transferability of SSR markers varies greatly between species, genera and families of plant species (Pratik *et al.*, 2016). This survey was done using heterologous primers (safflower and pulses) to know whether these primers will work in cotton or not and their transferability. Out of 88 heterologous SSR primers, 41 primers were effectively amplified cotton genome. Among 41, 24 from safflower and 17 from pulses were found to be monomorphic or polymorphic between DS-28 and SBYF-425 on 3 % agarose gel (Fig.1&2). Among these 37 safflower SSR markers, those based on mono-nucleotide repeates constituted 40.54% while those based on di-, tri- and complex- nucleotide repeat motif constituted 24.32%, 16.22% and 18.92%, respectively.

Those markers which are amplified SSR priming regions of DNA of cotton genotypes (DS-28 and SBYF 425) and produced single and specific bands of reported expected product size were considered as transferable markers. Based on this criterion, *per cent* transeferability was calculated as [(Number of markers amplified/ Total number of markers) x 100]. *Per cent* transferabilty of each type of nucleotied repeats motifs- based SSR markers was estimated. To take into account variable number of SSR markers with different types of motifs, conditional probability that a given transferable marker is based on a particular motif was estimated as (Number of transferable markers based on a particular motif/ Total number of transferable markers).

Forty one of 88 SSR primer pairs constitutes 46.59 per cent (27.27% from safflower and 19.32% from pulses) were found transferable as they amplified SSR priming genomic regions of both the genotypes. Among all markers, 27 (30.68 %) were shown monomorphism and 14 (15.91 %) were shown polymorphism (Table 1 and Fig.1&2). While 24 SSR markers from safflower (64.86 %) and 17 markers from pulses (33.33 %) were transferable. Among amplified markers, total monomorphic and polymorphic were 65.85 per cent and 34.15% respectively. 45.83 per cent and 17.65 per cent polymorphism was observed by these heterolgous markers of safflower and pulses respectively. Such polymorphic markers were also

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amplified in cotton RILs obtained from the cross between the DS-28 and SBYF-425 (These genotypes react differently for disease reaction). Their association with disease resistance in RILs is suggested for the future line of work (Table 2). Out of 37 safflower SSR markers mono-nucleotide repeats- based markers had highest transferability (86.67 %) followed by those based on complex- (85.71 %), tri- (50 %) and di- nucleotide repeat motifs (22.22 %) (Table 3 & Fig. 3). The conditional probability that a given cross genera transferable SSR marker is based on mono-nucleotied repeat motif was higher than that based on di-, tri and complex nucleotide repeat motifs (Table 3).

Thus, extent of transferability of cross genera SSR markers to cotton depends on their number and nature of repeat motifs. Chandra (2011) reported tranferability of *Medicago truncatula* EST-SSR markers to forage legumes. Shivakumar *et al.*, (2015) observed 100 per cent transferability from soybean, 78.57 per cent from greengram and 77.77 per cent from chickpea to dolichos bean. Naturally out crossing species, such as maize, tend to have high levels of DNA polymorphisms and virtually any cross that does not involve related individuals will provide sufficient polymorphism for mapping (Helentjaris, 1987). However, levels of DNA sequence variation are generally lower in naturally inbreeding species and finding suitable DNA polymorphisms may be more challenging (Miller and Tanksley, 1990). In guava, off the 23 SSR loci, 18 (78.2 %) gave cross-amplification in *Eucalyptus citriodora*, 14 (60.8 %) in *E. camaldulensis* and 17-17 (73.9 %) in *Callistemon lanceolatus* and *Syzygium aromaticum* (Manoj *et al.*, 2013).

Pratik *et al. (*2016) assessed confamiliar transferability of SSR markers from cotton (*Gossypium hirsutum*) and jute (*Corchorus olitorius*) to 22 species distributed in different taxonomic groups of Malvaceae. Of the 14 cotton SSR loci tested, 13 (92.86 %) amplified in *G. arboreum* and 71.43 % exhibited cross-genera transferability. Nine out of 11 jute SSRs (81.81 %) showed cross-transferability across genera. At tribe level, transferability of jute SSRs (41.04 %) was higher than that of cotton SSRs (33.74 %). Transferability of wheat SSR markers to rye was 17%, whereas 25% of rye markers were amplifiable in wheat. In triticale, 58% and 39% transferability was achieved for wheat and rye markers, respectively (Kuleung *et al.*, 2004). In the process of identification of molecular markers linked to economically important traits, use of heterologous EST markers like markers derived from disease resistant analogues will be most useful. Therefore in this study, markers developed from disease resistant analogues from pulses and safflower was tried in cotton.

The present study provides adequate evidence for transferability of heterologous SSR markers to genomic resources of cotton. These transferable markers could be used in cotton for (1) Assessing genetic variability for working germplasm/breeding material, (2) fingerprinting germplasm for identification of duplicate accessions, (3) selection of genetically diverse parents for planned crossing to generate variability and (4) Constraction of linkage map for chromosomal localization and unraveling mode of action of genes controlling desired traits. The linked DNA markers serve as powerful surrogates for selection of desirable genotypes for complex traits and aid in enhancing pace and efficiency of cotton breeding.

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Table 1. List of monomorphic and polymorphic loci detected using heterologous primers in cotton

Sl. No.	Trait/loci	Monomorphic	Polymorphic
1	acetolactate synthase – dr	√	
2	vamp721 F	√	
3	solute carrier family 7	√	
4	protease inhibitor	√	
5	cold-specific protein	√	
6	GB-CT-023 microsatellite	√	
7	photosystem I subunit PsaD	√	
8	chloroplast outer envelope 24 kD protein (omp24)	√	
9	drought stressed subtracted	√	
10	NADH dehydrogenase subunit		√
11	G protein-coupled receptor	√	
12	stearoyl-acyl carrier protein desaturase		√
13	oleoyl-acyl carrier protein thioesterase	√	
14	Small heat shock protein		√
15	GB-CT-022 microsatellite	√	
16	G protein-coupled receptor		√
17	protease inhibitor		√
18	MYB133	√	
19	Pistil-specific extensin-like protein		√
20	NADH dehydrogenase subunit 3		√
21	carbonic anhydrase 3		√
22	photosystem I subunit PsaD		√
23	chloroplast outer envelope 24 kD protein (omp24)		√
24	Aquaporin		√
25	CLRR	√	
26	VuRS01J11	√	
27	VuRS01P23V	√	
28	VuRS02F19V	√	
29	VuRS01N9V	√	
30	VuRS02L24R	√	
31	VuRS03A23V	√	
32	VuRS03O19V	√	
33	VuRS02B24R	√	
34	S2	√	
35	CLRR-INV1	√	
36	Pto kin3		√
37	NLRR-INV1		√
38	VuRS01J7V		√
39	RLK	√	
40	S1	√	
41	S2-INV	√	

Table 2. Per cent transferability of heterologous SSR markers to cotton genotypes

Crop	Total number of markers used	Number of markers amplified			% Transferability
		Monomorphic	Polymorphic	Total	
Safflower	37	13	11	24	64.86
Pulses	51	14	3	17	33.33
Total	**88**	**27**	**14**	**41**	**46.59**

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Table 3. Per cent transferability of heterologous SSR markers from safflower to cotton by number and nature of repeat motifs

Number and nature of repeat motifs of markers	Total number of markers used	Number of markers amplified	% Transferability	Conditional probability that a given transferable marker is based on a particular motif
Simple mono -nucleotide repeats	15	13	86.67	0.54
Simple di - nucleotide repeats	9	2	22.22	0.08
Simple tri- nucleotide repeats	6	3	50.00	0.12
Complex- nucleotide repeats	7	6	85.71	0.25

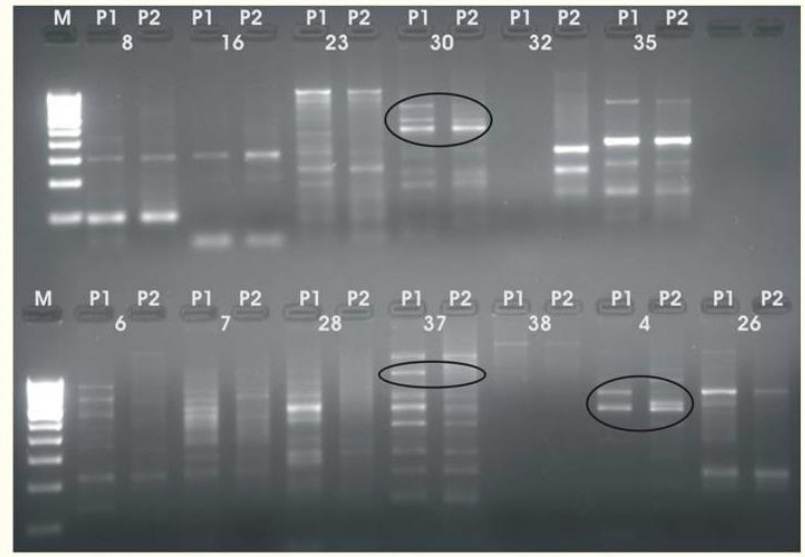

Fig. 1. Polymorphism for heterologous primers from safflower

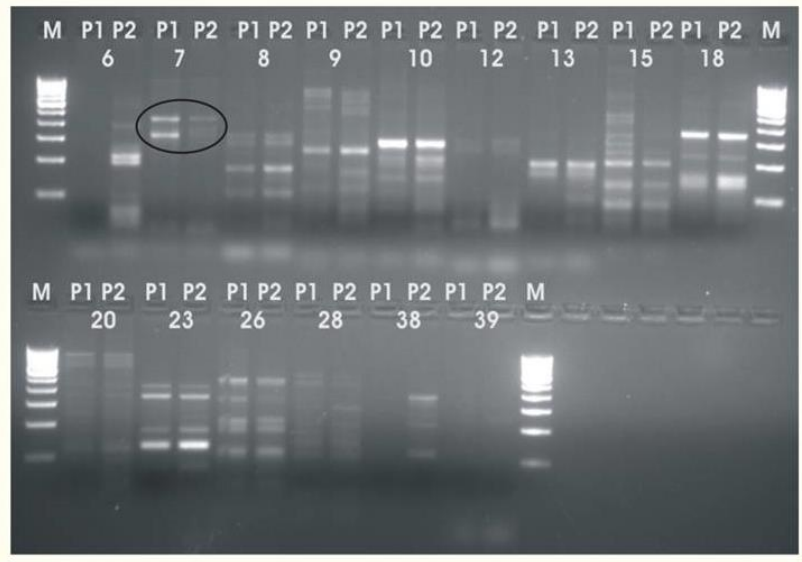

Fig. 2. Polymorphism for heterologous RGA from Pulses

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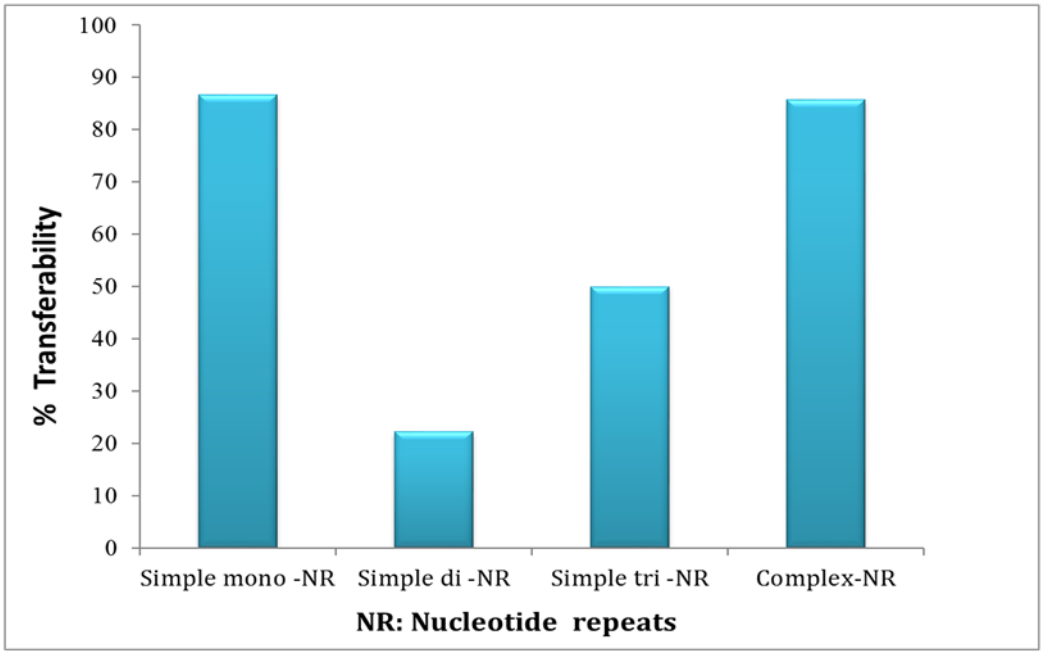

Fig. 3. Per cent transferability of heterologous SSR markers from safflower to cotton by number and nature of repeat motifs