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Comparative study of EST-SSR, SSR, RAPD, and ISSR and their transferability analysis in pea, chickpea and mungbean

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Abstract:

In this study, we have used different molecular markers to test their amplification potentiality in pea and transferability of EST-SSR primers generated from pea EST, in three legumes. In transferability analysis EST derived SSRs produced 50-80% amplification in mungbean, chickpea, and pea respectively. In another experiment, EST derived SSRs generated 80% amplification in pea followed by genomic SSR (75%), RAPD (55%), ISSR (50%). The RAPD and ISSR markers showed high amplification but they contained little information in comparison to EST-SSR markers. These results showed that EST-SSR primer have high potentiality for amplification and also transferability in different legumes. Further these primers will be used as universial primers for linkage mapping, diversity and transferability study and they can provide a rich source of information.

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Key words: Pea, RAPD, ISSR, EST-SSR, Transferability

INTRODUCTION

Locus which shows experimental (polymorphism) variation between the parents and the individuals of the mapping population is considered as a genetic marker. The genetic markers linked to agronomic traits are most important for accelerating traditional breeding programmes. This process is known as marker-assisted selection (Zheng et al. 1995). The most important properties for good quality of a genetic marker are: (a) highly polymorphic nature; (b) co-dominant inheritance; (c) frequent occurrence in genome; (d) selective neutral behavior; (e) easy and fast assay; (f) high reproducibility; and (g) low or null interaction with other markers allowing the use of many markers at the same time in a segregating population (Joshi et al 1999).

Molecular markers are considered to be the most reliable tools to identification of germplasm, to estimate the relationships between genotypes at the DNA level and also for marker assisted selection (Raddova et al. 2003). Three types of markers have been used in genome analysis: (1) morphological, (2) isozyme/protein based and (3) DNA based. When proteins and isozymes are taken as biochemical markers, they reveal polymorphism at protein level. The most common protein markers are isozymes, which are variant forms of the same enzyme (Vodenicharova 1989). Protein markers reveal differences in gene sequences and functions and are co-dominant in nature. DNA markers reveal polymorphism at DNA level. DNA markers are promising tools to evaluate genetic diversity among germplasm (Chao-Zhi et al. 2003). These can be categorized as hybridization based and PCR based. Hybridization based polymorphisms include RFLP

(Restriction Fragment Length Polymorphism) (Sambrooke et al. 1989) and VNTR (Variable Number of Tandem Repeats) loci (Rogstad 1993, 1996; Weising et al. 1992, 1998), where probes such as random genomic clones, cDNA clones, and probes for microsatellite and minisatellite sequence are hybridized to filters containing DNA which has been digested with restriction enzymes (Kumar 1999). PCR based DNA markers are RAPD, ISSR, EST-SSR, Microsatellite, CAPS etc (Table 1).

The molecular marker has been used previously for construction of genetic maps in many plants species. The objective of the present work was to evaluate amplification and polymorphic properties of different markers in legumes.

MATERIALS AND METHOD

Commercial variety of pea, chickpea and mung bean seeds available were purchased from local markets and growing them in laboratory condition. The genomic DNA was isolated from leaves of individual plants. The leaves were stored at -80°C for any further of DNA isolations. DNA was isolated using a modified version of the protocol described in Doyle and Doyle (1987). For max preparations approximately 3 g of leaf material was used for extraction of DNA. The purity and concentration of isolated DNA sample was estimated by taking OD (absorbance) at 260 nm and 280 nm and then again rechecked by loading 2 μl of each sample, in 0.8% agarose gel, along with standard λ DNA (30ng, 60ng, 120ng, 150 ng, 300ng, 500ng and 1000ng).

RAPD and ISSR profiling

The A to C, RAPD primers (OPA, OPB, OPC) and 10 ISSR primers were used for amplification study. For RAPD profiling, each polymerase chain reaction (PCR) was set up in 25 μL

volume containing 1unit of *Taq* polymerase (Invitrogen Corporation, USA), 25 ng of genomic DNA, 0.8 mM of primer, 0.1 mM of each of four dNTPs (Amersham Bioscience), 2.5 µL of 10X PCR reaction buffer [500 mM KCl, 200 mM Tris-HCl (pH 8.4)], and 3 mM $MgCl_2$. DNA amplifications were carried out in a iCycler (Bio-Rad). The steps used to generate RAPD markers were: 1 cycle consisting of 60 s at 94°C, 30 s at 36°C and 60 s at 72°C followed by 45 cycles of 5 s at 94°C, 15 s at 36°C, and 60 s at 72°C, and a final cycle of 7 min at 72°C. The ISSR amplification were carried out with a preliminary cycle of 120 s at 94°C, followed by 35 cycles of 20 s at 94°C, 50 s at 50°C, and 90 s at 72°C, and a final cycle of 7 min at 72°C. The amplification products were resolved on 1.2 % (for RAPD) and 1.5% (for ISSR) agarose gels (Sigma-Aldrich USA).

SSR and EST-SSR marker analysis

Around 20 genomic SSR and 10 EST-derived SSR primers were used for amplification study in pea. The amplification reactions were carried out in a 20 µL volume containing 1unit of *Taq* polymerase (Invitrogen Corporation, USA), 25 ng of genomic DNA, 0.80 µM of each primer (Forward and Reverse), 0.2 mM of each dNTPs, 2.0 µL of 10X PCR reaction buffer and 3 mM $MgCl_2$. DNA amplifications were carried out in a iCycler (Bio-Rad). The amplifications parameter were as follows: 3 min at 94°C, followed by 45 cycles of 60 s at 94°C, 60 s at 50°C, and 120 s at 72°C, and a final step of 10 min at 72°C. The amplification products were resolved on Metaphore agarose gel.

RESULT AND DISCUSSION:

Presently EST-SSR markers developed by *in silico* method are more acceptable and were frequently used in several plant systems like legumes, cereals and medicinal plants. Here we

have used 10 EST-SSR markers (developed from EST data of pea available on NCBI), in amplification of 3 legumes plants (Table 2). All 10 EST-SSR markers showed more amplification capability in all 3 legumes, contained better capacity for quantifying the genetic diversity through total number of effective alleles. Around 80% of EST-SSR showed amplification in pea, followed by 60% in chickpea and 50% in mung bean.

For the comparative study of amplification, we were used first and second generation of markers. In this experiment we have used EST-SSR primers along with RAPD, ISSR and SSR primers. A comparison of the levels of amplification and the discriminating capacity of 60 RAPD, 10 ISSR, 20 SSR and 10 EST-SSR molecular markers are shown in Table 3. The levels of amplification and polymorphism capacity of all primers were high in the analyzed samples however higher amplification was obtained with EST-SSR markers. We obtained 80% amplification with EST-SSR primers followed by genomic SSR (75%) and RAPD (55%). The lowest percentage of amplification reported in ISSR primers (50%). EST-SSR markers have been proven to be more informative than previously reported markers and should be used in subsequent fingerprint analysis.

In conclusion, present study showed the potentiality of EST derived SSR primers in legumes. The amplification generated by these EST-SSR in legumes showed we can use these primers as a universal marker in legumes. The use of EST-SSRs have 3 advantages over previously reported primers: (1) After DNA isolation, no further manipulations are needed with EST-SSR; (2) In case of other markers like SSR we need to generate cDNA Llibrary to generate SSR, are time-consuming and require clean DNA. (3) the additional restriction, ligation, and pre amplification steps make SSR more expensive than EST-SSR. Additionally, we have demonstrated that EST-SSR markers generate better amplification and transferability.

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Table 2: Amplification study of EST derived SSRs primers in legumes

Primer name	Pea	Chickpea	Mung bean
GR41	+	+	+
GR42	+	-	+
GR43	-	+	-
GR44	+	-	-
GR45	+	+	-
GR46	+	+	-
GR47	-	-	+
GR48	+	+	+
GR49	+	-	-
GR50	+	+	+

Table 3: Molecular markers used in the amplification study of pea DNA

Marker type	Number of primers/ primer pair screened	Number of primers/ primer pair amplified	Number of amplified bands	Percentage of amplification
RAPD	60	33	111	55%
ISSR	10	5	27	50%
microsatellite	20	15	48	75%
EST_SSR	10	8	20	80%
Total	100	61	206	

Fig. 1 Amplification pattern generated by EST derived SSR primers in legumes