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DEVELOPMENT AND CROSS-SPECIES AMPLIFICATION OF GRASS PEA EST-DERIVED MARKERS

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ABSTRACT

Expressed Sequence Tags (ESTs) deposited in the public domain are providing a cost-effective means of developing sequence based molecular markers. In this study, publicly available grass pea (*Lathyrus sativus*) EST sequences were used to develop 21 molecular markers for *L. sativus* (grass pea). Out of these, thirteen markers were polymorphic. Evaluation of the transferability of the newly developed markers to five other legume species resulted in transferability percentage of 95.23% in *L. cicera,* 61.9% in field pea, 52.38% in faba bean, 38.1% in chickpea, and 33.33% in lentil. The markers developed will be useful for genetic studies in grass pea and other legume species.

Key Words: Expressed Sequence Tag, *Lathyrus sativus*

RÉSUMÉ

Les marqueurs exprimés de séquence Tag (ESTs), disponibles dans le domaine publique fournissent des moyens moins chers de développement des marqueurs moléculaires. Dans cette études, les séquences EST publiquement disponibles de la gesse (*Lathyrus sativus*) étaient utilisés pour dsvelopper 21 marqueurs moléculaires de la gesse *L.sativus*. Parmi ces derniers, treize marqueurs étaient polymorphiques. L'évaluation de la transferabilité des marqueurs nouvellement développés en cinq autres espèces de légumineuses ont induit un pourcentage de transferabilité de 95.23% dans *L. cicera*, 61.9% dans le pis, 52.38% dans la fève, 38.1% dans le pois chiche, et 33.33% dans les lentilles. Les marqueurs développés seront utiles dans des études génétiques de la gesse et autres espèces de légumineuses.

Mots Clés: Séquence Tag exprimée, *Lathyrus sativus*

INTRODUCTION

Molecular markers are useful tools in genetic analysis of plants. Currently, different types of molecular markers are available, which differ in abundance in the genome, reproducibility, degree of polymorphism, locus specificity and technical demand (Agrawal *et al*., 2008). Development of highly informative sequence based molecular markers is expensive; hence, in plants such markers have been developed mainly for species of high economic importance. To overcome this, cross species transferability of markers have been studied and utilised in different species (Erpelding *et al*., 1996; Varshney *et al*., 2005; Chandra and Dubey, 2007; Jensen *et al*., 2007). Additionally, large-scale sequencing projects that aim at discoveries of novel genes have generated large amount of expressed sequence tags (EST) deposited in publicly available sequence databases, offering a potential resource for the development of genetic markers (Gupta and Rustagi, 2004). EST based markers are derived from transcribed regions of the DNA and, hence,

it is expected that these markers would be more conserved and have a higher rate of transferability than genomic markers (Scott *et al*., 2000). Transferable EST based markers have been used in phylogenetic studies (Rouf *et al*., 2005), characterisation of allelic diversity on a set of accession of different species (Zang *et al*., 2007), and genetic diversity studies (Gupta *et al*., 2003). Lack of genomic resources is a characteristic of most legume crops. However, recent efforts by various research groups on different model legumes and crops are providing information that has implications on their improvement (Varshney *et al*., 2009). Understanding the conservation of genome structure among legumes assures the transfer of technology from well studied or model legume species to others (Fredslund *et al*., 2006). Conserved and cross species transferable markers have many applications in this aspect. They are useful in constructing comparative genetic maps, thus facilitating the study of synteny conservation and collinearity among related genomes (Gupta and Rustgi, 2004). In legumes, comparative mapping have been utilised to illustrate relationship between model species and other legumes (Aubert, 2006; Phan *et al*., 2007; Ellwood *et al*., 2008).

Grass pea (*Lathyrus sativus* L.) is a food and forage legume cultivated in Central, South and Eastern Europe, West Asia, North Africa and Ethiopia. The crop is tolerant to extremely dry conditions in areas susceptible to drought and it is also tolerant to excessive floods (Campbell, 1997). Grass pea has been recommended as a potential source of disease resistance genes for use in grain legume breeding programmes (McCutchan, 2003). Like most crop legumes, genetic information available for grass pea is minimal. There are few published sequence derived markers which were developed by converting *L. sativus* defense-related ESTs into mapable genetic markers (Skiba *et al*., 2003), of which some have been used for generating a linkage map of *L. sativus* (Skiba *et al*., 2004).

The genus, *Lathyrus,* is a member of the Vicieae tribe, together with *Pisum*, *Lens* and *Vicia*, which are important food legumes, with whom it could share genes, physiological processes and defense mechanisms. For this reason, the *Lathyrus* spp. may serve as potential sources of new and useful genetic traits, such as resistance genes, for closely related genera.

The objective of this study was to develop molecular markers useful for genetic studies of grass pea, and examine the cross species amplification of the newly developed markers in other legume species.

MATERIALS AND METHODS

DNA extraction. DNA was extracted from leaf tissues of two weeks old seedlings of grass pea (*L. sativus)*, lentil (*Lens culinaris*), field pea (*Pisum saivum*), faba bean (*Vicia faba*), chickpea (*Cicer arietinum*), and *Lathyrus cicera*, using GenElute Plant Genomic DNA Miniprep Kit (Sigma-Aldrich, St. Louis, MO).

Primer design. Forty sequence tagged site (STS) primers were designed from *L. sativus* EST sequences available in Genbank (http://www.ncbi.nlm.nih.gov) using Primer3.0 software (Rozen and Skaletsky, 1998). The parameters used for designing the EST-STS primers were: primer length of 18-24 bp with an optimum of 20 bp, annealing temperature of 50-60 °C with an optimum of 55 °C, percentage GC in the range of 40-50% and amplification of 100-400 bp fragment length. Similarly, using Batchprimer3 software which allows detection of simple sequence repeats (SSRs) (You *et al*., 2008), nineteen primer pairs flanking a microsatellite region were designed. SSR-containing sequences consisting of di-, tri- and tetranucleotide repeats with a minimum of seven, four, or three subunits, respectively were selected.

PCR condition. For EST-STS markers, PCR amplifications were performed in 20 µl reactions containing 5 µl 5x PCR buffer, 0.2 mM of each dNTPs, 0.4 µM of the forward and reverse primers 1 U of GoTaq® polymerase (Promega), and 30 ng of genomic DNA. Cycle conditions were initial denaturation step at 94 °C for 5 minutes followed by 30 cycles of 94 °C for 45 s, annealing at optimum annealing temperature (Ta) for 60 s, extension at 72 °C for 60 s with a final extension at 72 °C for 5 minutes. Amplified products were run on 2% agarose gel stained with Ethidium bromide. For STS markers that gave monomorphic bands with

amplicons more than the expected fragment size restriction enzymes were used to convert them to polymorphic markers (Table 1).

For EST-SSR markers, PCR was performed in a final reaction volume of 15 µl containing 30 ng genomic DNA, 5X PCR buffer, 0.2 mM each of dNTPs, 0.5 unit Go*Taq*® polymerase (Promega), 0.3 µl each of forward and reverse primers and 0.02 mM labeled M13 primer (6-FAM/VIC/PET/NED). PCR was performed in two phases: first with a 5 min initial denaturation step at 94 °C, followed by the first 30 cycles with conditions at 94°C for 30 s, 30 s at the optimal annealing temperature and extension at 72 °C for 45 s; and second with 15 cycles with conditions 94 °C for 30 s, 61 °C for 30 s, and 72 °C for 45 s, with a final extension at 72 °C for 10 minutes. PCR products (2 - 2.5 µl) were mixed with 10 µl of formamide (Hi-Di™ Formamide) and 0.3 µl labeled size standards (GeneScan™-500 LIZ® Size Standard) per reaction, denatured for 5 min at 94 °C and analysed using an ABI3130xl genetic analyser (Applied Biosystems).

Marker transferability. To test the potentials for cross-species amplification of *L. sativus* derived markers to other legume species, the newly designed primer pairs were used to amplify genomic DNA of lentil, field pea, faba bean, chickpea and *L. cicera* under the same PCR-conditions applied for amplifying genomic DNA of grass pea. For each primer pair amplification success was scored as positive only if a clear and reproducible band was observed.

RESULTS

Marker development and polymorphism evaluation. Out of the 40 EST derived primer pairs screened for amplification in genomic grass pea DNA, 80% gave amplification products. After optimisation, 12 markers (30%) gave amplification of a single fragment. Of these, nine of the primer pairs directed amplified products larger than the predicted size. Digestion of seven of these single monomorphic PCR products with a range of restriction endonucleases resulted in 6 polymorphic STS markers (Table 1). Amplification product from marker Ls175 was digested with six different types of restriction

TABLE 1. EST-STS markers, primer sequences, PCR conditions, product sizes and results of restriction endonuclease digestions

NCBI accession No.	Primer name	Primer sequence	Expected size (bp)	Amplified fragment size (bp)	Ta (°C)	Enzymes resulting in polymorphism
DY396274	Ls048*	F: GGTTGGGTCAAGCAAGAAAAR:TGGGTATTCTTGAGCGCAGT	384	700	57.5	*RsaI*
DY396277	Ls060*	F: GCCTTGTCCTTCCTCTTCCTR:TTTTGCCCAGTTCCCATAAA	413	500	57.5	*HaeIII, AluI, ApoI*
DY396284	Ls093	F: GAGTACGCGGGGAAAGACTC R:GGCATCCAGCAAAACAATTT	345	350	57.5	NA
DY396285	Ls094	F: GGTTGCAAAGCAAGAAGGTC R:CCTACCAGACCAAACCGATCT	406	550	57.5	NA
DY396289	Ls109*	F: GAATCTGGTCATTGGGATCG R:TGGGTTTTTCTTTTCGAACG	403	1000	57.5	*HaeIII, HinfI, RsaI, ApoI, DdeI*
DY396292	Ls125*	F: GACACCGGAATCTGGTCATT R:TTTTCTTCTCGAACGCATCC	400	1000	57.5	*HaeIII, HinfI, RsaI, ApoI, DdeI*
DY396294	Ls137*	F: CCATCAACTTTCCCACTTCA R: AGCACCTTTCGCAGTTTTTG	402	1350	57.5	*HaeIII, HinfI, AluI, RsaI, ApoI,*
DY396296	Ls148	F: ACGGGGGATATCAAACACAC R:CACGATGAGCACACACATAACTT	395	550	59	NA
DY396300	Ls160*	F: AACATGGGTGTGGGTCAGAT R:CGAGCCTCAACAATGTTTCA	412	950	59	*AluI*
DY396301	Ls162	F: CCTCTACTGTGCCTCCTGCT R: GGCTTGAAAAGAGCATCACC	406	350	59	NA
DY396304	Ls175	F: TCGCTCTCTTCGTCCTCTTC R: CATTCCCTGGGCTATCGTAA	397	1000	59	None
DY396361	Ls619	F: GAGCTGAGCGATTAACAGATA R: CATCTGCTGAAGAAGATTTTG	148	700	57.5	NA

NA = not analysed, * = polymorphic markers

enzymes (*HaeIII, HinfI, RsaI, ApoI, Dde, AluI I*), but none of them resulted in polymorphism.

Among 19 primer pairs flanking microsatellite fragments, 12 (63%) gave successful amplicon of the expected range. Blast analysis revealed that amplified fragments from primers Ls848 and Ls792 aligned as identical fragments and Ls709, Ls481 and Ls459 also aligned in a similar manner showing that these were redundant sequences. Hence, only two of these EST-SSRs (Ls848 and Ls709) were used for further analysis. Screening of the nine EST-SSRs for polymorphism on grass pea genotypes resulted in seven polymorphic markers (Table 2).

Cross-species amplification. For evaluating cross-species amplification, all the 21 EST derived primer pairs were screened in five legume species (lentil, field pea, faba bean, chickpea, and *L. cicera*) and resulted in various level of transferability. A marker was considered to be transferable if it gave a comparable fragment size with that obtained for *L. sativus*. The majority of the markers in this study yielded good quality PCR products in all the legumes tested (Fig. 1). Five of the primer pairs (23%) amplified across all five species, and five other primer pairs amplified only within the genus (*L. cicera)*. The average transferability success for all markers was 56.19%. One marker (Ls694) was specific to grass pea. Considering all the 21 markers, amplification success revealed by species was 95.23% in *L. cicera,* 61.9% in field pea, 52.38% in faba bean, 38.1% in chickpea, and 33.33% in lentil (Table 3). The highest transferability rate was detected in *L. cicera* and the lowest in lentil. The highest transferability outside the genus was detected in field pea (61.9%). Considering only EST-SSR markers, the highest transferability was detected in *L. cicera* (100%) and the lowest in chickpea (44%).

DISCUSSION

Publicly available *L. sativus* EST sequences were used to develop sequence based molecular markers for grass pea. Twenty-one markers were developed and 13 of them were polymorphic within *L. sativus*. Seven of these primer pairs were

TABLE 2. Primer sequences, PCR conditions, product sizes and characteristics of EST-SSR markers analysed

NCBI accession No.	Marker name	Repeat motif	Size (bp)	Forward F and Reverse R primer sequence	Ta (°C)
DY396423	Ls989*	(GT)8	157-165	F: GGGCTTGTTACACTGATATGTR: AACAGCATAAATACCCCTTTT	55
DY396279	Ls074*	(TC)7	160-168	F: ATGCTTCAGATGCAAAAGATR: TCAAAAGTTGAAACAGAGAGC	56
DY396360	Ls617*	(GTTG)3	164-172	F:ATGGTTGATTGCTGGTATTTAR: GAAGCAAATGCAGGTTTTAAT	56.5
DY396353	Ls576*	(ATG)5	173-179	F: GGAAAACCTGAAGAAGATAGCR: TAACTATCCTTGGGACTCCTC	57
DY396382	Ls744*	(TTC)4	132-147	F: CAGAAAAAGATGAAAACGATGR: ACAAGAATCTCTTACGCTTCA	57
DY396400	Ls848*	(CAT)4	158-173	F: TTGAAACTATTGAGGCAACATR: AGACTTTCAAGGAAAATGCTT	59.8
DY396411	Ls942*	(CCAA)3	137-145	F: GAATTCGATTAAGCAGTGGTAR:ACCTTTCTTCACTCTGTTTCC	51
DY396380	Ls733	(AGA)4	172	F:AAGATTGTGGATGTTTTAGGCR: GTTGAAGAGAAACCTTCTTCC	55
DY396377	Ls709	(ATGG)3	174	F: GCTCATCCTTGAAGTAAGTGAR:GCCAAGATTAGCATCAATATC	55

* = polymorphic markers for grass pea

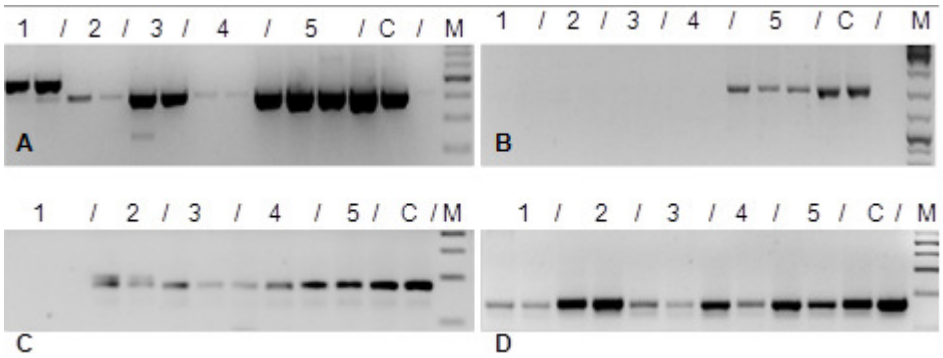

Figure 1. Example of agarose gel image of EST-STS (A & B) and EST-SSR (C & D) bands amplified by primer pairs (A) Ls162, (B) Ls137, (C) Ls744, (D) Ls709 on genomic DNA of: 1-lentil, 2-field pea, 3-faba bean, 4-chickpea, 5-*L. cicera*, C-grass pea (control), M-Molecular weight marker.

TABLE 3. Results of cross-species amplification of *L. sativus* EST-derived markers in five legume species

Genbank accession number	Primer name	Lentil	Field pea	Faba bean	Chickpea	*L. cicera*	Overall % of transferability
DY396274	Ls048	-	+	+	+	+	80
DY396277	Ls060	-	-	-	-	+	20
DY396284	Ls093	-	-	-	-	+	20
DY396285	Ls694	-	-	-	-	-	0
DY396289	Ls109	-	+	+	-	+	60
DY396292	Ls125	-	+	+	+*	+	80
DY396294	Ls137	-	-		-	+	20
DY396296	Ls148	-	+	-	-	+	40
DY396300	Ls160	+*	+*	+*	-	+	80
DY396301	Ls162	+	+	+	+*	+	100
DY396304	Ls175	-	+	-	-	+	40
DY396361	Ls619	-	+	-	+	+	60
Overall STS		16.6	66.6	41.6	33.3	91.6	50
DY396423	Ls989	-	-	-	-	+	20
DY396411	Ls942	-	-	-	-	+	20
DY396400	Ls848	+	+	+	+	+	100
DY396382	Ls744	+	+	+	+	+	100
DY396380	Ls733	-	-	-	-	+	20
DY396377	Ls709	+	+	+	+	+	100
DY396360	Ls617	+*	+*	+*	+*	+	100
DY396353	Ls576	+	+	+	-	+	80
DY396279	Ls074	-	-	+	-	+	40
Over all SSR		55.5	55.5	66.6	44.4	100	64.44
STS+SSR		33.33	61.9	52.38	38.1	95.23	56.19

+ = Successful amplification; – = no amplification; * = showed amplicon size difference with *L. sativus*

useful in genetic diversity assessment of grass pea (Shiferaw *et al*., 2012).

One of the advantages of markers developed from EST sequences is the high transferability between related species. In this study, primer pairs resulted in amplified fragments of comparable size in different legume species. This could be due to the conservation of the target sequence and flanking regions in the legume species assayed. However, the level of conservation has to be confirmed by sequence analysis. In a few cases, the intensity of the right sized fragment was weak which probably is due to the use of similar PCR parameters in all species and hence optimisation might be necessary in using these markers for further study.

EST-SSR markers showed higher rate of transferability in all legumes, with transferability rate ranging from 44% (lentil) to 100% (*L. cicera*). A review by Peakall *et al*. (1998) reported that cross-species amplification of microsatellite loci in plants could range from moderate to complete primer conservation (50–100%) within genera. This complete primer conservation explains the highest rate of transferability observed between *L. sativus* and *L. cicera*. It is hypothesized that *L. sativus* was derived from the genetically nearest wild species *L. cicera* (Hopf, 1986). All the legume species tested for marker transferability here belong to the subfamily Papilionoideae, and all except chickpea belong to the tribe viceae (Steele and Wojciechowski, 2003).

Prior studies on cross-species amplification between legumes using SSRs derived from total genomic DNA showed various levels of sequence conservation. Peakall *et al*. (1998) reported the transferability of up to 65% of soybean derived SSR markers within the genus *Glycine,* and 3–13% of the SSRs outside the genus. Gutierrez *et al*. (2005) reported transferability of *Medicago truncatula* EST-SSRs to faba bean (40%), chickpea (36%) and field pea (37%) while within genus transferability of *M. truncatula* derived EST-SSRs was 74% (Eujayl *et al*., 2004). Considering all the 21 markers developed for grass pea, the average rate of successful transferability outside and within the genus was 46 and 95% respectively which is consistent with earlier findings.

CONCLUSION

This study has demonstrated the value of sequence data stored in public database for developing molecular markers in species that lack pre-existing genomic information. The cross-species transferability of 33-61% of the markers shows the potential of these markers to be used in comparative mapping and other genetic studied among different species. Since genomic resources are scarce in most legume crops, cross-transferability also allows the use of markers developed in one species for others.

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REFERENCES

Agrawal, M., Shrivastava, N. and Padh, H. 2008. Advances in molecular marker techniques and their applications in plant sciences. *Plant Cell Reports* 27:617-631.

Aubert, G., Morin, J., Jacquin, F., Loridon, K., Quillet, M.C., Petit, A., Rameau, C., Lejeune-He´naut, I., Huguet, T. and Burstin, J. 2006. Functional mapping in pea, as an aid to the candidate gene selection and for investigating synteny with the model legume *Medicago truncatula*. *Theoretical and Applied Genetics* 112:1024-1041.

Campbell, C.G.1997. Grass pea. *Lathyrus sativus* L. Promoting the conservation and use of underutilised and neglected crops. 18. Institute of Plant Genetics and Crop Plant Research, Gatersleben/International Plant Genetic Resources Institute, Rome, Italy. 7pp.

Chandra, A. and Dubey, A. 2007. Transferability of STS markers for studying genetic diversity within the genus *Cenchrus* Poaceae. *Current Science* 92:7 961-967.

Ellwood, S.R., Phan, H.T.T., Jordan, M., Hane, J., Torres, A.M., Avila, C.M., Cruz-Izquierdo, S. and Oliver, R.P. 2008. Construction of a comparative genetic map in faba bean *Vicia*

faba L.; conservation of genome structure with *Lens culinaris*. *BMC Genomics* 9:380.

Erpelding, J.E., Blake, N.K., Blake, T.K. and Talbert, L.E. 1996. Transfer of sequence-tagged site PCR markers between wheat and barley. *Genome* 39:802-810.

Eujayl, I., Sledge, M.K., Wang, L., May, G.D., Chekhovskiy, K., Zwonitzer, J.C. and Mian, M.A.R. 2004. *Medicago truncatula* EST-SSRs reveal cross-species genetic markers for *Medicago* spp. *Theoretical and Applied Genetics* 108:414-422.

Fredslund, J., Madsen, L.H., Hougaard, B.K., Sandal, N., Stougaard, J., Bertioli, D. and Schauser, L. 2006. GeMprospector-online design of cross-species genetic marker candidates in legumes and grasses. *Nucleic Acids Research* 34:670-675.

Gupta, P.K., Rustgi, S., Sharma, S., Sing, R., Kumar, N. and Balyan, H.S. 2003. Transferable EST-SSR markers for the study of polymorphism and genetic diversity in bread wheat. *Molecular Genetics and Genomics* 270:315-323.

Gupta, P.K. and Rustgi, S. 2004. Molecular markers from the transcribed/expressed region of the genome in higher plants. *Functional & Integrative Genomics* 4:139-162.

Gutierrez, M.V., Vaz Patto, M.C., Huguet, T., Cubero, J.I., Moreno, M.T. and Torres, A.M. 2005.Cross-species amplification of *Medicago truncatula* microsatellites across three major pulse crops. *Theoretical and Applied Genetics* 110:1210 - 1217.

Hopf, M. 1986. Archaeological evidence of the spread and use of some members of the Leguminosae family. pp. 35-60. In: *The origin and domestication of cultivated plants*. Barigozzi, C. (Ed.). Elsevier, Oxford, New York and Tokyo.

Jensen, L.B., Holm, B.B. and Lübberstedt T. 2007. Cross-species amplification of 105 *Lolium perenne* SSR loci in 23 species within the Poaceae. *Molecular Ecology Notes* 7:1155-1161.

McCutchan, J.S. 2003. A brief history of grass pea and its use in crop improvement. *Lathyrus Lathyrism News letter* 3:19-23.

Peakall, R., Gilmore, S., Keys, W., Morgante, M. and Rafalski, A. 1998. Cross-Species amplification of soybean *Glycine max* Simple Sequence Repeats SSRs within the genus and other legume genera: Implications for the transferability of SSRs in plants. *Molecular Biology and Evolution* 15:1275-1287.

Phan, H.T.T., Ellwood, S.R., Hane, J.K., Ford, F., Materne, M. and Oliver, R.P. 2006. Extensive macrosynteny between *Medicago truncatula* and *Lens culinaris* ssp. *Culinaris. Theoretical and Applied Genetics* 114:549-558.

Rouf Mian, M.A., Saha, M.C., Hopkins, A.A. and Warnke, S.E. 2005. Use of tall fescue EST-SSR markers in phylogenetic analysis of cool-season forage grasses. *Genome* 48:637-647.

Rozen, S. and Skaletsky, H. 1998. Primer3. Code available at: http://www-genome.wi.mit.edu/genome_software/other/primer3.html.

Scott, K.D., Eggler, P., Seaton, G., Rosetto, M., Ablett, E.M., Lee, L.S. and Henry, R.J. 2000. Analysis of SSRs derived from grape ESTs. *Theoretical and Applied Genetics* 100:723-726.

Shiferaw, E., Pe', M.E., Porceddu, E. and Ponnaiah, M. 2012. Exploring the genetic diversity of Ethiopian grass pea (*Lathyrus sativus* L.) using EST-SSR markers. *Molecular Breeding* 30(2):789-797.

shiferaw e [PubMed] [Google Scholar] pè me [PubMed] [Google Scholar] porceddu e [PubMed] [Google Scholar]

Skiba, B., Ford, R. and Pang, E.C.K. 2003. Amplification and detection of polymorphic Sequence- Tagged Sites in *Lathyrus sativus*. *Plant Molecular Biology Reporter* 21:391-404.

Skiba, B., Ford, R., and Pang, E.C.K. 2004. Construction of a linkage map based on a *Lathyrus sativus* backcross population and preliminary investigation of QTLs associated with resistance to Ascochyta blight. *Theoretical and Applied Genetics* 109:1726-1735.

Steele, K.P. and Wojciechowski, M.F. 2003. Phylogenetic analyses of tribes Trifolieae and Vicieae, based on sequences of the plastid gene, *mat*K Papilionoideae: Leguminosae. pp. 355-370. In: Advances in legume systematics, part 10, Higher level systematics. Klitgaard, B.B. and Bruneau, A. (Eds.). Royal Botanic Gardens, Kew.

Varshney, R.K., Sigmund, R., Borner, A., Korzun, V., Stein, N., Sorrells, M.E. and Langridge, P. and Graner, A. 2005. Interspecific transferability and comparative mapping of barley EST-SSR markers in wheat, rye and rice. *Plant Science* 168:195-202.

Varshney, R.K., Close, T.J., Singh, N.K., Hoisington, D.A. and Cook, D.R. 2009. Orphan legume crops enter the genomics era! *Current Opinion in Plant Biology* 12:202 -210.

You, F.M., Huo, N., Gu, Y.Q., Luo, M.C., Ma, Y., Hane, D., Lazo, G.R., Dvorak, J. and Anderson, O.D. 2008. BatchPrimer3: A high throughput web application for PCR and sequencing primer design. *BMC Bioinformatics* 9:253.

Zhang, L.Y., Bernard, M., Ravel, C., Balfourier, F., Leroy, P., Feuillet, C. and Sourdille, P. 2007. Wheat EST-SSRs for tracing chromosome segments from a wide range of grass species. *Plant Breeding* 126:251-258.