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Characterization of novel soybean derived simple sequence repeat markers and their transferability in hyacinth bean [*Lablab purpureus* (L.) Sweet]

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Abstract

Simple Sequence Repeat (SSR) markers were developed from soybean expressed sequence tag (EST) sequences obtained from The European Molecular Biology Laboratory (EMBL) database. Of the 1517 loci considered 764 (50.4%) and 573 (37.8%) constituted tri and dinucleotide and, 73 (4.8%), 44 (2.9%), 38 (2.5%) and 25 (1.6%) represented mono, hexa, tetra and pentanucleotide repeats, respectively. Fifty of these SSR makers exhibited 100% transferability in *Lablab purpureus*. Eight out of 50 loci (16%) displayed length polymorphisms. BLAST results showed that the function of only 15 EST sequences (30%) of the selected 50 SSR sequences were known. The novel EST-SSR will be useful for developing new molecular markers of *L. purpureus*.

Key words: Soybean; *Lablab purpureus*; EST-SSR; transferability

Introduction

The hyacinth bean (*Lablab purpureus*) is widely distributed in China, South-East Asia, India, Australia and eastern areas of Africa [1]. It has a strong capacity for nitrogen fixation and large biomass, and has been used as food resource for more than 3500 years. In Australia and America, it plays an important role as fodder and as green manure in mixed crop-livestock systems [2, 3]. *L. purpureus* possesses tolerance to drought and salinity stress [1, 4]. However, information related to its genome is very poor compared with other leguminous crops such as *Glycine max* and *Medicago sativa*. Recently, the genetic map of *L. purpureus* is still being constructed using amplified fragment length polymorphism (AFLP), restriction fragment length polymorphism (RFLP) and random amplification polymorphic DNA (RAPD) markers [5-8].

Development of new markers using whole genome sequences and expressed sequence tag (ESTs) has been extensively applied in recent years. Microsatellites or simple sequence repeats (SSRs) possess favorable genetic attributes including hypervariability, multi-allelic nature, co-dominant inheritance, reproducibility, and are extensively distributed in genomes and ESTs [9]. A vast amount of publicly available ESTs can be mined to develop SSR as genetic markers. Those markers are derived from transcripts, and can be easily used for assaying the functional diversity of related species because of higher level of transferability between related species [10, 11]. EST databases are an important source of candidate genes, and EST-derived SSR markers are not only effectively applied in mapping of useful genes, construction of linkage maps, marker assisted selections and backcrosses *per se*, they may also be transferable between closely related genera [12].

Till now, approximately 1000 EST-SSR markers have been developed [13, 14]. These markers were mainly generated from non-redundant ESTs of soybean and were derived from over 50 different cDNA libraries available in public databases. These markers, however, have not yet been used to analyze the hyacinth bean [15]. The paucity of EST expression databases for *L. purpureus* makes it difficult to develop SSR markers for this species. Based on the rich information that is

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available in soybean EST databases, we here exploited novel soybean EST-derived SSR markers and analyzed their transferability to, and polymorphism in, *L. purpureus*. These data would provide a basis for the construction of high-density genetic maps for *L. purpureus*, for gene mapping and molecular marker-assisted breeding.

Materials and methods

Acquisition of soybean EST data

Plant EST sequences were downloaded from The European Molecular Biology Laboratory (EMBL) database (updated March 13, 2009) of the European Bioinformatics Institute (EBI) (ftp://ftp.ebi.ac.uk/pub/dababases/embl/). Soybean EST sequences were filtered according to species source. A total of 1296222 soybean EST sequences were collected and the quality of sequences (uncertain bases were less than 3%, and the sequence length was more than 100 bp) was confirmed with seqclean (http://compbio.dfci.harvard.edu/tgi/software/). Sequences that did not match these quality criteria were excluded.

Cluster analysis of EST

Cluster analysis of EST was performed with TGICL (TGI Clustering tools) [16]. In order to explore the polymorphism of the SSRs, sequence similarity was set to more than 94% and the overlap was more than 100 bp.

EST-SSR polymorphism screening

Sequence cluster composed of 2 to 5000 sequences in EST cluster analysis were screened by a modified sputnik analysis for SSRs with 1 to 6 base repeats. The detection threshold was set to a repeat sequence length of more than 8 bp and compared to complete repeat sequences of not more than 10% variation. Considering that poly(A) tails may be present in EST sequences, only C/G repeat sequences were tested. SSR cluster fulfilling the above criteria were tested for Indels (insert/delete mutation), and length polymorphism markers were developed in the region with Indel mutations using SSR sequence alignments. The processes described above were carried out by use of programs written with perl script.

SSR primer design

Primers for polymorphic SSRs were designed using the software 'primer3' (http://primer3.sourceforge.net/) to conserved sequence stretches of ESTs. The length of the primers ranged from 18 to 25 bp, and the size of the amplified products ranged from 100 to 500 bp. All primers were synthesized by Shanghai Biotech Co. Ltd. (Shanghai, China).

Plant material and DNA extraction

The transferability of the newly developed soybean EST-SSR molecular markers was investigated in two varieties of *L. purpureus,* Nanhui 23 and Meidou 2012, with different agronomic characters, and their F_2 population. Nanhui 23 and Meidou 2012 were obtained from Nanhui District of Shanghai, and Henan province, China, respectively. The DNA of young leaves of these two varieties was extracted using the hexadecyl trimethyl ammonium bromide (CTAB) method.

PCR amplification

A total of 50 primer pairs for the novel SSR markers were randomly selected; their suitability and potential to detect polymorphisms in *L. purpureus* were investigated. PCR assays were performed by using PCR amplifier 2720 Thermal cycler (Applied Biosystems, Foster City, CA, USA). Ten µl reactions were used consisting of 30 ng of DNA, 1.5 mM Mg^{2+}, 0.15 mM dNTPs, 0.4 µM primers, 1 U *Taq* polymerase (Takara, Shiga, Japan). The PCR conditions were as follows: a denaturation step at 95°C for 2 minutes (min) was followed by 33 cycles of denaturation at 95°C for 1 min, annealing at 60°C for 1 min, extension at 68°C for 1 min. Following a final extension step at 68°C for 10 min, the samples were kept at 4°C. PCR products were examined by electrophoresis with 6% PAGE. DNA of the two varieties, Nanhui 23 and Meidou 2012, and their F_2 population, was used as template in the PCR reactions.

Results and discussion

A total of 63791 EST clusters were obtained from 1296222 soybean ESTs, each cluster consisting of at least two EST sequences. A 75402 SSR sites were detected within the sequences obtained by EST cluster analysis, each of these consisted 1.18 SSR on average. A total of 1517 loci were detected using alignments of SSRs which contained length polymorphisms as a result of Indel mutations. Out of all these loci, 764 (50.4%) and 573 (37.8%) were trinucleotide and dinucleotide repeats respectively, while 73 (4.8%), 44 (2.9%), 38 (2.5%) and 25 (1.6%) represented mononucleotide, hexanucleotide, tetranucleotide and pentanucleotide repeats, respectively.

Table 1. SSR primers designed from EST sequences of *G. max*

Locus name	Primer sequences(5'→3')	SSRmotif	Expected size in *G. max* (bp)	Tm (^{o}C)	Amplicon size in *L. purpurues*
Gm000011	F:GAAACAGCACATGCTGAGGA R:CACCACAATCATGCATCTCC	(AGGAAA)4	324	59	280-350
Gm000097	F:AGAGGTACAGGCTGAAGGCA R:GGGAGCACCGAAAAGTTGTA	(TG)7	261	60	250
Gm000182	F:CTGCTTCCGCTGGATTAAAG R:GGTGGGCTTCACGAAATCTA	(CCCAAA)7	314	59	300
Gm000195	F:TAAATCCGAAAACCTCGTCG R:CCGTTACCAACAAAGGCTGT	(CT)6	462	60	500-350
*Gm000240	F:CTTCACAGAGAGAGGTGCCC R:CTATTGGGTGGAAGGGTTGA	(TC)5	171	59	150-160
Gm000264	F:TTATCTCTTTGGCAGTGGGG R:CAAGCCACACCAACATTGTC	(CCAGCA)6	456	60	450-500
Gm000272	F:TAATTGGTGGAAGCCAAAGG R:CCAGCATCAAAGTGGAGGAT	(TG)4	458	59	450-500
Gm000288	F:GAGCAGGTGTGTGCAAGTGT R:GCAAGAATAAGGGGAGGGAG	(CT)12	416	59	450-500
*Gm000332	F:GAAACTTGGGCAACAGGAAA R:AGTTCGCTTCAGACCCAAGA	(AG)7	151	60	150
Gm000352	F:TGCAAGAAGCAAGTAATCCCT R:CTCCACCACTCTGCTCTTCC	(AT)16	177	58	200-250
Gm000403	F:CAAGACCACACTGCTCTCCA R:AGACGCAACTGATTCAGGAAA	(AT)8	185	60	150
Gm000425	F:GGTTGCACCAGGAAGACATT R:AATGTATGGTCCCATCCCAA	(AT)4	318	59	300
Gm000448	F:GAAGTCTGGAAAGACCAGCG R:ACAATTGAGGATTCAACGCC	(GA)9	113	59	150-200
*Gm000499	F:GGAAGAGCTGAGAGGGGAGT R:CCAGATCTGAGAACCCCAAA	(AG)5	124	59	150
Gm000534	F:TGGAAAACGGAAGGAAGATG R:AGCACCCTTCTTCTTGAGCA	(AG)4	328	60	450-300
Gm000539	F:AACGAGAATCCCCCTCCTTA R:GTTCGTCGGTGGACATTTCT	(TC)19	435	59	400
Gm000587	F:TGACTGGATTACACAAGGACCA R:GGAAATGACGGAAACGAAGA	(AG)7	209	60	200-300
Gm000625	F:TACTTTGCCCAATGATGCAC R:GCAGGGTCATCCAATCTAGC	(TC)10	481	59	400-550
*Gm000659	F:GATCATGGGCCAGCTTAAAA R:AAACTGCTATGGGACCTCGT	(GA)9	242	60	245
*Gm000664	F:GGTGCTGTTCGTGCTGTTAC R:ACCGTCACAAAGCAAAAAGG	(TG)7	461	59	470
Gm000724	F:GACAATGGGTCCGAGAAGAA R:TGTGTGTGCAACTTGACCTTT	(GA)5	220	60	200-250
Gm000740	F:AGCGATGCAATTATTCCTGG R:AGGGTGATAGCCACCACAAG	(CT)18	389	60	400
*Gm000742	F:CTTCACAGAGAGAGGTGCCC R:CTATTGGGTGGAAGGGTTGA	(TC)5	481	59	460
Gm000857	F:CGGAATGCAATCAAAAAGGT R:AAAGCCACAAAGCAGCTATCA	(TG)4	396	59	300-400

Gm000904	F:TGCATTGGAAGCTATAGGGG R:TTTTCCGACATGCATAAACG	(AT)7	141	60	150
Gm000971	F:CTTGTCTTCGCAAGAGGGTC R:GCTCAGACCTGAAACCATCA	(AT)7	261	59	250-350
Gm000982	F:CCTAGCTCTGTCGTTCCGTC R:AGCGTCTCCATTCCATTGAC	(TC)6	232	60	250-300
Gm001010	F:AGCAATGTGTGATGGTGGAA R:TCGCTTTAAGCAAAATGATACAGA	(CA)8	126	59	150
Gm001011	F:GCTGGAGCAGATCCTAATGC R:GCTTACAAGCCAAGAGCACC	(CT)4	393	59	400-500
Gm001050	F:AGTATAAAGCCGGCATCGTG R:AGAGGTTGAGGTGCGTCTGT	(CG)5	291	60	300
Gm001064	F:TGCTTGTGTCAAGATGCTTTG R:TGTGCAGAGGTGGTTGTAGC	(TA)18	390	60	400
Gm001105	F:GTAGGTGCTGCCCAAAACAT R:TGTGTGCCCCTGCTACAATA	(AT)23	203	60	250-300
Gm001130	F:AGGAAGAGTGGGTGTTGGTG R:AGTTGGAGGTGAAATCGTGG	(ACCCTA)4	223	60	200
Gm001143	F:AACCATGCCTCTGCCAATAC R:CGTGAGATGAGACCACACCA	(AT)8	369	59	400
Gm001152	F:TTAGGGCAGGGATTGATGAG R:GGCCATAATTGATTTTGCAG	(TG)5	334	60	300-350
Gm001156	F:CCCTCAAACTCCATTTCACTC R:CAAGAAAAACTCTGGCTCCG	(AG)5	450	58	400-500
Gm001161	F:ATCAGATCAGAATCCCACCG R:TGTGAAATCTCTGCCAGCAC	(AG)4	180	59	200-250
*Gm001168	F:TGTGGTCCGATTGTTTGCTA R:ACACCAAGCTCGAAAACCAC	(TC)16	240	60	210
Gm001180	F:CTCACTCCCACAATTCCCAC R:CAACGCTTGAAAAGAAAGGC	(AC)11	438	60	500-550
Gm001187	F:CGGAAAGCTTGTCTCCTACG R:TTAGCAATAAAGCCGCACAA	(AT)11	417	60	400
Gm001197	F:AGGGAGTAGCGACGAACTCA R:GAAACGGACTTTTCTCAGCG	(GA)6	481	60	450-500
Gm001236	F:ACGTTGAGGCTCGAGAACAT R:CACGCCATATGAGTGTGAGG	(AT)12	489	59	500
Gm001243	F:GAGGGTGGTGCAATACTCGT R:TTCCAAGCTCAAACTCAAATCA	(AT)19	380	59	400
Gm001328	F:GTGGGGAGGCTGCTGTATTA R:CGATGGAAACCTGAACGAAT	(GACCAT)6	408	60	400-450
Gm001360	F:TGAAGCTTCGGTCTTGTGTG R:CGAGAAGAAACACTCCTCGG	(CA)4	482	60	500
*Gm001362	F:ATCCACCGGTGTTGTGGTAT R:GGTGGATCAAATGGTTGGAC	(AG)5	269	59	550
Gm001364	F:CAACACAAAGCTCCCACCTT R:CACCGTAGATCTTGCCCAAT	(GA)13	407	60	400
Gm001416	F:CACGAAATGAAACCTCCTCC R:AGGCTTTTCTGCTGCATTGT	(TC)5	276	59	250-300
Gm001444	F:AATTGGGAAGCATCATCAGC R:TTGTCTTTATGCAAGGAAAAGTTG	(TA)5	200	60	200
Gm001471	F:TTTTTCAAGCTCCACCATCC CCAATCCCTCTTCCTCTTCC	(TC)10	464	60	450-500

* = The loci contained length polymorphisms in *L. purpureus*

The most common repeat type in EST-SSR are trinucleotide repeats (TNRs), which were 54%-78% followed by dinucleotide repeats (DNRs), as reported, in wheat and rice [17, 18].

Primers were designed for 50 randomly selected EST-SSR showing length polymorphisms, and their ability to amplify the regions in Hanhui 23, Meidou 2012 and a F_2 population was tested. The results of PCR experiments showed that all 50 primer pairs generated amplification products in soybean and field bean, Nanhui 23, Meidou 2012 and their F_2 population (Fig. 1). This demonstrated that the transferability was 100%, and 8 out of 50 loci (16%) contained length polymorphisms. All primers revealed clear bands in Nanhui 23 and Meidou 2012 although some displayed multi-bands in soybean than expected. The results also demonstrated that some of the EST-SSR in *L. purpureus* showed the distinct amplicons but differed in length between *L. purpureus* and soybean (Table 1).

Transferability of SSR markers across taxa has been successfully demonstrated in many species [19, 20]. Although both genic and genomic SSR markers can be transferred across species, genic SSR markers are expected to have a higher transfer rate among related species due to conservation of transcribed regions. The transfer rate of a given marker will correspond to the phylogenetic distances and the extent of sequence conservation between species. Comparative mapping of transferable SSR markers from related species will help to get a genetic map in minor species and better information on chromosomal regions with fewer markers. In addition, SSR marker from a gene with known function can be used for identification and cloning of homologous genes in related species [10].

In order to explore the potential practicability of transferable EST-SSR, the 50 SSR sequences with transferability in *L. purpureus* were used to performed homology comparison by BLAST in GenBank to find out the putative functions. The BLAST results showed

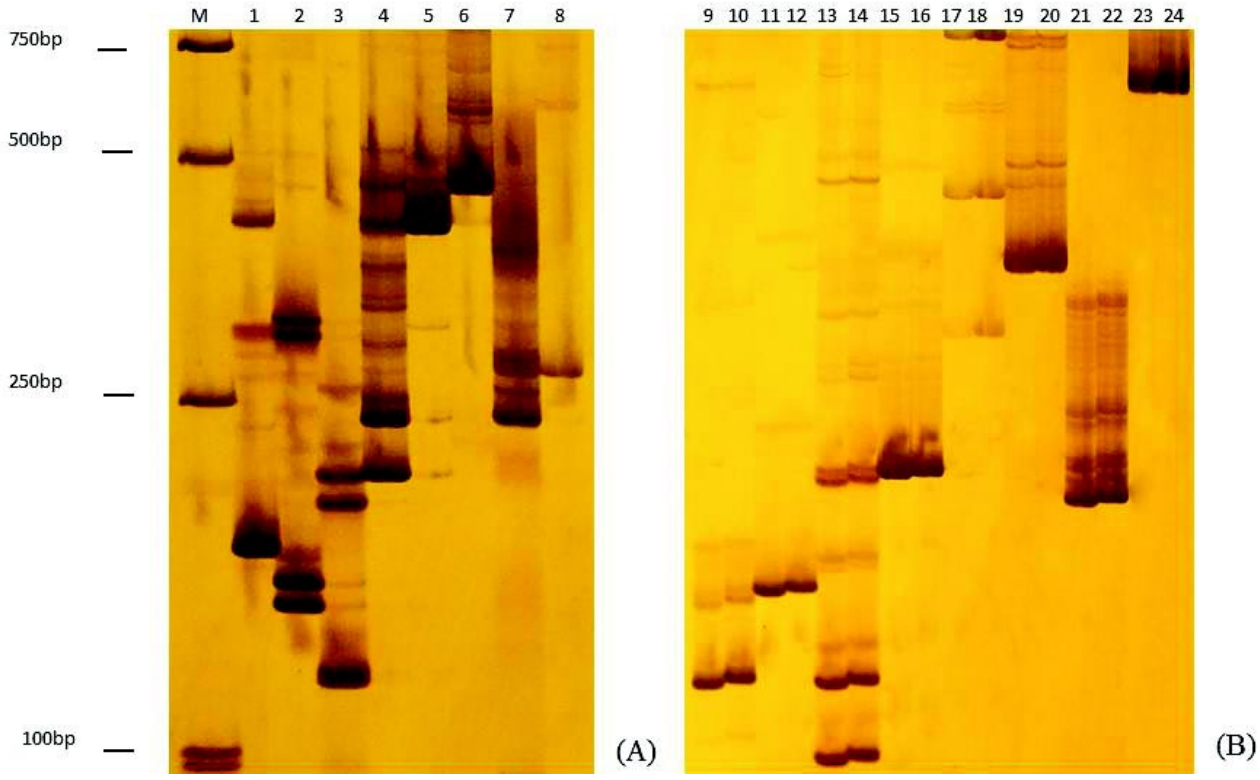

Fig. 1A, B. The amplicon in soybean (A) and field bean (B) used by developing SSR primers.
M: marker, Lane 1-8: SSR loci Gm000240, Gm000332, Gm000499, Gm000659, Gm000664, Gm000742, Gm001168, Gm001362 in soybean. Lane 9, 11, 13, 15, 17, 19, 21, 23: SSR loci Gm000240, Gm000332, Gm000499, Gm000659, Gm000664, Gm000742, Gm001168, Gm001362 in Meidou 2012. Lane 10, 12, 14, 16, 18, 20, 22, 24: SSR loci Gm000240, Gm000332, Gm000499, Gm000659, Gm000664, Gm000742, Gm001168, Gm001362 in Nanhui 23.

Table 2. Putative function of the 50 EST-SSR markers developed in the present study

Locus name	Top e-value	Putative function
Gm000011	1.00E-35	unknown [*Glycine max*]
Gm000097	0	unknown [*G. max*]
Gm000182	3.00E-71	WRKY42 [*G. max*]
Gm000195	7.00E-69	RNA helicase [*Vigna radiata*]
Gm000240	2.00E-37	hypothetical protein [*Phaseolus vulgaris*]
Gm000264	1.00E-69	unknown [*G. max*]
Gm000272	9.00E-151	aspartyl-tRNA synthetase, putative [*Ricinus communis*]
Gm000288	3.00E-48	predicted protein [*Populus trichocarpa*]
Gm000332	1.00E-35	hypothetical protein [*Vitis vinifera*]
Gm000352	1.00E-38	unknown [*G. max*]
Gm000403	1.00E-136	agamous-like 1 protein [*G. max*]
Gm000425	2.00E-64	5-enolpyruvylshikimate-3-phosphate synthase [*Gossypium hirsutum*]
Gm000448	2.00E-150	unknown [*G. max*]
Gm000499	3.00E-117	unknown [*G. max*]
Gm000534	2.00E-115	ATP binding protein, putative [*R. communis*]
Gm000539	2.00E-25	unknown [*G. max*]
Gm000587	—	No homology
Gm000625	—	No homology
Gm000659	3.6	hypothetical protein bthur0009_6110 [*Bacillus thuringiensis* serovar andalousiensis BGSC 4AW1]
Gm000664	3.00E-35	unnamed protein product [*Vitis vinifera*]
Gm000724	2.00E-77	unnamed protein product [*V. vinifera*]
Gm000740	6.00E-53	unknown [*G. max*]
Gm000742	6.00E-130	unknown [*G. max*]
Gm000857	0	unknown [*G. max*]
Gm000904	0	carbonic anhydrase [*Phaseolus vulgaris*]
Gm000971	3.00E-138	ATP-binding cassette transporter, putative [*R. communis*]
Gm000982	0	calreticulin-1 [*G. max*]
Gm001010	6.00E-163	unknown [*G. max*]
Gm001011	0	unknown [*G. max*]
Gm001050	2.00E-144	pollen allergen-like protein [*Pisum sativum*]
Gm001064	3.00E-42	unknown [*G. max*]
Gm001105	3.00E-46	unknown [*G. max*]
Gm001130	0	unknown [*G. max*]
Gm001143	1.00E-52	unknown [*G. max*]
Gm001152	3.00E-134	Pectinesterase precursor, putative [*R. communis*]
Gm001156	7.00E-102	unknown protein [*G. max*]
Gm001161	2.00E-38	unknown [*G. max*]
Gm001168	7.00E-46	hypothetical protein [*V. vinifera*]
Gm001180	1.00E-160	homeodomain-leucine zipper protein 57 [*G. max*]
Gm001187	5.00E-104	unknown [*G. max*]

Gm001197	3.00E-41	predicted protein [*Populus trichocarpa*]
Gm001236	2.00E-69	unknown [*G. max*]
Gm001243	4.00E-16	hypothetical protein [*V. vinifera*]
Gm001328	7.00E-126	t-snare [*Medicago truncatula*]
Gm001360	0.012	GL22562 [*Drosophila persimilis*]
Gm001362	5.00E-13	hypothetical protein [*V. vinifera*]
Gm001364	2.00E-75	predicted protein [*Populus trichocarpa*]
Gm001416	4.00E-135	unknown [*G. max*]
Gm001444	5.00E-50	unknown [Glycine max]
Gm001471	2.00E-05	hypothetical protein [*V. vinifera*].

that the function of 15 EST sequences (30%) were known (Table 2), while the function of the remaining 35 EST sequences (70%) were unknown.

Enriching genome information of a species by resorting to existing or newly developed molecular markers from other species can not only accelerate research progress, but it can also confirm the transferability of a certain marker between different species. Studies on the transferability of EST-SSR from *Phaseolus vulgaris* to other leguminous crops demonstrated that 82% of markers are transferable in at least one kind of leguminous crops [21]. Reports on the transferability of SSR marker derived from wheat, corn and sorghum to *Paspalum vaginatum* indicated that 67.5%, 49.0% and 66.8% of markers, respectively, have transferability while polymorphisms noted in *P. vaginatum* reached 51.5% [22]. The degree of transferability of SSR markers developed in one species to another species is possibly related to the genetic relationship among species. A recent study has shown that the transferability of *Medicago truncatula* and non-leguminous crops SSR markers are 53-71% and 33-44%, respectively [23]. This study showed that the transferability of soybean EST-SSR to *L. purpureus* was 100%. This is probably attributable to the fact that both, soybean and *L. purpureus,* belong to the leguminous crop family, and that they have a high degree of homology. Those developed EST-SSR markers would be useful for molecular approaches to breed new varieties of *L. purpureus*.

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