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Cross-species Amplification of Common Bean (*Phaseolus vulgaris*) EST-SSRs within Hyacinth Bean, Pea and Soybean

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ABSTRACT

Background: The emerging expressed sequence tag-derived simple sequence repeats (EST-SSRs) offer an important approach to investigate plant genetic diversity.

Methods: A total of seventy common bean polymorphic EST-SSRs were utilized for assessing genetic diversity among 19 hyacinth, 20 pea and 21 soybean accessions, respectively. The genetic statistics and principal coordinates analysis (PCoA) were conducted by GenAlEx 6.5.

Result: The transferability rates of common bean EST-SSRs in hyacinth, pea and soybean were 27.1%, 20.0% and 21.4%. And the ratios of polymorphic SSR markers in these legumes were 42.1%, 85.71% and 100.0%, respectively. The hyacinth, pea and soybean accessions could be assigned to three distinct clusters for the germplasm types greatly depending on the geographic distributions. The present results revealed that the common bean EST-SSRs are highly transferable to hyacinth bean, pea and soybean. Moreover, these transferable markers would provide a set of inexpensive and effective tools for future research on molecular breeding, taxonomy and comparative mapping.

Key words: Common bean, EST-SSRs, Genetic diversity, Hyacinth bean, Pea, Soybean, Transferability.

INTRODUCTION

Legumes crops, including soybean, pea, hyacinth bean, common bean and cowpea, are the important food and cash crops worldwide (Singh, 2005). However, the possible worth of these crops is not comprehensively adopted in genetic breeding yet, which is due to the limited research on the genetic relationship between germplasm resources. In this regard, research on the genetics excavation and utilization in legume crops is crucial to the development of their production.

With the development of molecular biology research, the molecular markers, including restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), simple sequence repeats (SSR) and single nucleotide polymorphism (SNP), have been identified as the efficient tools in germplasm identification and plant breeding (Kour *et al*., 2011). Among them, the EST-SSR is frequently applied in variety identification and genetic diversity analysis based on high-throughput sequencing. The SSR markers were regarded as the molecular markers with the highest potency due to their advantages of abundant information, reproducibility, effectiveness, simplicity, easy detection through polymerase chain reaction (PCR) and co-dominant inheritance. Nonetheless, it is time- and money-consuming to develop conventional SSRs on the basis of the genomic DNA library (Ellis and Burke, 2007). Besides, its poor transferability also results in the limited application of those tightly associated varieties (Ma *et al*., 2009). According to our previous study, the transferability of SSR markers were less than 30% between the legume family, which significantly restricts cross-species application within the legume family (Gong *et al*., 2010).

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Expressed sequence tags (ESTs) represent the short and unedited sequences gained through single-pass sequencing of the cDNA library-derived cDNA clones. Due to the high level of transferability in EST-SSRs, developing SSR by retrieving related species in database of EST (dbEST) is an effective method for species with no available EST database. However, there is little research regarding the transferability level for EST-SSRs (ranging from the extensively investigated crops to species with distant relation). Thus, it is necessary to understand whether these EST-SSRs markers play roles as the new markers for the rarely investigated crops only, or also offer new information to carry out comparative mapping between related crops. Various researchers have used ESTs for developing the SSR markers of many pulse crops. Zhang *et al*., (2013) developed 22 EST-SSRs based on 459 ESTs of hyacinth bean.

As suggested by phylogenetic tree analysis, EST-SSRs were successfully used for assessing the structure and genetic diversity in a population across hyacinth bean local varieties. In addition, 15 novel markers of pea EST-SSR were also established based on the public ESTs and the target loci among a total of seven *Pisum sativum subsp. sativum* accessions were amplified successfully (Teshome *et al*., 2015).

This study reported the processes for developing and characterizing 70 EST-SSRs from the common bean as well as the cross-species transferability of EST-SSRs using hyacinth bean, pea together with soybean. These EST-SSRs will add novel markers to hyacinth bean, pea and soybean and might be helpful to investigate the population-based genetic diversity within hyacinth bean, pea and soybean.

MATERIALS AND METHODS

Nineteen hyacinth bean (*Lablab purpureus* L.), 20 pea (*Pisum sativum* L.) and 21 soybean (*Glycine max* L.) landraces were used in the study. Leaves were collected from all individuals at the four-leaf stage to extract the total genomic DNA with the CTAB method. Scanning UV spectroscopy was applied to determine DNA concentration. The experiment was carried out at Zhejiang Academy of Agricultural Science in 2019.

Seventy pairs of EST-SSR primers in common bean were obtained as described previously (Xu *et al*., 2014). The GenAlEx 6.5 was employed to calculate genetic statistics, such as polymorphic observed heterozygosity (H_O), expected heterozygosity (H_E) and allele number (N). Meanwhile, the formula put forward by Anderson *et al*., (1993) was utilized to calculate the polymorphism information content (PIC). The GenAlEx 6.5 program was used to determine the inbreeding index (Fis) and allele frequency of a population. For inferring genetic diversity distribution, PCoA was conducted by GenAlEx 6.5. The species tree of 10 legume family members including hyacinth bean, pea, common bean, soybean, broad bean (*Viciafaba*), cowpea (*Vigna unguiculate*), adzuki bean (*Vigna angularis*), sword bean (*Canavalia gladiate*), groundnut (*Arachis hypogaea*) and pigeon pea (*Cajanus cajan*), was got from Open Tree of Life (https://opentreeoflife.github.io/). *Vitis vinifera* was used as the outgroup.

RESULTS AND DISCUSSION

Transferability of common bean EST-SSRs in hyacinth bean, pea and soybean

In hyacinth bean, 19 (27.14%) of the 70 EST-SSRs were functional, among which, 8 (42.11%) EST-SSRs displayed polymorphism. In addition, fourteen (20.00%) among the 70 EST-SSRs were functional in pea, with 12 (85.71%) primer pairs showing polymorphism. Fifteen (21.43%) EST-SSRs were functional and all of them showed clear polymorphic patterns in soybean (Fig 1A).

Among the 70 EST-SSRs, 40 loci yielded fragments from one or more species. However, among those40 functional primers, only 5 displayed transferability to two among those three tested legume species. And successful amplification of fragment was only observed in 2 EST-SSRs among those three tested legume species (Fig 1B).

Polymorphism of common bean EST-SSRs in hyacinth bean, pea and soybean

Among the 8 polymorphic EST-SSRs, altogether 19 alleles were discovered among the 20 hyacinth bean landraces. In addition, the values of H_O and H_E were 0.000-0.350 and 0.051-0.710, separately, whereas the PIC value was 0.046-0.658 (mean, 0.232) (Table 1). Among the 12 polymorphic EST-SSRs, altogether 33 alleles were identified among the 20 pea landraces. The value of H_O was 0.000-0.870 (mean, 0.239) and that of H_E was 0.087-0.662 (mean, 0.353). The value of PIC was between 0.082 (PV019 and PV062) and 0.601 (PV061) (mean, 0.313) (Table 2). In soybean, 15 EST-SSRs exhibited polymorphism and totally 85 alleles (mean, 5.7) were identified. The value of PIC was 0.136-0.835 (mean, 0.576), while the value of H_O was 0.000-0.500 (mean, 0.175) and the value of H_E was 0.141-0.852 (mean, 0.615) (Table 3).

Application of common bean EST-SSRs on genetic diversity analysis of hyacinth bean, pea and soybean

PCoA was conducted based on the EST-SSR data in three legume species. The first and second PCoA components in

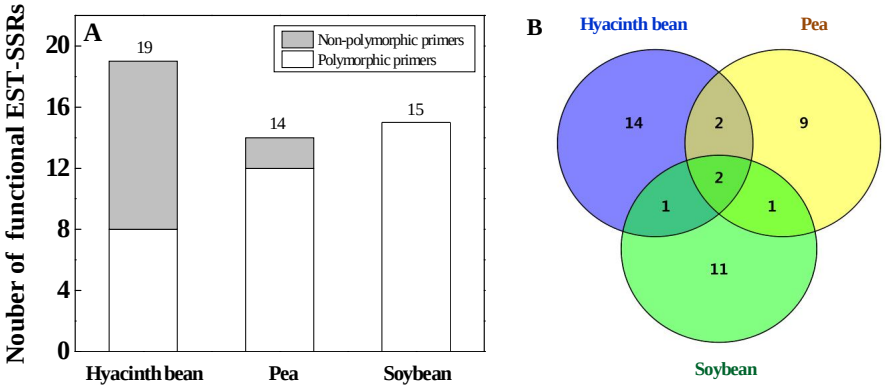

Fig 1: The PCR amplification of 70 pairs of common bean EST-SSR primers in hyacinth bean, pea and soybean (A) and Venn diagram of working EST-SSR primers between hyacinth bean, pea and soybean (B).

hyacinth bean took up 37.14% and 19.30%, respectively, of total variations, revealing three diverse clusters with regard to germplasm types among the 21 hyacinth bean landraces (Fig 3). Meanwhile, the first and second PCoA components in pea occupied 29.34% and 19.01%, respectively, of total variations. Those 20 pea landraces showed the highest distributions among three areas in the plot, which were consistent with the respective distribution in natural regions. The first and second PCoA components in soybean took up 22.71% and 20.14%, respectively, of total variations. Those 21 soybean landraces were classified into three diverse clusters in terms of germplasm types (Fig 3).

Table 1: Characteristics of the 8 EST-SSR markers and the diversity detected in the 19 hyacinth bean landraces.

	Na	H_O	H_E	Pis
PV-09	3	0.350	0.464	0.425
PV-017	2	0.000	0.455	0.351
PV-027	2	0.000	0.180	0.164
PV-030	2	0.000	0.180	0.164
PV-033	2	0.042	0.046	0.046
PV-037	2	0.000	0.111	0.105
PV-042	2	0.045	0.051	0.049
PV-064	4	0.000	0.710	0.658
Mean	2.4	0.049	0.259	0.232

Table 2: Characteristics of the 12 EST-SSR markers and the diversity detected in the 20 pea landraces.

	Na	H_O	H_E	Pis
PV-01	3	0.286	0.357	0.325
PV-011	4	0.083	0.476	0.453
PV-017	2	0.870	0.491	0.371
PV-019	2	0.091	0.087	0.082
PV-024	2	0.526	0.465	0.357
PV-037	3	0.421	0.555	0.469
PV-049	3	0.167	0.559	0.470
PV-058	3	0.053	0.234	0.217
PV-059	2	0.552	0.454	0.351
PV-061	5	0.056	0.662	0.601
PV-062	2	0.000	0.062	0.082
PV-064	2	0.000	0.087	0.194
Mean	2.8	0.239	0.353	0.313

Table 3: Characteristics of the 15 EST-SSR markers and the diversity detected in the 21 soybean landraces.

	Na	H_O	H_E	Pis
PV-02	5	0.063	0.580	0.512
PV-03	5	0.059	0.358	0.344
PV-05	8	0.267	0.818	0.798
PV-06	9	0.357	0.852	0.835
PV-011	6	0.235	0.811	0.784
PV-017	8	0.211	0.659	0.637
PV-018	3	0.000	0.553	0.472
PV-022	5	0.500	0.742	0.698
PV-024	8	0.350	0.735	0.696
PV-025	3	0.000	0.405	0.368
PV-029	4	0.333	0.471	0.392
PV-032	5	0.200	0.711	0.661
PV-041	8	0.000	0.792	0.760
PV-042	4	0.000	0.598	0.551
PV-046	4	0.050	0.141	0.136
Mean	5.7	0.175	0.615	0.576

The EST-SSR markers are widely used for genetic research of economic crops, such as, sweet potato (Wang *et al*., 2011), maize (Galvao *et al*., 2015) and soybeans (He *et al*., 2015). However, mining for the EST-SSR markers is restricted to plants with genome databases and this limits EST-SSR marker mining and use for species with currently known genomic information (Wang *et al*., 2013). Some study has been carried out on EST-SSR transferability among legumes crops. Dutta *et al*., (2011) developed numerous functional EST-SSR markers based on those assembled transcriptome sequences for pigeon pea. In the present study, altogether 70 EST-SSR markers were screened from common bean, which were identified as the efficient markers of hyacinth bean, pea and soybean. Among these EST-SSR markers, a total of 19 loci were identified to be functional within hyacinth bean. Additionally, in pea and soybeans, we obtained 14 and 15 primer pairs that had clear amplification products, respectively. Among the 70 EST-SSRs, 40 loci yielded fragments from one or more species. It was suggested that the EST-SSRs of common bean can be highly transferable into hyacinth bean, pea as well as soybean, which may serve as the possible source to develop these legume species EST-SSR markers.

The amplified SSRs number within a specific variety is found to show positive correlation with the phylogenetic relatedness for that specific variety, as well as the variety to design marker (Pan *et al*., 2018). Thus, we explored the phylogenetic relationships among 10 common cash crops included in the legume family. As we expected, the common bean and hyacinth bean showed the closest relationship in ten tested species, the soybean was identified as the sister group of hyacinth bean and common bean, they all belonged to Phaseoleae. While *Pisum sativum* and *Viciafaba* belonged to the different tribe Vicieae (Fig 2). The close phylogenetic relationship between common bean and hyacinth bean seems like one reason why hyacinth bean showed a significant higher transferability rate of common bean EST-SSRs than those of soybean and pea. The transferability rates of common bean EST-SSRs in hyacinth bean, pea and soybean were positively correlated with the phylogenetic relatedness among these legumes crops. It was suggested that the transferability of EST-SSRs markers of common beans reflected the sequence conservation and genetic distance of the tested species.

Polymorphism is one of the key indexes for evaluating the EST-SSR markers. In general, He, Ho, PIC and Na represent the vital indexes for the EST-SSRs polymorphism. This work calculated the polymorphism ratios of common

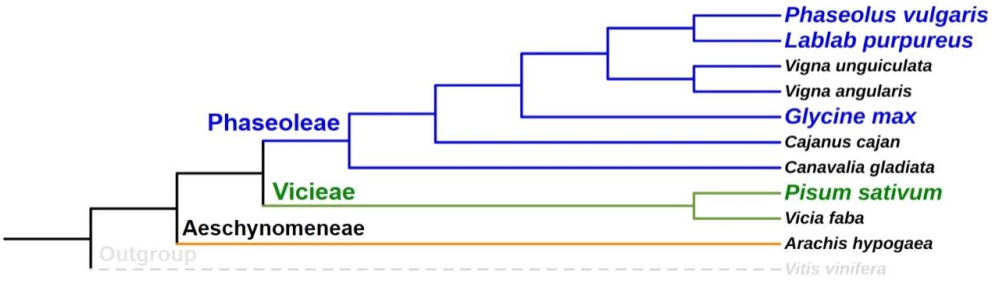

Fig 2: Phylogenetic analysis among 10 species from legume family. *Vitis vinifera* was used as the outgroup.

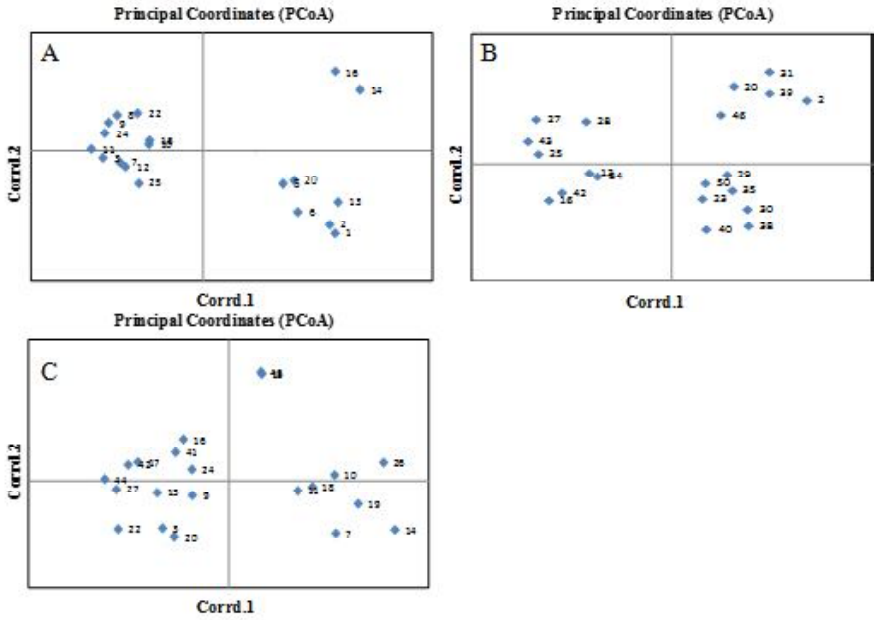

Fig 3: Principal coordinates analysis (PCoA) of hyacinth bean (A), pea (B) and soybean (C) based on results with common bean EST-SSR primer pairs. Coord.1 and Coord.2 refer to the first and second principal components, respectively.

bean EST-SSRs markers in hyacinth bean, pea and soybean to be 42.11%, 85.71% and 100%, respectively. Meanwhile, the average PIC values of polymorphic markers were 0.232, 0.313 and 0.576 and the average Na of polymorphic markers were 2.4, 2.8 and 5.7, respectively, indicating the significantly higher population diversity of soybean than those in hyacinth bean and pea. This might be related to the relatively large number of selected soybean. In addition, the polymorphism of molecular markers has a certain relationship with the sequence position.

The random genomic DNA markers are widely adopted to investigate economic crop genetic diversity, including SAMPL (Roy *et al*., 2002), AFLP (Sun *et al*., 2017), along with SSRs (Suman *et al*., 2019) markers. While the genetic diversity identified through the above markers may be unable to stand for the real genetic diversity. On the contrary, the functional markers, including EST-SSRs, can detect the polymorphism related to the genomic coding regions (Thiel *et al*., 2003). Several studies on EST-SSRs amplification among different species have been reported. Zhang *et al*., (2006) reported that the transferable EST-SSRs from bread wheat might be adopted in phylogenetic research across various *Triticeae* species. Moreover, 30 faba bean germplasms were classified into two major clusters with Pea EST-SSRs (Gong *et al*., 2011). In present study, common bean EST-SSR markers were used to analyze genetic diversity in hyacinth bean, pea and soybean. Based on PCoA, varieties that had identical origin were more likely to be clustered, which indicated that such newly developed markers were effective on the analysis of hyacinth bean, pea and soybean accessions. In addition, the genetic relationships among hyacinth bean, soybean and pea accessions were greatly associated with the respective geographic distribution and such results were consistent with those obtained in wheat (Li *et al*., 2008).

CONCLUSION

The present work firstly reported the cross-species amplification for common bean EST-SSR within the hyacinth bean, pea and soybean. A total of 19, 14 and 15 EST-SSR markers derived from common bean show transferability in hyacinth bean, pea and soybean, respectively and they can be adopted for investigating genetic diversity across hyacinth bean, pea and soybean accessions, respectively. These

results reveal that, common bean EST-SSRs are highly transferable to hyacinth bean, pea as well as soybean. The above novel EST-SSR markers are convenient and valuable genetic tools for cultivar identification, germplasm conservation, marker-assisted selection and molecular mapping of legume crops.

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