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Primer Note

Isolation and characterization of novel EST-derived genic markers in *Pisum sativum* (Fabaceae)[1]

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- *Premise of the study:* Novel markers were developed for pea (*Pisum sativum*) from pea expressed sequence tags (ESTs) having significant homology to *Medicago truncatula* gene sequences to investigate genetic diversity, linkage mapping, and cross-species transferability.
- *Methods and Results:* Seventy-seven EST-derived genic markers were developed through comparative mapping between *M. truncatula* and *P. sativum* in which 75 markers produced PCR products and 33 were polymorphic among 16 pea genotypes.
- *Conclusions:* The novel markers described here will be useful for future genetic studies of *P. sativum*; their amplification in lentil (*Lens culinaris*) demonstrates their potential for use in closely related species.

Key words: comparative mapping; expressed sequence tags; lentil; marker-assisted selection; pea; synteny.

Pea (*Pisum sativum* L.) is an important grain legume grown in temperate regions of the world because its seeds are a cheap and rich source of protein and contribute to the nutritional quality of human and animal diets. Marker-assisted selection (MAS) for agronomic traits such as yield, quality, and tolerance to abiotic and biotic stresses is not widely applied in pea due to unavailability of a reference pea genome and the limited number of molecular markers for tagging of agronomically important genes in pea improvement programs (Jain et al., 2012; Smykal et al., 2012). Pea expressed sequence tag (EST) sequences (http://www.ncbi.nlm.nih.gov/dbEST/dbEST_summary.html) are valuable tools for developing breeder-friendly markers from coding regions of genes and have been used in the past to develop a modest number of simple sequence repeat (SSR) markers in pea (Xu et al., 2012; Mishra et al., 2012; DeCaire et al., 2012; Zhuang et al., 2013). Genomic resources of the sequenced model legume *Medicago truncatula* Gaertn. (http://gbrowse.jcvi.org/cgi-bin/gbrowse/medicago/) also offer a wealth of information for developing EST-derived genic markers in closely related species using a comparative genomics approach (Smykal et al., 2012). Genic markers developed in this study using the conserved sequences between the two legumes are valuable because they can add density to gene-rich linkage maps of pea, establish macro- or microsynteny between *M. truncatula* and pea, and have higher chances of transferability between closely related species. This information can help in identifying markers that are tightly linked to the genes of interest or candidate gene/quantitative trait locus for agronomic traits.

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Investigation of conserved regions in different studies has provided strong evidence for sequence correlations between *M. truncatula* and pea (Choi et al., 2004a; Aubert et al., 2006; Bordat et al., 2011). This information can be used to develop genic markers based on sequence homology between the related species. Choi et al. (2004b) developed EST-based intron-targeted primers after aligning *M. truncatula* ESTs with the homologous genomic sequences of *Arabidopsis* (DC.) Heynh. and used them to construct a genetic map of *M. truncatula*. The basic assumption for this strategy is that introns or noncoding regions contain more DNA polymorphism than exons or coding regions (Brauner et al., 2002). A similar strategy—one that allows amplification of genomic DNA fragments covering two or more exons and bracketing polymorphic intron regions between those exons—was used in this study to develop pea EST-derived genic markers. Markers developed in this study are also available as cross-species markers within the legume family.

METHODS AND RESULTS

Primers were designed from pea EST sequences having significant similarity (score ≥100; *E*-value ≤e^{-50}) using the BLASTn search with *M. truncatula* gene calls from the contig assembly (Mt3.0) of *M. truncatula*. Approximately 1200 *M. truncatula* gene calls were searched for presence of introns. One or more introns were present in 510 of the 1200 *M. truncatula* gene calls and were aligned with the available pea ESTs (n = 18576) in the database. Seventy-seven primers were designed from the pea ESTs having well-conserved sequences with *M. truncatula* gene calls spanning one or more introns. Primers were designed by importing sequences into Primer-BLAST (www.ncbi.nlm.nih.gov/tools/primer-blast/) and selecting primers 18–24 bp long with annealing temperatures of 55–65°C. New primers were designed to amplify fragments from 150 to 1200 bp.

Genomic DNA of 16 pea genotypes including widely grown cultivars and plant introduction lines (i.e., Shawnee, Melrose, Medora, Lifter, Radley, PI 179449, Green Arrow, Frolic, A778-26-6, Sparkle, JI73, Bohatyr, ICI12043, PI 240515, PI 103709, PI 169603) was extracted from leaf tissue using a modified cetyltrimethylammonium bromide (CTAB) extraction protocol (Rogers and Bendich, 1985). PCR amplifications were performed in 25-μL reaction mixtures with 50 ng of template DNA, 0.2 μM of each forward and reverse primers, 200 μM

