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Genetic diversity and population structure of *Pisum sativum* accessions for marker-trait association of lipid content

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ABSTRACT

Field pea (*Pisum sativum* L.) is an important protein-rich pulse crop produced globally. Increasing the lipid content of *Pisum* seeds through conventional and contemporary molecular breeding tools may bring added value to the crop. However, knowledge about genetic diversity and lipid content in field pea is limited. An understanding of genetic diversity and population structure in diverse germplasm is important and a prerequisite for genetic dissection of complex characteristics and marker-trait associations. Fifty polymorphic microsatellite markers detecting a total of 207 alleles were used to obtain information on genetic diversity, population structure and marker-trait associations. Cluster analysis was performed using UPGMA to construct a dendrogram from a pairwise similarity matrix. Pea genotypes were divided into five major clusters. A model-based population structure analysis divided the pea accessions into four groups. Percentage lipid content in 35 diverse pea accessions was used to find potential associations with the SSR markers. Markers AD73, D21, and AA5 were significantly associated with lipid content using a mixed linear model (MLM) taking population structure (Q) and relative kinship (K) into account. The results of this preliminary study suggested that the population could be used for marker-trait association mapping studies.

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1. Introduction

Field pea (*Pisum sativum* L.) is a valuable cool-season pulse crop grown worldwide for its seed and soil fertility benefits [1]. Numerous improved pea cultivars have been developed with increased yield potential, modified maturity, lodging resistance (e.g. afila type) and increased nutritional value. Pea, being a leguminous crop, fixes its own nitrogen, and therefore could become an excellent candidate for bio-energy especially in temperate regions. Various studies around the world are being conducted to find non-conventional feedstock for biodiesel. In Canada, canola oil is currently the major feedstock for biodiesel production. The cost is a major barrier to the development and economic survival of a canola biodiesel industry in Canada. Therefore, efforts are being made to develop alternative low cost and sustainable oilseed crops. Field pea is one of the promising crops for such alternatives. The potential development of pea as an oil

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source for biodiesel is motivating for many reasons: 1) Canada is one of world's largest producers of pea. Therefore, no market penetration and promotion of the crop is needed. 2) Pea fixes nitrogen in the soil, thereby reducing the requirement for nitrogen fertilization. 3) Pea, as a feedstock for biodiesel, could open a new market for farmers. 4) From December 2010 the *Renewable Fuels Regulations* in Canada requires an average of 2% of diesel and heating oil to come from renewable feedstock. To date, there has been little research conducted to assess lipid content in peas, but preliminary data indicate that enough genetic diversity is available in field peas to identify appropriate genotypes for the biodiesel industry. The prospect of developing an oilseed pea has not been thoroughly investigated.

Analysis of lipids in vegetable samples is fairly intricate because of the high degree of complexity and heterogeneity of the components [2]. Previous studies reported percentage lipid contents of *Pisum* seeds ranging from 0.9% to 5.0% [3]. Compared to soybean and canola (14.0% and 41.0%, respectively) such low lipid contents would require effective breeding programs to develop field peas as a competitive oil source.

Market-driven intensive breeding for higher crop performance and increased crop uniformity often leads to unwanted loss of genetic diversity and genetic erosion [4]. In addition, self-pollinating crops such as *P. sativum* L. end up with increased homozygosity and increasing loss of genetic variation [5].

Generally, geneticists and plant breeders have emphasized the need for further improvement in capturing and harnessing genetic diversity [6]. Several methods are available to assess diversity in diverse genotypes. Examples include, allele mining, a sequence-based allelic mining technique that entails detection of variation in DNA sequences of diverse lines following PCR amplification of alleles. Another method to detect DNA sequence polymorphism is targeted induced local lesions in genomes (TILLING). However, these methods for capturing genetic diversity are expensive and time consuming. Alternatively, molecular markers can be used effectively to study genetic diversity in crops. Microsatellites, also known as simple sequence repeats (SSR), have been commonly used for assessing genetic diversity in peas due to their accuracy, reliability, co-dominance, reproducibility, and high polymorphism [7–10]. Genetic diversity and population structure of 164 pea accessions was determined using a combination of microsatellites, retrotransposons and morphological markers [11]. A recent study reported a genetic map with the positions of 37 new markers identified by using a SNP set for genotyping a pea mapping population [12].

This study was planned to determine the population structure and preliminary marker trait associations in pea. We assessed the genetic diversity of 35 diverse pea genotypes using 100 SSR markers, 50 of which generated unambiguous and highly reproducible banding patterns. The same pea accessions were also used in a previous study [13] involving only fifteen SSR markers. In this study we increased the number of markers for further investigation of population structure and marker-trait association. Our results indicate that the accessions form five major clusters. These groups can be valuable to derive parental lines for pea breeding. Having determined the diversity of the 35 pea accessions and the informativeness of the SSR markers, we next looked to identify marker-trait associations that may further facilitate the process of developing an oilseed pea. Lipid content estimations in the *Pisum* seeds for its use in marker-trait associations were obtained from Khodapanahi et al. [3]. This study is among the first to perform marker-trait associations in field pea.

2. Materials and methods

2.1. Plant materials and DNA extraction

Pea (*P. sativum* L.) seeds were acquired from Plant Gene Resources of Canada (Saskatoon, SK) and the U.S. Department of Agriculture (Pullman, WA). Choice of the 35 diverse accessions (Table 1) was based on plant characteristics, such as cotyledon color, cotyledon shape (wrinkle or round), flower color, and country of origin. The accessions were grown in a greenhouse located at Macdonald Campus of McGill University, Ste-Anne-de-Bellevue, Quebec. The average day temperature was kept at about 20 °C with a 24 h average of 19 °C. DNA was extracted from each plant, selecting fresh, young leaves at the 8–10 leaf stage. A standard phenol:chloroform DNA extraction protocol with minor changes was used to isolate DNA from each pea accession. The tissues were homogenized using TissueLyser II (Qiagen, Toronto, ON) with natural extraction buffer and 10% sodium dodecyl sulfate (SDS) followed by incubation at 65 °C for 15 min [14]. Subsequently, 200 μL of 5 mol L^{-1} potassium acetate (KOAc) was added, mixed and centrifuged at 14,000 r min^{-1} for 5 min. The supernatant was extracted with 450 μL of 1:1 phenol:chloroform and the DNA was precipitated with isopropanol and centrifuged for 5 min. The pellet was washed with 70% ethanol before dissolving in 65–70 μL Tris EDTA buffer with RNaseA (TER). The DNA quality and quantity was analyzed using a nanodrop spectrophotometer.

2.2. SSR primers for PCR

About 100 SSR primer pairs located on different pea linkage groups were assayed. These were selected from primers used by other workers [9]. Only 50 SSR loci (Table 2) showed polymorphism, therefore, used for subsequent analysis while, those amplifying ambiguous, unclear, and faint bands were not considered. PCR amplifications were conducted [15] in total volumes of 25 μL comprising 1 μL of template DNA (25–35 ng), 0.4 μmol L^{-1} each forward and reverse primer and 5 μL of 5 × C *Taq*-& LOAD Mastermix (MP Biomedicals; 1.5 μmol L^{-1} $MgCl_2$, 200 μmol L^{-1} dNTP final concentration). Amplifications were performed on a C1000 Thermocycler (Bio-Rad, Missisauga, ON) with the following profile: 95 °C initial denaturation for 2 min, followed by 36 cycles of 30 s at 95 °C, annealing at 50 °C for 45 s and 1 min at 72 °C. PCR products on 2% agarose gels stained with ethidium bromide (EtBr) in Tris Borate EDTA (TBE) buffer were analyzed under UV light. To determine the size of each amplified product a 1 kb DNA ladder (Invitrogen, USA) was used.

2.3. Marker analysis

Amplicon profiles produced by microsatellites were compiled onto a binary data matrix with each band scored "1" for presence

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