Utilizing synteny between common bean and soybean for molecular characterization of key genes for folate synthesis in common bean

Weilong Xie[1,2], Youn-Seb Shim[1], Alireza Navabi[1,2] and K. Peter Pauls[1]

[1] Department of Plant Agriculture, University of Guelph and [2]Agriculture and Agri-Food Canada, Guelph, Ontario, Canada, N1G 2W1

INTRODUCTION

- Common bean (*Phaseolus vulgaris* L.) is an excellent source of folates, but levels of these compounds can vary among bean genotypes (Khanal et al. unpublished).
- High levels of folate content in bean genotypes are known to be associated with high levels of expression of dihydroneopterin aldolase (DHNA) and aminodeoxychorismate synthase (ADCS) (Y-S Shim et al. unpublished data), which encode enzymes in the folate synthesis pathway.

OBJECTIVE

- Molecular characterization of DHNA and ADCS genes, may facilitate the development of new tools for selecting bean genotypes with enhanced levels of folates.

MATERIALS AND METHODS

- Common bean BAC libraries of the cultivar OAC Rex (Gepts et al. 2008, In Legume Genomics. pp. 113-143) and the genotype G19833 (kindly provided to us by Clemson University Genomics Institute), from Mesoamerican and Andean gene pools, respectively, were screened with a DIG-labeled fragment of DHNA gene (Roche, Mannhein, Germany).
- The plasmids of positive clones were extracted using Large-Construct Kit (Qiagen, Mississauga, Canada), and sequenced at Plant Biotechnology Institute, Saskatoon, Canada. Sequences were assembled and aligned using CLC Main Workbench, CLC bio. Common bean DHNA and ADCS gene sequences were compared to the soybean sequence database (http://www.phytozome.net/soybean) with BLAST (McClean et al. 2010; BMC Genomics 11: 184) for synteny mapping.
- A Sequence Characterized Amplified Region (SCAR) marker, based on the DHNA gene sequence and its adjacent upstream sequence of positive BAC clones was developed for the DHNA gene between the two core map parents, Bat 93 and Jalo EEP558. A single-nucleotide polymorphism (SNP) marker was identified between ADCS gene fragments of Bat 93 and Jalo EEP558 (Y-S Shim et al. unpublished data). The common bean core mapping population (Nodari et al. 1992; Theor Appl Genet 84: 186), which contains 70 RILs of a cross between Bat 93 and Jalo EEP558 was genotyped with the SCAR marker for DHNA gene and with the SNP marker for ADCS gene. The chromosome locations of these two genes on the bean linkage maps were determined by constructing a linkage map using JoinMap (Stam, 1993; The Plant Journal 3: 739).

RESULTS

- Positive clones of DHNA were identified from both libraries (Fig. 1).
- Full length DHNA sequences were obtained after sequencing positive clones from the G19833 and OAC Rex libraries. There is one SNP, between OAC Rex and G19833, in the 393 bp coding region (Fig. 2). The translated sequences (130 amino acids) are identical between the two cultivars. The deduced amino acid sequence of DHNA in common bean is closest to a DHNA sequence (ACU16784) from soybean.

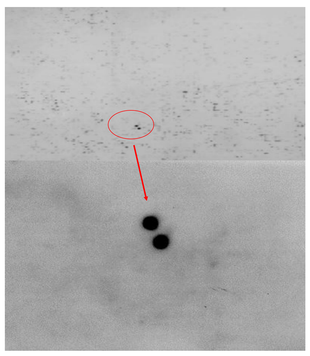

Fig. 1. Positive clone 125G13 was identified from G19833 BAC library with DIG-labeled DHNA probe.

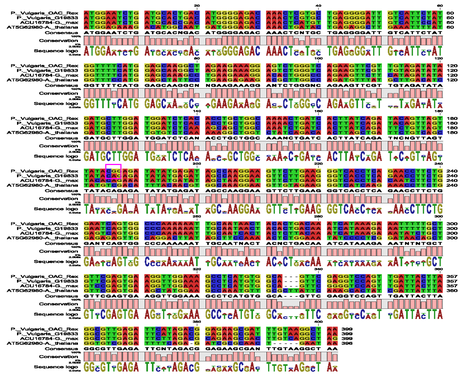

Fig. 2. Alignment of DHNA homologs from common bean, soybean and Arabidopsis.

- The 157 kb contig assembled from two G19833 BAC clone sequences contained approximately 20 genes (including DHNA) which had an order that was highly syntenic with a segment of soybean chromosome 3 that is linked to (400 Kb away) but does not include DHNA (Fig. 3). Based on the synteny mapping (McClean et al. 2010), DHNA was predicted to be located on *P. vulgaris* chromosome 1.

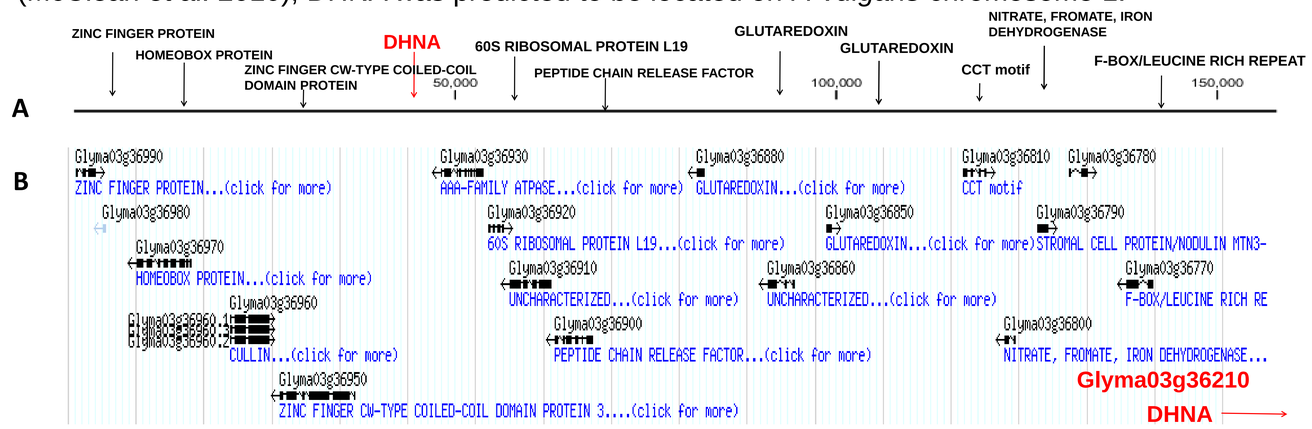

Fig. 3. Annotated genes in a 157 kb contig assembled from two G19833 BAC clone sequences of common bean (A) syntenic region on chromosome 3 of soybean (B).

- BLAST searches of bean ADCS against the soybean sequence database showed that the closest matches occurred with ADCSs on chromosomes 10 and 20 of soybean. Synteny mapping (McClean et al. 2010) predicted that ADCS is located on *P. vulgaris* chromosome 7.
- A SCAR marker for DHNA and a SNP marker for ADCS were developed between Bat 93 and Jalo EEP558, and used to map the DHNA and ADCS genes on *P. vulgaris* chromosome 1 and 7, respectively (Fig. 4).

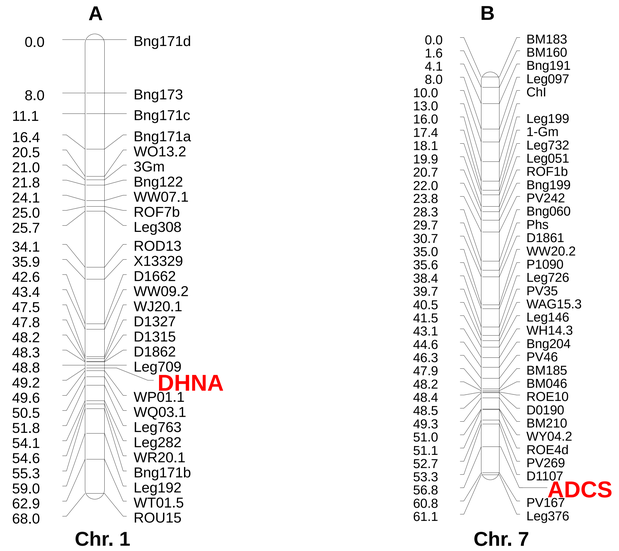

Fig. 4. DHNA (A) and ADCS (B) were mapped on chromosome 1 and 7 of *P. vulgaris*, respectively, by genotyping the core mapping population (Bat 93 x Jalo EEP558.

CONCLUSIONS

- The nucleotide sequences of DHNA from OAC Rex and G19833 of different gene pools differ slightly, while their amino acid sequences are identical.
- The DHNA and ADCS genes were mapped on chromosome 1 and 7 in *P. vulgaris,* respectively, by both a synteny analysis approach and by conventional mapping with core map RILS, demonstrating the usefulness of synteny mapping for characterizing genes in common bean.
- The markers might be useful for selecting beans with enhanced levels of folates.

Acknowledgments