Characterization of Common Bean (*Phaseolus vulgaris*) Dihydroflavonol 4-reductase (*DFR*) Gene Paralogs

Mahbuba Siddiqua, Zeinab Yadegari and K. Peter Pauls
Department of Plant Agriculture, University of Guelph, Guelph, Ontario, Canada N1G 2W1

Introduction

Some of the health benefits of common bean consumption are related to the phenolic compounds they contain. These compounds, including anthocyanins, and proanthocyanidins have antioxidant properties. DFR (Dihydroflavonol 4-reductase) is the first committed enzyme of the flavonoid pathway leading to the production of anthocyanins and proanthocyanidins (Figure 1).

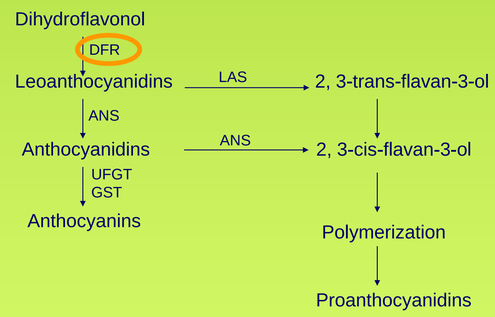

Figure 1. Partial flavonoid biosynthetic pathway

The current work was initiated to obtain information about *DFR* paralogs in common bean to facilitate the development of genomic tools to select varieties with high levels of beneficial antioxidant compounds. The objectives of the work were to: 1) identify *DFR* paralogs in common bean, 2) clone cDNAs of *DFR* paralogs, express the genes in a heterologous system and study the catalytic activities of the enzymes, and 3) identify correlations between *DFR* gene expression and anthocyanin and proanthocyanidin levels in beans.

Materials and Methods

The common bean seedlings were drought treated by withholding water for ten days. Three-day old seedlings were treated with continuous white light for 4 days and four-day old seedlings were treated with UV light for 7 hrs. In each case, the plant materials were frozen in liquid nitrogen and kept at –80^{o}C until RNA was extracted.

PvDFR paralogs were cloned from cDNA prepared from the RNA of seeds, seed pods, and stress treated seedlings of common bean by degenerate primers. Degenerate primers were designed from available DFR DNA sequences of several legumes including soybean. 5' and 3' ends were amplified by RACE.

Bioinformatics Analysis was done using SDSC Biology Workbench 3.2 and CLC Genomics Workbench 3.6.5. .

Results

One full length *DFR* paralog was cloned from a common bean BAC library and two others were cloned from cDNA fractions of common bean. The paralogs were sequenced and named *PvDFR-1*, *PvDFR-2*, and *PvDFR-3,* respectively.

1. PvDFR-1 is expressed only in seeds at very low level. PvDFR-2, and PvDFR-3 are expressed mostly in seeds and seed pods but also in white light, UV light, and drought treated seedlings (Figure 2).

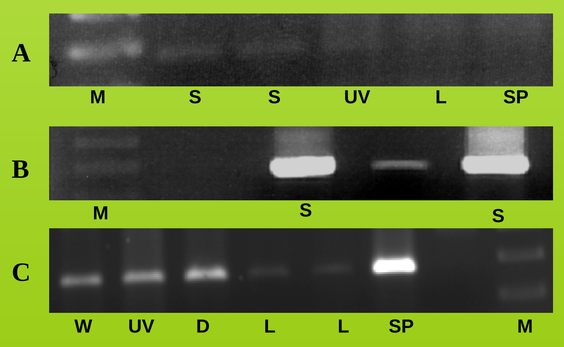

Figure 2. Panel A, B, & C represent expression of mRNA for PvDFR-1, PvDFR-2 & 3 and PvDFR-2 & 3 respectively. Here M = Marker; S= Immature Seed; UV= UV light treated seedlings; L= Leaf grown in normal growing condition; SP= Immature Seed pod; D= Drought treated seedlings.

Results

2. PvDFR1, PvDFR-2, and PvDFR-3 are predicted DFR proteins

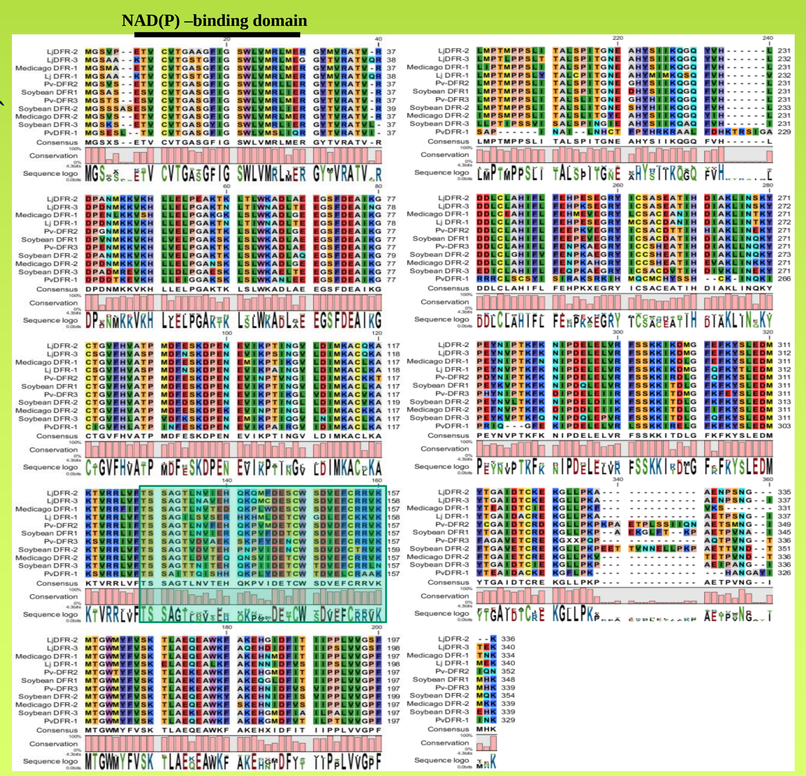

Figure 3. Alignment of deduced amino acid sequences of DFRs. Note that Lj = *Lotus Japonicus*; Pv = *Phaseolus vulgaris*. The boxed region is postulated to control the substrate specificity of DFR.

3. The amino acid sequences were also compared with those from soybean. An unrooted tree showed that PvDFR-1 grouped with soybean DFR-3 (65%), PvDFR-2 grouped with soybean DFR-1 (87%) and PvDFR-3 grouped with soybean DFR-2 (86%)

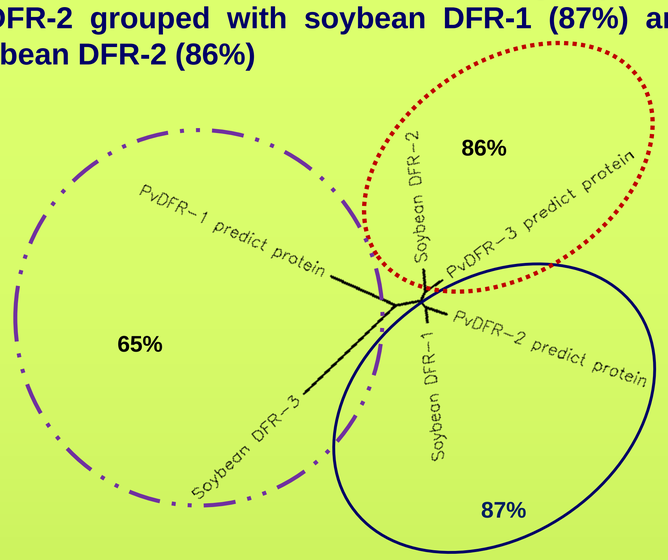

Figure 4. Comparison of PvDFR-1, 2, & 3 full length proteins with those from Soybean DFR-1, 2, & 3

Conclusion and Future Directions

1. Common bean has three paralogs, named as PvDFR-1, PvDFR-2, and PvDFR-3.

2. PvDFR-1 is expressed at very low level whereas PvDFR-2 and PvDFR-3 are expressed at higher level in seeds.

3. Three distinct clusters formed in the uprooted tree confirm that common bean is descended from soybean.

4. The differential expression patterns of the *DFR* paralogs suggest that they might have tissue- and developmental- specific roles in common bean and may differ in their catalytic activities.

5. Therefore, PvDFR-2, and PvDFR-3 were cloned into pBAD expression vector for heterologous expression and measurement of their catalytic activities.

Acknowledgement

This research was supported by grants from OMAFRA