

A.M. González¹, F.J. Yuste-Lisbona², A. Fernández-Lozano², C. Capel², O. Muñoz¹, A.P. Rodiño¹, R. Lozano², M. Santalla¹

¹ Grupo de Biología de Agrosistemas. Misión Biológica de Galicia-CSIC. P.O. Box 28. 36080 Pontevedra, Spain.

² Centro de Investigación en Biotecnología Agroalimentaria (CIAMBITAL). CeIA3. Universidad de Almería. 04120 Almería, Spain.

ABSTRACT. The reduction of pod shattering represents a key domestication syndrome in the domesticated common bean (*Phaseolus vulgaris* L.), similar to what happens in other legume crops. Seed dispersal in the wild common bean dehiscent pod seems to undergo through an explosive rupturing along a dehiscence zone in the ventral suture of the pod. Both valves of the pod detach due the tensions established by the specific mechanical properties of drying cells of the endo and exocarp. Aspects which are shared among families producing dry dehiscent fruit, such as Fabaceae and Brassicaceae (Grant 1996; Dong and Wang 2015). Understanding the genetic variation of common bean in relation to pod shattering will provide breeders with key tools to improve this trait, thus reducing yield losses. In this study, we identified quantitative trait loci (QTLs) controlling pod fibers along the pod valves (string) and shattering in a recombinant inbred line (RIL) population derived from a cross between a cultivated common bean and wild nuña bean. Genes underlying QTL regions could be potential targets for improving shattering resistance performance through marker assisted selection and could provide useful information for isolating candidate genes.

Anatomical and histological differences between pod valves of cultivated and wild common bean

The analysis conducted with 20-days post anthesis (DPA) pods showed obvious differences between the indehiscent PMB0225 (Fig. 1A, C) and dehiscent PHA1037 (Fig 1B, D) genotypes, revealing changes in the cell wall thickness as a key factor generating required tension. Indeed, the proportion of a thick secondary cell-wall formation was observed in both pod sutures and much more pronounced in the shattering type (Fig. 1F, H) than the non-shattering type (Fig. 1E, G).

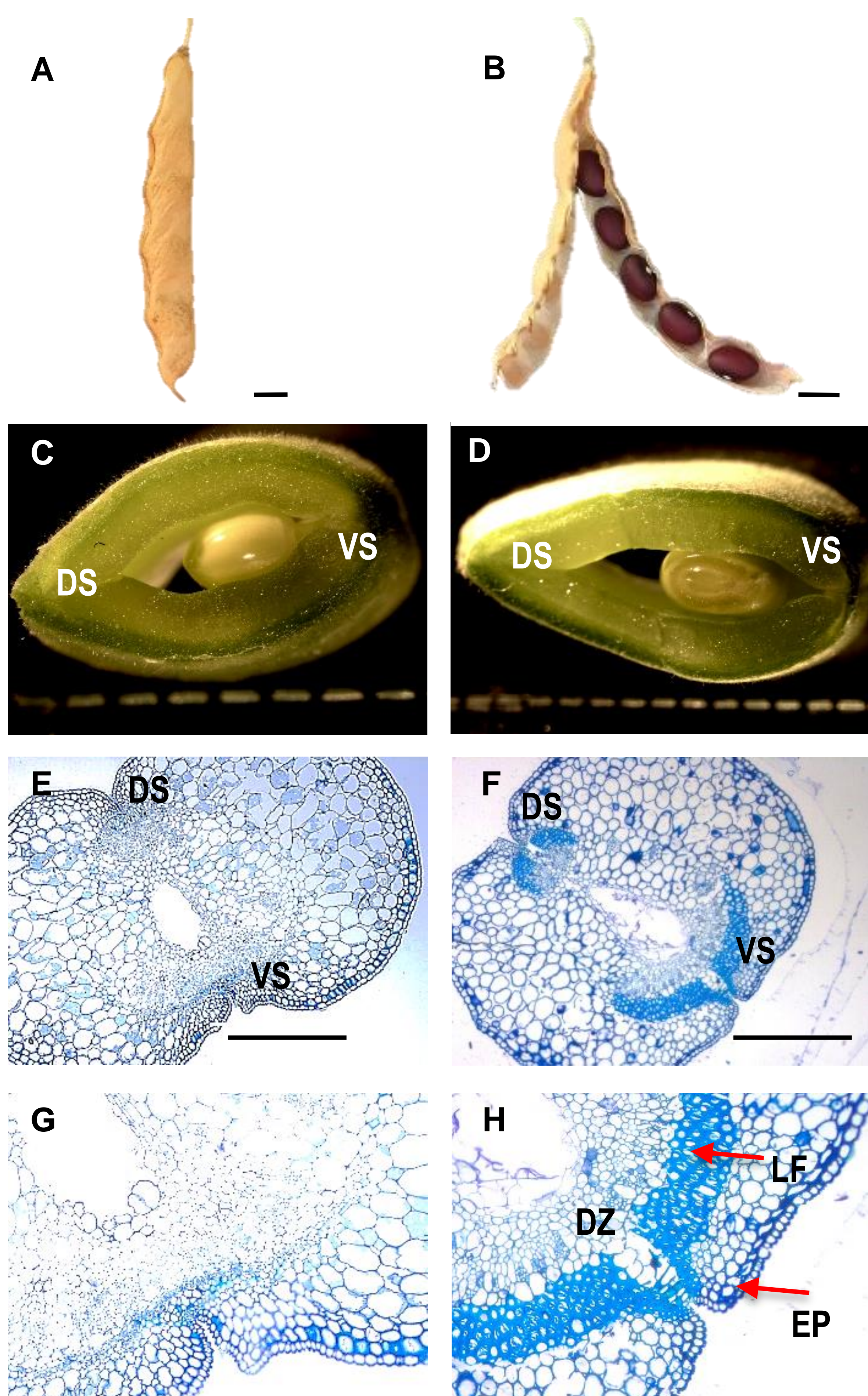


Figure 1. Representative anatomical and histological images of ventral suture of the common bean indehiscent and dehiscent pod. (A) Mature pod of indehiscent line (PMB0225). (B) Mature pod of dehiscent line (PHA1037). Scale bar 1 cm. (C, D) Magnified views of mature pod 20 DPA of indehiscent and dehiscent, respectively. Scale bar 1 mm. (E, F) Transverse pod sections stained with toluidine blue of indehiscent and dehiscent, respectively. Scale bars 500µm. (G, H) Sections of ventral suture of indehiscent and dehiscent, respectively. DS, dorsal suture, VS, ventral suture, EP, epidermis; LF, lignified fiber.

This observation is consistent with Murgia et al. (2017) and Prakken (1934), who indicated this anatomical and histological difference is the basis of the presence/absence of pod strings, and the basis of the shattering/non-shattering phenotypes. This suggests that the role of the cell wall thickness in the shattering might be more relevant (or at least different) in common bean compared to soybean (Dong et al. 2014).

Functional category analysis of pod string and shattering QTLs

Multi-associated QTL mapping revealed a major QTL on chromosome 02 (Chr) associated to pod string (PST) and explained up to 59% of the phenotypic variance. QTLs for PST and pod shattering (PIP) co-localized on Chr01 and explained 9% of the phenotypic variance (Fig. 2). However, the control of string (which depends on the characteristics of the ventral suture) and parchment (which depends on the layer between the inner and outer parenchyma of the pod wall) was suggested as independent in common bean (Prakken 1934).

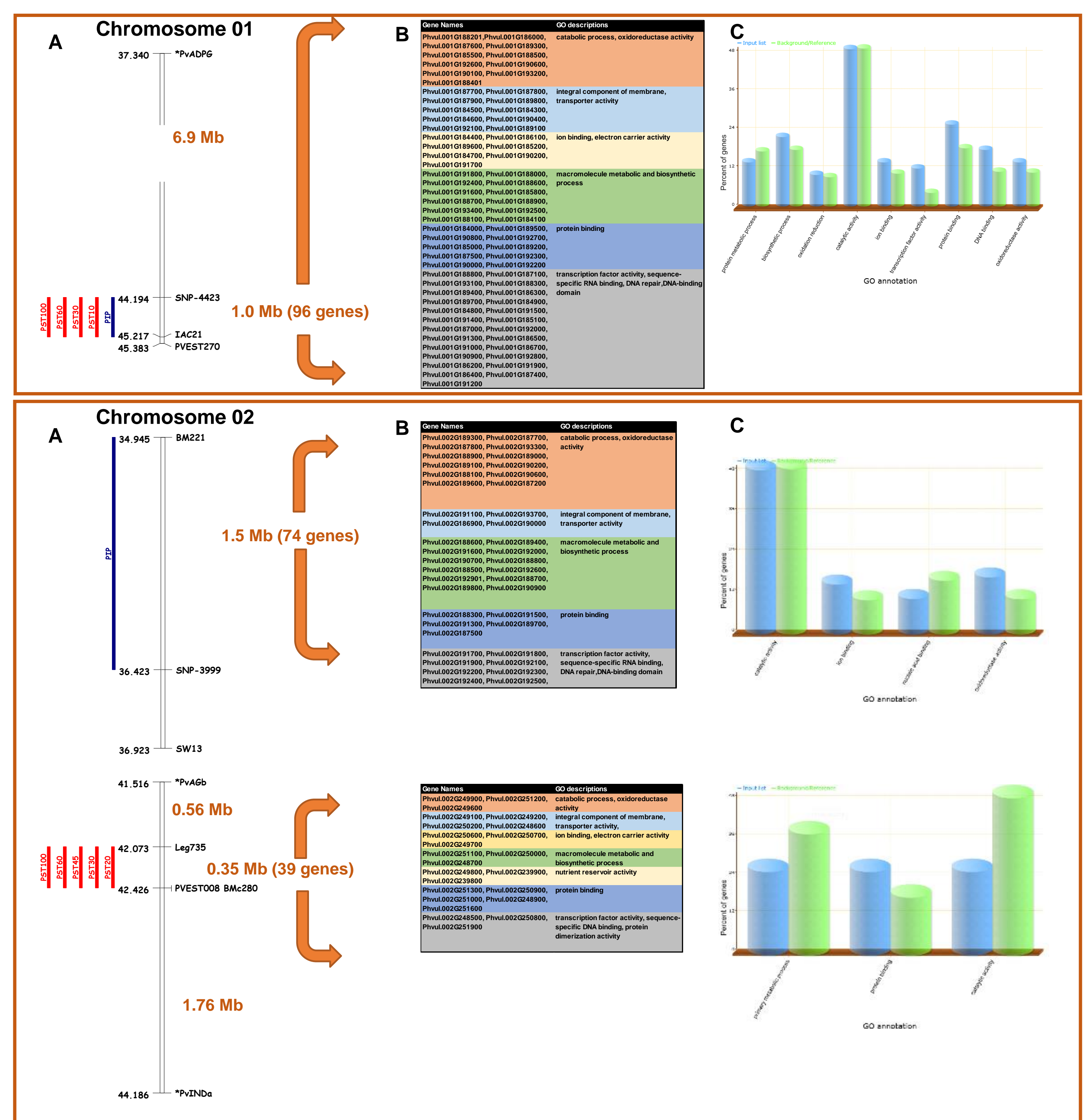


Figure 2. (A) Physical common bean map of Chrs 01 and 02 showing the positions of markers on the left and Arabidopsis homologues genes indicated with asterisks on the right. QTLs are depicted as vertical bars to the left (red and blue for PST and PIP, respectively). Positions are indicated in Mega base pairs (Mb). (B) Identified candidate genes ranked by GO terms within the QTLs. (C) Global analysis of gene annotation.

Neither of Arabidopsis homologues for genes involved in shattering were located within the QTL regions. Only *PvIND* and *PDH1* involved in pod shattering in Arabidopsis and soybean, respectively, were located on Chr02 (Gioia et al. 2013; Funatsuki et al. 2014). Genomic region on Chr01, which included 96 annotated genes, was significantly enriched for protein and DNA binding GO terms. In this region, 25 transcription factors (TFs) were identified, highlighting the genes *Phvul.001G187100* and *Phvul.001G185100* that encode the TFs-*APETALA2* (*AP2*) and *MYB*. *AP2* was involved in shattering in rice (Zhou et al. 2012), while *MYB* was an important regulator in the secondary cell wall biosynthesis in Arabidopsis (Zhong et al. 2015). Two adjacent genomic regions on Chr.02, which included 74 and 39 annotated genes, respectively, were significantly enriched for primary metabolic and catalytic activity GO terms. These regions harbor putative cell wall candidate genes: polygalacturonases (*Phvul.002G188700* and *Phvul.002G189400*), cellulose synthase (*Phvul.002G188600*), phospholipase-D (*Phvul.002G248700*), and 6-P gluconate proteins (*Phvul.002G251100*). Based on these data, the genes involved in the regulation of the secondary cell-wall deposition and fiber-cell differentiation could be considered as candidate genes for shattering in common bean.

References

Dong et al. (2014) Nat. Commun. 5:3352; Dong and Wang (2015) Front Plant Sci. 6: 476; Grant (1996) Can J Plant Sci 76:447; Gioia et al. (2013) J Hered 104:273; Funatsuki et al. (2014) Proc Natl Acad Sci USA 111:17797; Murgia et al. (2017) Front Plant Sci. 8: 251; Prakken (1934) Genetica 6:174; Zhong et al. (2015) Natl Cell Physiol 56:195–1214; Zhou et al. (2012) Plant Cell 24(3):1034.



Acknowledgements

This work was financially supported by supported by the Ministerio de Economía y Competitividad (proyectos AGL2014-51809-R and AGL2015-64991-C3-1-R) and UE-FEDER Program.