

**ARCELIN-LIKE AND α -AMYLASE-LIKE INHIBITOR DNA SEQUENCES
COSEGREGATE WITH A NOVEL SEED STORAGE PROTEIN IN *PHASEOLUS
VULGARIS* X *P. ACUTIFOLIUS* HYBRIDS**

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Wild tepary beans (*P. acutifolius* A. Gray) are a potentially valuable source of desirable traits, including drought tolerance, disease and insect resistance. So far, only common bacterial blight resistance has been transferred from tepary beans (Singh and Munoz, 1999; Munoz et al., 2004). Several accessions of wild tepary beans confer strong resistance to the two major bruchid pests of common bean. The main objective of this project is to develop common bean cultivars resistant to the bean bruchid *Acanthoscelides obtectus* through interspecific transfer of resistance from tepary bean.

The resistance mechanism in tepary beans is not well understood. Various types of lectin family proteins (arcelin, α -amylase inhibitor and phytohemagglutinin) may play a role in bruchids resistance (Kornegay & Cardona, 1991; Mirkov et al., 1994; Finard-Filho, 1996; Yamada et al., 2005). In this work, the wild tepary bean accession G40199 identified by CIAT as resistant to *A. obtectus* was hybridized to 'ICA Pijao' and F₁ plants were obtained via embryo rescue after 22 to 28 days. Seeds from the first backcross were used to screen for the presence of novel protein profiles corresponding to those found in the *P. acutifolius* accession G40199. A small portion of the seed were scratched on sand paper to obtain a fine powder for total protein extraction and electrophoresis of proteins fragments on SDS – PAGE followed by standard staining with coomasie blue in 10% acetate and 40% methanol. A 33 – 35 kDa seed storage protein (similar in size to arcelins) was stably integrated after two backcrosses to 'ICA Pijao'. The protein has also been successfully introgressed into 'Rojo', an improved Tanzanian cultivar, via bridge interspecific hybrid lines. More than one seed storage protein may be responsible for conditioning resistance to bruchids. The unique arcelin-like isoform and α -amylase inhibitor-like proteins appear to be tightly linked. Other storage proteins with potential insecticidal properties are also likely to be co-transferred into *P. vulgaris*.

BLAST search and screening of related storage protein gene sequences for phytohemagglutinins, α -amylase inhibitors and arcelins isoforms was conducted. DNA sequence alignment and primer designing for different genes was performed manually. These primers were used for screening for DNA amplifications from leaf tissues of tepary G40199, cultivated brown tepary bean and the interspecific hybrid lines. Only sets of primers that generated polymorphic DNA fragment with stable and repeatable size after optimization of PCR conditions were used to screen for the presence of the introgressed novel protein among interspecific hybrids. The DNA fragment generated by primers for the co segregating proteins was sequenced and their DNA sequence homology aligned and compared by the BLASTP alignment. Two DNA markers associated with arcelin-like and α -amylase inhibitor-like proteins, respectively, co-segregate with seed storage proteins. An arcelin like (ARL) DNA fragment with molecular size of ~800bp was only

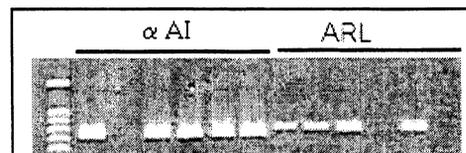


Figure 1 DNA PCR fragments amplifications for α -amylase-like (α -AI) and arcelin-like (ARL) proteins from leaf tissues of *P. acutifolius* and *P. vulgaris* interspecific hybrids. Lane 1: 100 bp ladder; Lanes 2 & 12: G40199; Lanes 3 & 11: ICA Pijao; Lane 4: Cultivated brown tepary; Lanes 5-9: Interspecific backcross lines.

detectable in genomic DNA from G40199 and among interspecific backcross lines that inherited the equivalent size of the storage protein (**fig. 1**). An additional DNA fragment corresponding to α -amylase inhibitor-like (α -AIL) protein is found in both tepary accessions (G40199 and cultivated brown tepary), but not in *P. vulgaris* cultivar ICA Pijao (**fig. 1**). This fragment of ~750bp also co-segregated with the novel storage protein inherited among interspecific hybrids.

The ARL and α -AIL genomic DNA sequence alignment in tepary bean shows a 93% and 80% homology to ARL-2 arcelin-like and α -AI-2 pa, respectively. (**fig. 2**). The two proteins ARL and α -AIL shares a high percent sequence homology and they may be tightly linked into a single super gene family.

We are in the process of associating the presence of one or more of the isoforms of seed storage proteins and resistance for bruchids in our backcross lines. Determining the actual sequence of the open reading frame for these storage proteins in our materials will be necessary so as to target a full sequence homology of the proteins to those in the data base and consequently cloning of the genes.

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ARL G: 669 TDYFNFDFFKQNDADTNRLILQRDATISSGRLRLTGVGSNEDPWVDSMGRAFYSDPIQ 490
          TDYFNFDFFKQNDADTNRLILQRDATISSGRLRLTGVGSNEDPWVDSMGRAFYSDPIQ
ARL 2: 23 TDYFNFDFFKQNDADTNRLILQRDATISSGRLRLTGVGSNEDPWVDSMGRAFYSDPIQ 82

ARL G: 489 IRDSTGNLASFHINFTFIIIRANNAGHSAYGLAFSLVPVGSQPKRKREYLGFLPDAHTVAV 310
          IRDSTGNLASFHINFTFIIIRANNAGHSAYGLAF+L PVGSQPKRKRE LGFLPDAHTVAV
Sbjct: 63 IRDSTGNLASFHINFTFIIIRANNAGHSAYGLAFALFPVGSQPKRKRENGLFLPDAHTVAV 142

ARL G: 309 AFNTLNNSIDIDVNSNSPSHTGFCDFNKHNGEKIDVQIITYESPKKNLRVVLHFKSNVQY 130
          FNT++N + + GF HNGE TDVQIITYESPKKNL++VL T SNVQ
ARL 2: 143 -FNTVSNVMSSTSTPRLAQRGFAISTNHNGETTIDVQIITYESPKKNLKIVLPSTNSNVQ- 200

ARL G: 129 EYDFNAPLYLENDVDRSVKRWVGFSAATSGLKEETTETHDILS 4
          YDFNAPLYLEN+vDR+v VGFSAATSGL EETTETHDILS
ARL 2: 201 -YDFNAPLYLENEVDNRVS--VGFSAATSGLEETTETHDILS 239

AMYLG: 641 THANSASDT-FNFHFSFNETNLIQQDATVSSNGNLQLHIMDSMCSAFYSAPIQIRDSTTG 465
          THANSA DT FNFHFSFNETNLIQQ ATVSSNG L+L+T DSMCSAFYSAPIQIRDSTTG
AILpa: 20 THANSACDTSFNFHFSFNETNLIQQQATVSSNGKLKNTYDSMCSAFYSAPIQIRDSTTG 79

AMYLG: 464 NVASFHINFTMNIITRYKANSVGLDFALVPVQPKSKGRLLGLFKTPDYDRNAGIVTVEF 285
          VASF TNFTMNIIT NSA+GLDFALVPVQPKSKG FKIPDYDRNAG VITVEF
AILpa: 80 KVASFDTNFTMNIITNYKNSAIGLDFALVPVQPKSKGH----FKIPDYDRNAGTIVTVEF 135

AMYLG: 284 DTLRRRISIDGNVNDIESVPPWVDDYDQQAQAEVRITYNSSTKVLAVSLLNPSTGKSNMVS 105
          DT R+ ISID N+ND+ SVPWVW DYD Q EVRITYNSSTKVLAVSLLNP TGKSN VS
AILpa: 136 DTRKRCISIDSNHNDLNSVPPWVLDYDRQNTVEVRITYNSSTKVLAVSLLNPITGKSNVS 195

AMYLG: 104 ARMELEKLLDDWWSVGFIGTSGVHEYSKRE---TWS 3
          ARMELEK LDDWWSVGF TSG +++ + +WS
AILpa: 196 ARMELEKILDDWWSVGFSAATSGAYQWGFETNEVLSWS 232

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Figure 2. BLASTP -alignment of protein sequences (translated DNA sequences) of two storage proteins from *P. acutifolius*: arcelin-like and α -amylase inhibitor-like from G40199 (ARL G & AMYLG) demonstrate homology with ARL 2 (AAF71744) and AILpa (BAB72259), respectively, from the NCBI Enterz database (<http://www.ncbi.nlm.nih.gov/>).

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