SEED STORAGE PROTEINS ARL2 AND ITS VARIANTS FROM THE APA LOCUS OF WILD TEPARY BEAN G40199 CONFERS RESISTANCE TO ACANTHOSCELLIDES OBTECTUS WHEN EXPRESSED IN COMMON BEANS

P.M. KUSOLWA[1] AND J.R. MYERS[2]

[1]Department of Crop Science and Production, Sokoine University of Agriculture, P. O. Box 3005, Chuo Kikuu , Morogoro, Tanzania

[2]Department of horticulture, Oregon State University, 4017 ALS Bldg, Corvallis, OR 97331-7304, USA

Corresponding author: kusolwap@gmail.com, kusolwa@suanet.ac.tz

ABSTRACT

Wild tepary beans (*Phaseolus acutifolius*) contain arcelins and arcelin-like (ARL2) proteins that are co-expressed with other proteins of the arcelin, α-amylase inhibitors and phytohemagglutinins (APA) locus. Arcelin and ARL2 protein from a wild tepary bean accession G40199 were transferred into common bean cultivars ICA Pijao and Rojo. Inbred backcross lines were developed and selected for segregation of the proteins at $BC_2F_{2:3}$ generations. Seeds from homozygous, segregating lines expressing the arcelin and ARL2, plus those without the proteins, were used for screening against resistance to *A. obtectus*. Significant delay of 63 days for 50% adult emergence and reduced number of emerged F_1 of *A. obtectus* adults were observed in backcross lines with homozygous expression of arcelin and ARL2 tepary bean proteins. Furthermore, a reduction in size and weight of emerged adult insects to almost half was observed. This work demonstrates the superior resistance common bean backcross lines to *A. obtectus* conferred by the presence of the APA proteins introgressed from tepary bean.

Key Words: Arcelin, *Phaseolus acutifolius*

RÉSUMÉ

Les haricots sauvages tepary (*Phaseolus acutifolius*) contiennent des protéines arcelines et les protéines semblables aux arcelines (ARL2) co-exprimées avec d'autres protéines arcelines, tels les inhibiteurs α-amylase et les locus phytohémagglutinines (APA). Les protéines arcelines et ARL2 provenant des accessions de haricots sauvages tepary G40199 étaient transférés dans les cultivars du haricot commun ICA Pijao et Rojo. Des lignées issues de croisement en retour étaient développées et sélectionnées pour la ségrégation des protéines aux générations BC2F2:3. Des graines des lignées homozygotes ségrégantes exprimant les protéines arcelines et ARL2, plus celles sans protéines, étaient utilisées pour la sélection contre la résistance au *A. obtectus*. Un retard significatif de 63 jours pour 50% de l'émergence des adultes et un nombre réduit de F1 émergées des adultes *A. obtectus* étaient observés dans le croisement reour avec expression des homozygotes des protéines arcelines et ARL2 des haricots tepary. En outre, une réduction jusqu'à la moitié en taille et poids des insects apparus était observée. Cette étude démontre la résistance supérieure des lignées de croisement en retour au *A. obtectus* conférée par la présence de protéines APA introgressées du haricot tepary.

Mots cles: Arceline, *Phaseolus acutifolius*

INTRODUCTION

In the tropics, dry bean (*Phaseolus vulgaris* L.) production is constrained by several biotic and abiotic factors. Some of the major biotic constraints involve post-harvest losses caused by two major bruchid species *Acanthoscellides obtectus* and *Zabrotes subfasciatus*. The bean weevil, *A. obtectus* is a predominant post-harvest pest in all tropical highland bean producing

regions, attacking beans in the field and in storage. The pest causes quality degradation and poor germination of damaged seeds.

In an effort to find sources of resistance to these pests, evaluation of bean germplasm has identified seed proteins of the lectin-like family, called arcelins, found in wild common beans (Osborn *et al*., 1988) associated with resistance to bruchids. Highest level of resistance to *Z. subfasciatus* was described to be found in in wild common bean accessions and is associated with the expression of arcelin 5, arcelin1, arcelin2 and arcelin 4 in order of effectiveness (Cardona *et al*., 1990; Kornegay *et al*., 1993). Breeding strategies to improve resistance of commercial cultivars of dry bean have involved backcrossing method to introgression of arcelin proteins from wild genotypes of common beans (Osborn *et al*., 1986; Osborn *et* al., 1988; Cardona *et al*., 1990; Kornegay and Cardona, 1991; Kornegay *et al*., 1993; Misangu, 1997).

High levels of resistance to two major bruchid species were demonstrated in tepary bean genotypes by Shade *et al*. (1987) and recommended for use as potential source of resistance. Further reports demonstrated that lectins present in some tepary bean accessions conferred high resistance to the bean weevil *Acanthocellides obtectus* (Pratt *et al*., 1984; 1990). Arcelin and arcelin-like proteins have been reported in cultivated accessions of tepary bean *P. acutifolius* (Mirkov *et al*., 1994; Gerhardt *et al*., 2000), but their resistance to *A. obtectus* was not characterised. Arcelins and ARL proteins present in tepary beans are part of the lectin-like proteins which may exist as different alleles and variants (Lioi *et al*., 2003; 2006). Since different variants of arcelins or arcelin-like proteins may confer different levels of resistance to bruchids, a wild tepary bean accession G40199 from CIAT and preliminary studies to use it as a resistant source to bruchids was proposed by Myers *et al*. (2001). Detailed characterisation of total seed storage proteins from this accession demonstrated that it contains a complete APA locus of the lectin-like proteins. This accession demonstrated expression of arcelin, arcelin-like (ARL2) and phytohemagglutinins, lectin like proteins specific to tepary beans only. It was hypothesised that the transfer of these proteins

into common bean cultivars may improve resistance to *A. obtectus*. These proteins were transferred by interspecific hybridisation *via* embryo rescue (Mbogo *et al*., 2009). This work is aimed at evaluating the association of the introgressed APA genes from G40199 x common bean interspecific backcross lines selected for segregating locus with ARL2 seed proteins.

MATERIALS AND METHODS

Plant materials and genotype identification. A population of interspecific hybrid backcross families was used to screen for *A. obtectus* resistance. Interspecific hybrid lines from BC_2F_3 seeds from a cross between ICA Pijao and tepary bean accession G40199 were used in the bruchid screening trials.

A total of eleven single plants from each backcross family was randomly selected based on genomic DNA analysis for the presence of ARL2, á-AI and PHA by PCR amplification. However, it was not possible to separate homozygous from heterozygous plants for the genes at this generation. Therefore, seeds from $ARL2^+$ single plant selection (SPS) were harvested and used to progeny test the $BC_2F_{2:3}$ by protein gel electrophoresis to identify individual lines that do not segregate for the 33KDa tepary protein corresponding to the APA proteins. Total seed proteins were analysed on eleven seeds from each individual backcross lines using 15% tris-HCl SDS –PAGE. Three genotypes were identified: homozygous (ARL/ARL, 26 lines), heterozygous (ARL/arl, 64 lines), and homozygous null (arl/arl, 36 lines) based on the 33kDa storage proeins. Four samples containing bean seeds from each cultivars Rojo, ICA Pijao were used as susceptible checks for parents, while four vials with accession G40199 seeds were included as resistant control of the parent wild tepary bean.

Screening for resistance to *A. obtectus* among tepary bean hybrids. A spontaneous colony of *A. obtectus* was obtained from bean stocks in the Oregon State University (OSU) dry bean breeding programme. The colony was multiplied and maintained in susceptible Rojo and Pinto

bean seeds at OSU to obtain enough adult insects for inoculation of experimental materials.

A total of fifteen non-sexed *A. obtectus* adults were placed into the glass vials containing 30 seeds from interspecific backcross lines with ICA Pijao as recurrent parents. Glass vials were loosely closed to allow aeration but prevent escape of adult insects placed into incubation trays, kept undisturbed for 12 days at $25 \pm 3°C$. After 12 days, the number of laid eggs was recorded using a magnifying lens and then adults were removed. In cases where no laid eggs were visible, samples were re-inoculated with fresh adults. Vials were placed back into the incubation tray and left undisturbed but monitored until the first adult emerged. Powdery/flour appearance on the surface of the beans was noted to be sure of the larvae penetration into the seeds and identify for possible escapes. Following the observation of the first emergence of F_1 adults, each vial was inspected daily and emerging F_1 adults were counted and removed to avoid laying of new eggs for a period of 72 days after inoculation of adults. Further observation was continued to 100 days in some lines that showed a delayed emergence of F_1 adults, especially in samples of the parent G40199 tepary beans and some of the interspecific hybrids. In an alternative attempt, seed coat from G40199 seeds was removed by scratching to verify if the hard seed coat was or not a factor for resistance to development of *A. obtectus* larvae in these seeds.

Data collected included the total number of F_1 adults emerging after inoculation, number of days for first adult emergence (DAE), number of days for 50% of total F_1 adults emerged and susceptibility index (SI) calculated as the natural log.

$$SI = \frac{Log(\# F1 Adult\ \ emerged)}{DAE} X 100$$

Other variables evaluated were the number of perforated seeds, severity of damage expressed by the number of seeds with 5 or more holes and percent seed weight loss.

Frequency of adult emergence was determined by the total number of F_1 adults emerged per day for the period of 72 days. During the experiment, samples of emerging adults were collected from vials of beans with and without

the ARL2/arcelin proteins and weight of 10 adults taken for each group.

Variables were subjected to analysis of variance, regression analysis for correlation of the novel seed protein to resistance *Acanthocelides obtectus* using SAS 9.1.3 and S-PLUS 6.2 programmes (for graphics).
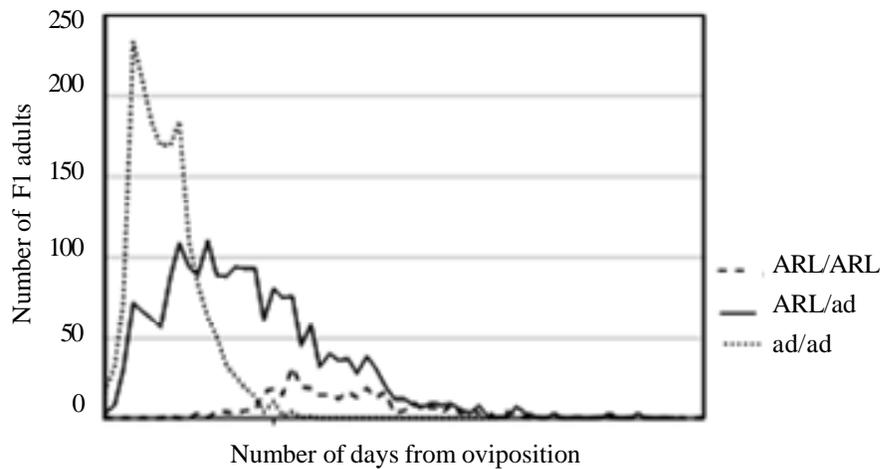
RESULTS

Delay for adult F_1 emergence. Significant delay for 50% F_1 adult emergence was observed in interspecific hybrid seeds containing ARL2 proteins (P <0.0001). Adult emergence was delayed to an average of 63 days for adults emerging from seeds that were homozygous for the expression of ARL2/arcelin seed proteins inherited from tepary bean G40199. While in the absence of the proteins, insect emergence was observed earlier (Table 1).

Reduced number of emerging F_1 adult bruchids. A significantly lowest number of emerging F_1 adults was observed in genotypes that were homozygous for the presence of ARL2 proteins (Table 1). Overall, only 18 F_1 adults were observed in these lines for a period of 72 days following oviposition. The highest number was observed in seed lots that did not express the ARL2 proteins. In addition, the frequency of F_1 adult emergence was low in seeds containing the ARL2 proteins (Fig. 1). A higher number of F_1 emerged early in susceptible materials without the ARL2 seed protein, followed by a fast drop a few days after the first adult emergence. While in homozygous ARL2 containing seeds, low frequency with fewer number of adult F_1 emerged late and slowly over long time implying a significant retardation of the reproduction cycle for the insect in this bean stock. The peaks for frequency of adult emergence corresponded to the mean number of days for 50% of F_1 adult emerged in each of the segregating /ARL2 genotypes (Fig. 1).

Seed damage. Significant differences occured in percentage perforated/ damaged of seeds (P <0.0001) as a result of larvae feeding the activities leaving holes and or dusty beans (Table 1). Bean seed lots containing arcelin and ARL2 proteins were less damaged with lowest number of

TABLE 1. Variables from genotypes of interspecific hybrid seeds segregating for the expression of ARL proteins subjected to A. obtectus feeding trial

Genotype	DAE-50% F_1	# of F_1 emerged	% Perforated	Severity >5 holes	SI	% weight loss
arl/arl	46.43	64.04	48.43	6.54	3.71	17.91
ARL/arl	52.87	36.39	29.49	2.69	2.7	8.45
ARL/ARL	62.68	17.81	26.45	1.57	1.6	7.84
LSD (0.05)	1.88	12.48	7.42	1.5	0.31	2.87

Figure 1. Frequency of emergence of F_1 A. obtectus adults in interspecific hybrid backcross lines with distinct genotypes for ARL2 seed storage proteins from tepary bean accession G40199.

perforated seeds. Very few seeds with 5 or more holes were observed in these seed lots, while up to 7 holes per seed could be observed in samples without this storage protein.

Susceptibility index. Susceptibility index (SI) was significantly lowest with a value of 1.6 for homozygous lines containing the ARL2 proteins, while the highest at 3.71 for backcross lines with no ARL2/arcelin proteins (Table 1). The SI was negatively correlated with number of days for F_1 adult emergence, whereby ARL2 containing seeds with higher DAE-50% and low SI (Fig. 2).

Susceptibility index also demonstrated exponential relationships with the number of emerging F_1 adults indicating that more susceptible seeds (high SI) had exponential increase in emergence of new adults (Fig. 3).

Homozygous backcross lines for arcelin and ARL2 proteins had less emerging adults with low SI, while in the absence of the proteins and higher SI resulted into exponential increase in total number of emerging adults and total damaged seeds.

Reduction of seed weight. Significant reduction in seed weight was observed as a result of larvae feeding on the seeds (P<0.0014). Genotypes from interspecific backcross families containing arcelin and ARL2 proteins had the lowest mean seed weight loss (7.84%) compared with 17.91% in genotypes within the same backcross family, but without the protein (Table 1). Seeds from segregating genotypes had no significant difference for seed weight loss when compared with homozygous ARL2 containing seeds.
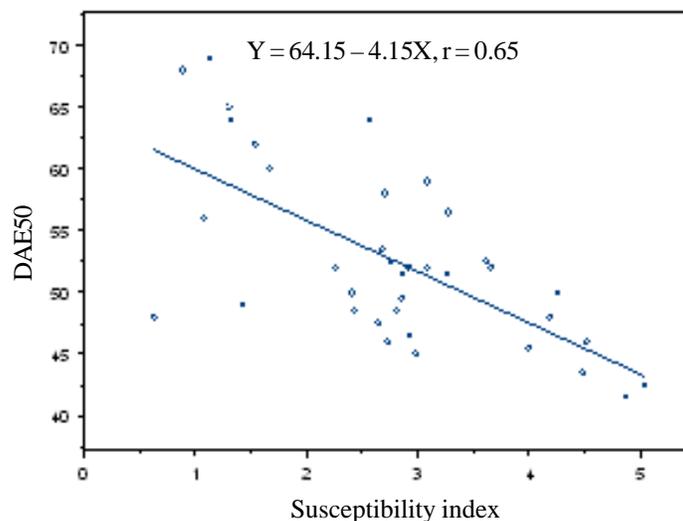
$$Y = 64.15 - 4.15X, r = 0.65$$

Figure 2. Relationships between days for 50% F_1 adult A. obtectus emergence and susceptibility index.
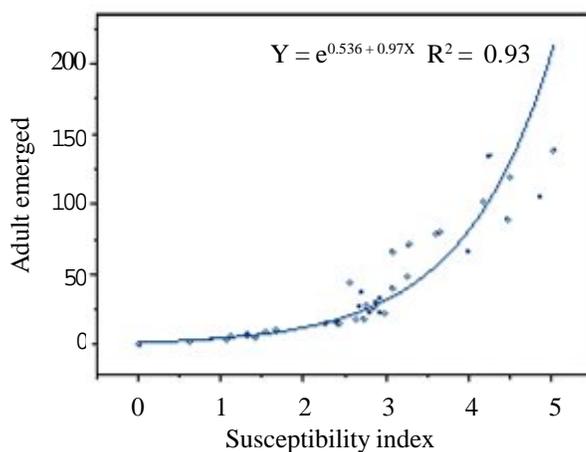
$$Y = e^{0.536 + 0.97X} \quad R^2 = 0.93$$

Figure 3. Relationships between total number of emerging F_1 adults and susceptibility index.

Seed weight loss was highly correlated with severity of damage (Fig. 4). Bean seed lots with severe perforation (more than 5 holes seed⁻¹) demonstrated high seed weight loss. Bean samples without ARL proteins had the highest number of severely damaged seeds, with more than 5 holes and thus suffered higher seed weight loss. Seed weight loss was also highly correlated to total number of perforated seeds.

No adults of *A. obtectus* that emerged from seed samples of the parent tepary bean G40199 after a period of 120 days. Larvae penetration into the seeds was clearly visible as powdery/floury appearance of the seeds and no larvae were observed to remain outside the seeds (not presented). But no adult emerged after another 90 days of observation following the oviposition. Similarly, no F_1 adults emerged from some of the backcross lines with homozygous presence of ARL2 of the APA proteins (ARL/ARL). These interspecific lines were kept for further screening and seed increase.

Weight and size of F_1 A. obtectus adults

Reduced fresh weight of F_1 adults. Significant differences in size of emerging adults were observed (Fig. 5). Earlier emerging F_1 adults from ICA Pijao, Rojo and backcross lines with null expression of arcelin and ARL2 proteins were consistently larger in size than the emerging adults from seeds with ARL2 of the APA proteins. The mean live weight of 10 F_1 adults emerging from arcelin plus ARL2 seeds were significantly low with mean insect weight almost twice lower than the adults emerging from non ARL protein seeds (Fig. 6).

DISCUSSION

The observed resistance to *A. obtectus* was consistently associated with the expression of a 33kDa seed storage lectin, like proteins among interspecific hybrids. This is a major Lectin-Like Protein (LLP) profile that cosegregates with the expression of arcelin and ARL2 genes in G40199 and interspecific hybrids as observed in a previous study (Mbogo *et al.*, 2009). Homozygous lines demonstrated higher resistance compared to the segregating genotypes in the two backcross populations. However, there were variations in number of
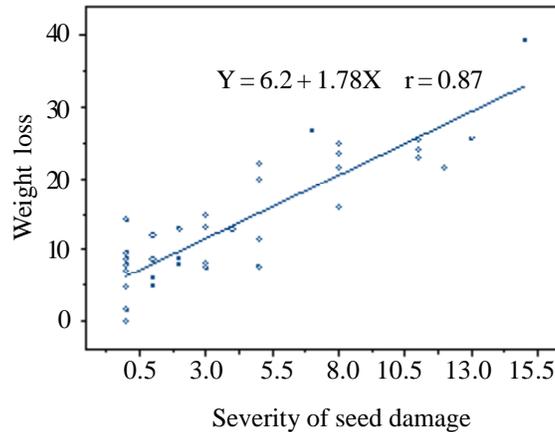

Figure 4. Relationships between percent seed weight loss and severity of damage (number of seeds with >5 holes) after A. obtectus damage.
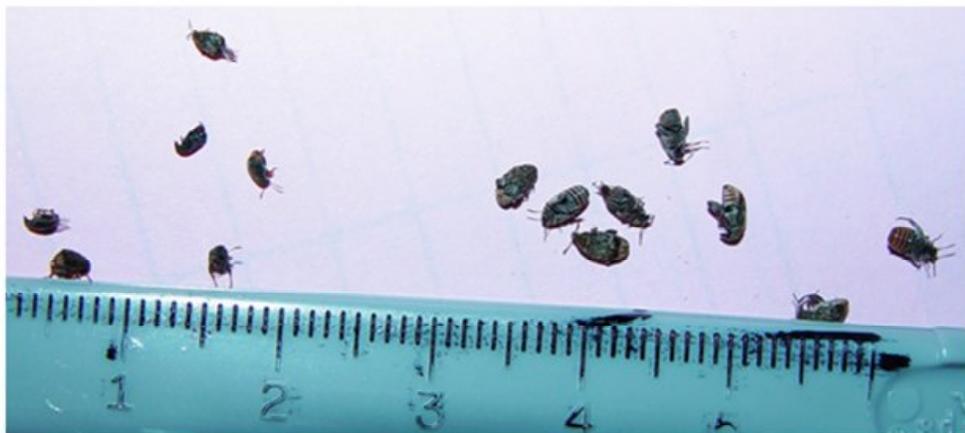

Figure 5. Size difference of F_1 A. obtectus adults emerging from interspecific hybrids seeds expressing ARL proteins (left) and from non ARL expressing seeds (right).
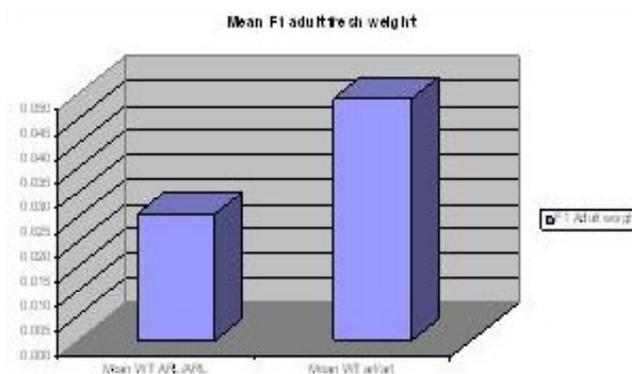
Figure 6.   Fresh weight of F_1 A. obtectus adults collected from seeds with and without arcelin-ARL2 proteins of interspecific hybrids during feeding trials.

F_1 adults that emerged from homozygous ARL/ARL containing seeds and delay in 50% adult emergence among individual backcross lines. If the resistance is highly associated with the presence of the 33kDa and the other linked subunits of LLP, then a variation in concentration, composition and conformation of the proteins may exist among the seeds of genotypes expressing the ARL2 proteins. These variations were some of the factors contributing to variations in means for variables observed during the screening trial. Some lines expressing the 33kDa proteins profiles were not at all damaged during feeding trial, and were re-inoculated for the second time then only few weak F_1 adults emerged late.

The delay in adult emergence observed among interspecific hybrids containing homozygous expression of the 33kDa protein profiles (Fig. 1) were remarkably high compared with susceptible cultivars Rojo and ICA Pijao used in the hybridisation. A mean delay for first adult emergence of 62.8 days (two months after infestation) among interspecific genotypes containing the protein is a significant factor for storage in tropical bean producing countries. Some of the backcross interspecific hybrids lines had as high as 69 days delay in emergence of first adult (Fig. 1). This means significant damage may occur very late during storage, farmers may be able to avoid this damage with minimum integration of pest management strategies like cultural, use of plant product (Schhonhoven and

Cardona, 1986; Mazzonetto and Vendramim, 2003) that will further prolong the DAE for new adults and increase adult mortality of bruchids. In addition to delayed emergence, emerging adults from these bean genotypes were fewer, small size and weak.

Full resistance to *A. obtectus* was only observed in samples of wild tepary bean G40199 where no single F_1 adult emerged from either beans with seed coat removed or beans with intact seed coat. The total absence of damaged seeds in the parent G40199 indicates that the 33kDa lectin-like proteins in this accession may not be the only factor responsible for its antibiosis. Alternatively, the concentration of LLPs in this accession may be significantly high to inhibit any development of larvae inside the seed. The 33kDa protein in G40199 was observed as the major protein almost replacing phaseolin content in the total seed storage protein (Kusolwa, 2007).

Backcross interspecific hybrid lines with the 33kDa proteins that demonstrated high antibiosis to the bruchids, may have a relative higher expression of the protein to the level comparable to that of G40199. Genomics and proteomics studies from Mbogo *et al.* (2009) and Kusolwa and Myers (2010) demonstrated that the accession G40199 contain the APA locus with variants arcelins and other APA locus proteins are expressed in seeds of this genotype. The tightly linked complex APA locus was transferred into common bean by interspecific hybridisation demonstrating the presence of expression of

arcelins alpha amylase inhibitors and phytohemagglutinins. It was, therefore, expected that the expression of APA locus in interspecific hybrids will confer the same level of resistance to bruchid similar to G40199. However, bruchid screening from interspecific hybrids shows that the introgressed APA locus highly increased resistance of common bean genotypes to *A. obtectus* though not to same level of resistant as of the parent source tepary bean G40199. These results indicate that the APA locus containing arcelin and ARL2 proteins is not the only factor associated with full resistance in G40199. Tepary beans are known to contain high quantities of other antinutritional proteins and other compounds (Idouraine *et al.*, 1992; Magdi *et al.*, 2003; Campos *et al.*, 2004) that may play an important role in antibiosis to bruchids.

These proteins and probably other factors yet to be identified were likely not transferred into common bean by interspecific hybridisation or might have been lost during backcrossing and bridge parent hybridisation process. Chromosome elimination and loss of quantitative traits among interspecific hybrids in common bean x tepary bean crosses are known (Rabakoarihanta *et al.*, 1979; Federici and Waines 1989; Mejia Jimenez *et al.*, 1994; Muñoz *et al.*, 2004). When the total seed storage proteins from interspecific hybrids and G40199 were compared by SDS-PAGE profiles, modification of the 26kDa and 28kDa were observed (Kusolwa, 2007). These may be part of factors that modify the composition of protein subunits of the APA locus in the interspecific hybrids resulting into difference in levels of resistance. To minimize this problem, we developed in bred congruity backcross lines of interspecific hybrids to G40199 containing two doses from *P. acutifolius* and *P. vulgaris* (ICA Pijao), followed by two selfing generations.

The reduction of fresh F_1 adult weight in insects emerging from seeds containing homozygous expression of the ARL2 proteins is an important supporting evidence that this protein is highly associated with resistance to *A. obtectus*. Poor digestibility of arcelin proteins by insect's larvae enzymes was observed in arcelin containing beans (Minney *et al.*, 1990; Goossens *et al.*, 2000; Paes *et al.*, 2000) this might have contributed to adult size and weight reduction of emerging adults. The arcelin and ARL2 proteins introgressed into interspecific hybrids may have contributed to the disruption of the digestive system of the larvae thus affecting its development. The weak-emerging adults may have suffered other physiological disabilities that contributed to the total delay in instar development, and possible reproductive disorders may also be expected. Consequently, the subsequent insect generations may experience low fecundity causing further weakening the entire bruchid colony in a given bean stock containing this ARL2 and its variants from the APA proteins.

Significant reduction in seed damage demonstrated by reduced seed weight loss and low number of severely perforated seeds with 5 or more holes was highly associated with these introgressed proteins among the backcross families. Low seed damage was highly correlated with the reduced adult F_1 fresh weight, low susceptibility index, and reduced number of emerging F_1 adults.

Although the full resistance observed in G40199 was not transferred into two common bean lines by interspecific hybridisation, the level of resistance reported in homozygous lines for the expression of ARL2 is highly significant compared with the susceptible parent common bean cultivars Rojo and ICA Pijao. Delayed F_1 adult emergence, reduced number of F_1 adults, and reduction in size and weight of adults are important factors for bruchid resistance common bean cultivars. Bruchid screening confirmed the stability of the introgressed ARL2 of the APA locus proteins into common beans as suggested by the genomic analysis for expression of APA genes in backcross lines (Kusolwa and Myers, 2010).

To our knowledge, this is the first work that describes the transfer, stable introgression into common bean cultivars and characterisation of arcelins and ARL2 proteins in tepary bean accession G40199. This highly resistant accession has been used by other researchers to study resistance to bruchid species and efforts to make interspecific hybrids with common beans have been attempted (Mejia Jimenez *et al.*, 2002; Cardona *et al.*, 2005). However, no stable self

reproductive plants have been developed with resistance to *A. obtectus*. Prior to this work, characterisation of antibiosis factors of the lectin-like protein variants in this accession and their association to resistance to *A. obtectus* was not documented. This is the second stable abiotic resistance factor transferred from tepary bean to common bean cultivars in addition to resistance to common bean bacterial blight (*Xanthomonas campestris* pv. *phaseoli*) (Singh and Muñoz, 1999).

REFERENCES

Campos, J.E., Whitaker, J.R., Yip, T., Hutchens, T.W. and Blanco-Labra, A. 2004. Unusual structural characteristics and complete amino acid sequence of a protease inhibitor from Phaseolus acutifolius seeds. *Plant Physiology and Biochemistry* 42:209-214.

Cardona, C., Kornegay, J., Posso, C.E., Morales, F. and Ramirez, H. 1990. Comparative value of four arcelin variants in the development of dry bean lines resistant to the Mexican bean weevil. *Entomology Experimental and Applicata* 56:197-206.

Cardona, C., Dick, K., Posso, C.E., Ampofo, K. and Nadhy, S.M. 1992. Resistance of a common bean (*Phaseolus vulgaris* L.) cultivar to post-harvest infestation by Zabrotes subfasciatus (Boheman) (Coleoptera: Bruchidae) 2. *Storage pests and Tropical Pest Management* 38:173-175.

Cardona, C., Valor, J.F., Mejia, A., Beebe, S. and Tohme, J. 2005. Developing germplasm with resistance to pests: Zabrotes, Acanthocelides – bruchids. CIAT – Annual Report. pp. 53-59.

Federici, C.T. and Waines, G. 1989. Interspecific hybridization of common beans with tepary beans: Efficacy of intraspecific hybrids vs inbred plants as female parents for interspecific hybrid formation. *Annual Reports Bean Improvement. Cooperative* 32: 70-71.

Gerhardt, I.R., Pappas, G., Chrispeels, M.J. and Grossi de Sá, M.F. 2000. Insecticidal arcelin genes of the common bean are subject to divergent selection. *Biotechnology* 130:87-98.

Goossens. A., Quitero, C., Dillen, W., De Rycke, R., Flower Valor J., De Clercq, J., Van Montagu, M., Cardona. C. and Angenon, G. 2000. Analysis of bruchid resistance in the wild common bean accession G02771: No evidence for insecticidal activity of arcelin 5. *Journal of Experimental Botany* 51:1229-1236.

Hartweck, L.M., Cardona, C. and Osborn, T.C. 1997. Bruchid resistance of common bean lines having an altered seed protein composition. *Theoretical Applied Genetics* 95:1018-1023.

Idouraine, A. and Sathe, K.S. 1992. Biological evaluation of flour and protein extract of tepary bean (*Phaseolus acutifolius*). *Journal of Agriculture and Food Chemistry* 40:1856-1859.

Kornegay, J.L. and Cardona, C. 1991. Inheritance of resistance to Acanthoscelides obtectus in a wild common bean accession crossed to commercial bean cultivars. *Euphytica* 52:103-111.

Kornegay, J., Cardona, C. and Posso, C.E. 1993. Inheritance of resistance to Mexican bean weevil in common bean, determined by bioassay and biochemical tests. *Crop Science* 33:589-594.

Kusolwa, P.M. and Myers, J.R. 2010. Phylogenetic relationship of lectin-like proteins expressed in tepary and common beans. *Aspects of Applied Biology* 96:52-57.

Kusolwa, P.M. 2007. Breeding for bruchid resistance in common bean (*Phaseolus vulgaris* L.): Interspecific introgression of lectin-like seed proteins from tepary bean (P. acutifolius A. Gray), genetic control and bruchid characterization. Ph.D. Thesis, Oregon State University, Corvallis, Oregon, USA.

Lioi, L., Sparvoli, F., Galasso, I., Lanave, C. and Bollini, R. 2003. Lectin related resistance factors against bruchids evolved through a number of duplication events. *Theoretical Applied Genetics* 107: 814-822.

Lioi, L., Galasso, I., Santantonio, M., Lanave, C., Bollini, R. and Sparvoli, F. 2006. Lectin gene sequences and species relationships among cultivated legumes. *Genetic Resource and Crop Evolution* 53:1615-1623.

Magdi, A.O., Phyllis, M.R. and Weber, C.W. 2003. The effect of feeding tepary bean *(Phaseolus acutifolius)* proteinase inhibitors on the growth and pancreas of young mice. *Pakistan Journal of Nutrition* 2 (3):111-115.

Mazzonetto, F. and Vendramim, J.D. 2003. Effect of powders from vegetal species on Acanthoscelides obtectus (Say) *(Coleoptera: Bruchidae)* in stored beans. *Neotropa Entomologica* 32:145-149.

Mbogo, P.K., Myers, J.R. and Davis, J. 2009. Transfer of the Arcelin-Phytohemmaglutinin-α Amylase inhibitor seed protein locus from tepary bean (Phaseolus acutifolius A. Gray) to common bean (*P. vulgaris* L.). *Biotechnology* 8:285-295.

Mejia-Jiménez, A., Muñoz, C., Jacobsen, H.J., Roca, W.M. and Singh, S.P. 1994. Interspecific hybridization between common and tepary beans: Increased hybrid embryo growth, fertility, and efficiency of hybridization through recurrent and congruity backcrossing. *Theoretical and Applied Genetics* 88:324-331.

Mejia Jimenez, A., Galindo, L., Criollo, A., Beebe, S., Cardona, C. and Tohme, J. 2002. Interspecific hybridization of common and tepary bean through double congruity backcrosses. CIAT Biotechemistry Annual Reports. pp. 6-11.

Minney, B.H.P., Gatehouse, A.M.R., Dobie, P., Dendy, J., Cardona, C. and Gatehouse, J.A. 1990. Biochemical bases of seed resistance to Zabrotes subfasciatus (bean weevil) in Phaseolus vulgaris (common bean); a mechanism for arcelin toxicity. *Journal of Insect Physiology* 36:757-767.

Mirkov, E.T., Wahlstrom, J.M., Hagiwara, K., Finardi-Filho, F., Kjemtrup, S. and Chrispeels, M.J. 1994. Evolutionary relationship among proteins in the phytohemagglutinins - arcelin and amylase inhibitor family of the common bean and its relatives. *Plant Molecular Biology* 26:1103-1113.

Misangu, R.N. 1997. Distribution of bean bruchid species in ten major bean growing regions of Tanzania and breeding beans for resistance to Acanthocelides obtectus (Say) and Zabrotes subfasciatus (Boh). Ph.D. Dissertation – Sokoine University of Agriculture- Tanzania. 215pp.

Muñoz, L.C., Blair, M.W., Duque, M.C., Tohme, J. and Roca, W. 2004. Introgression in common bean x tepary bean interspecific congruity backcross lines as measured by AFLP markers. *Crop Science* 44: 637-645.

Myers, J.R., Davis, J., Kean, D., Nchimbi-Msolla, S. and Misangu, R. 2001. Backcross Breeding to Introduce Arcelin Alleles into Improved African Bean Cultivars. Bean/Cowpea Collaborative Research Support Program. East Africa Proceedings: Bean Seed Workshop Arusha, Tanzania January 12-14.

Osborn, T.C., Blake, T., Gepts, P. and Bliss, F.A. 1986. Bean arcelin 2. Genetic variation, inheritance and linkage relationships of a novel seed protein of *Phaseolus vulgaris* L. Theoretical and Applied Genetics 71: 847-855.

Osborn, T.C., Alexander, D., Sun, S.S.M., Cardona, C. and Bliss, F.A. 1988. Insecticidal activity and lectin homology of arcelin seed protein. *Science* 240:207-210.

Paes, N.S., Gerhardt, I.R., Coutinho, M.V., Yokoyama, M., Santana, E., Harris, N., Chrispeels, M.J. and Grossi de Sá, M.F. 2000. The effect of arcelin-1 on the structure of the midgut of bruchid larvae and immuno-localization of the arcelin protein. *Journal of Insect Physiology* 46:393-402.

Pratt, R.C., Singh, N.K. and Bressan, R.A. 1984. Transfer of an apparent 30 kD seed polypeptide from tepary bean *(Phaseolus acutifolius)* to common bean (*Phaseolus vulgaris*) (abstract No. 795). *Plant Physiology* 75:5-141.

Pratt, R.C., Singh, N.K., Shade, R.E., Murdock, L.L. and Bressan, R.A. 1990. Isolation and partial characterization of a seed lectin from tepary bean that delays bruchid beetle development. *Plant Physiology* 93:1453-1459.

Rabakoarihanta, A., Mok, D.W.S. and Mok, M.C. 1979. Fertilization and early embryo development in reciprocal interspecific crosses of Phaseolus. *Theoretical and Applied Genetics* 54:55-59.

Schoonhoven, A.V. and Cardona, C. 1986. Main insect pests of stored beans and their control. Study guide CIAT.

Shade, E.R., Pratt, R.C. and Pomeroy, M.A. 1987. Development and mortality of the bean weevil, Acanthocelides obtectus (Coleoptera: Bruchidae), on mature seeds of tepary beans, Phaseolus acutifolius, and common beans *(Phaseolus vulgaris). Environ. Entomol.* 69: 1067-1070.

Singh, S.P. and Muñoz, C.G. 1999. Resistance to common bacterial blight among Phaseolus species and common bean improvement. *Crop Science* 39:80-89.