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Backcross Breeding to Introduce Arcelin Alleles into Improved African Bean Cultivars

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INTRODUCTION

Bruchids, or bean weevils are a major pest of dry bean seed around the world. Two bruchid species (*Zabrotes subfasciatus* and *Acanthoscelides obtectus*) are found in East Africa. In general, *Z. subfasciatus* is thought to prefer lower elevations and warmer temperatures, while *A. obtectus* is found in higher latitude environments with cooler temperatures. However, in Tanzania such environmental preference is difficult to detect, and species composition varies over time and across regions (R. Misangu, SUA, personal communication).

Traditional methods for control of these pests in East Africa include the use of ash, insecticides (both synthetic and natural), and physical barriers. Physical barriers are less likely to be effective against *A. obtectus* because this species will infest seeds in the field before harvest. The mixing of other substances with the beans is limiting in that the substances may be toxic to humans and thus are only suitable for preserving seed for planting; or the substances may be difficult to apply on a large scale. Another effective practice is to shake or tumble the seeds several times per day to disrupt larvae while they are burrowing into the seed (Quentin et al., 1991). Additionally, solar drying and drum storage technologies developed by the West African Bean/Cowpea CRSP may be transferable to improve bean storage in East Africa, although it remains to be seen if these techniques will work under the higher rainfall conditions of the bean growing regions.

Bean cultivars with resistance to both species of bruchids would be valuable in preventing infestations and eliminating the need for external applications of pesticides. Smallholder farmers are forced to sell their crop quickly, or risk substantial spoilage and yield loss within two months of harvest. As a result, the price of beans fluctuates in the market from a low price just after harvest, to a high price just before the beginning of the long rain planting season. Farmers with bruchid resistant beans would be able to keep their beans longer and would be able to sell them for a higher price later in the season (K. Ampofo, personal communication). Long-term storage is probably most important in areas where a single crop per year is produced, and beans must be stored for up to nine months, such as in southern Tanzania. Resistance combined with physical treatments and/or suitable containers is probably the best long-term strategy for reducing storage losses caused by bruchids. In this paper, we discuss progress towards developing improved bean varieties with bruchid resistance.

Arcelins are seed storage proteins of the phytohemagglutinin – arcelin – α-amylase inhibitor gene family, first discovered in wild common bean (*Phaseolus vulgaris* L.) accessions from Mexico (Osborne et al., 1988). Cultivated common bean cultivars lack these proteins that were presumably excluded by the genetic selections that occurred during domestication. Proteins in this gene family appear to have evolved the ability to deter vertebrates and invertebrates from feeding on mature bean seed. In particular, certain alleles of arcelin are toxic to bruchids and will reduce or prevent predation by these seed insects. Seven alleles are known in addition to the wild type allele, and are designated *arc1* – *arc7* (Osborne et al., 1986; Liloi and Bollini, 1989; Santino et al., 1991; Kornegay et al., 1993; Suzuki et al., 1995, Acosta-Gallegos et al., 1998; Goossens et al., 2000). Wild bean lines with the different arcelin types inhibit to different degrees the development of bruchid larvae of both species feeding on the seeds. *Arc1* has been shown to be very effective against *Z. subfasciatus* but not *A. obtectus.* Wild bean accessions with *arc4* confer resistance to both bruchid species. Other factors may be involved in conferring resistance to *A. obtectus* (Fory et al., 1996; Hardwick et al., 1997 Goossens et al., 2000).

Researchers at CIAT developed the RAZ lines, which possess the *arc1* allele (Cardona et al., 1992). Misangu (personal communication) used the RAZ lines to transfer *arc1* into several of the advanced lines in the SUA bean breeding program. One cultivar, a Rojo backcross line, is nearing final testing in on-farm trials, and should be released in 2001.

By combining a phaseolin null mutation with the arcelin genes, researchers have found enhanced resistance at least in the case of *arc1* (Hardwick et al., 1997). In phaseolin – arcelin + lines, a greater proportion of the seed storage protein is arcelin (Hardweck & Osborn, 1997). Germplasm was obtained from Tom Osborn at the University of Wisconsin that is phaseolin null, and possesses *arc1, arc2*, or *arc4*. In addition, CIAT discovered a wild tepary (*Phaseolus acutifolius)* accession with *A. obtectus* resistance. Seed of this accession was obtained from CIAT, tested at SUA, and resistance to *A. obtectus* was confirmed.

Arc^2 and *Arc^4* Alleles

At Oregon State University, we have been pursuing a backcross strategy to incorporate *arc2* and *arc4*, phaseolin null alleles into the Rojo background. The procedure consists of making crosses between the SMARC lines with the appropriate alleles and Rojo, allowing the F_1 to self-pollinate, and screening individual F_2 seeds for the required phenotype. Selection is carried out using sodium dodecyl sulphate poly acrylamide gel electrophoresis (SDS PAGE) to determine the seed storage proteins (Figure 1). A small portion of the cotyledon of a seed is removed and the crude protein mixture is extracted. The extract is run out on the gel and stained with Coomassie Blue to visualize the protein. Bands are compared to appropriate markers to determine seed phenotype. The selected seeds are grown in the greenhouse and crossed to the recurrent parent. Two backcrosses have been performed following the procedure outlined in Figure 2. One additional backcross will be needed to sufficiently recover the Rojo phenotype while maintaining the desired combination of seed storage protein alleles (Figure 3).

Resistance in Tepary

The resistance mechanism in tepary accession G40199 is unknown. In addition, transfer of bruchid resistance from tepary into common bean requires the use of embryo rescue for at least two generations. Therefore, a two-pronged approach was pursued where crosses were made to a cultivated tepary bean in order to study inheritance of the trait. At the same time we tested the possibility of using tepary – common bean congruency backcross lines as a bridge to transfer the trait without resorting to embryo rescue. We also examined the seed storage protein profile to investigate the hypothesis that the wild tepary accession possesses a variant arcelin allele.

The wild tepary accession was difficult to bring into flower, and it appeared that a combination of short days and warm temperatures were required. Crosses to the cultivated tepary were successful, and the F_1 was grown to produce F_2 seed for inheritance studies. These studies will be carried out with analysis of seed storage protein on an individual basis correlated with bruchid feeding tests.

The crosses to eleven congruency backcross lines were not successful. Pods and ovules developed for approximately two to three weeks before ovules aborted. Thus, embryo rescue will be needed to transfer the trait to common bean.

When seed storage proteins from G40199 were compared to the cultivated tepary and common bean varieties by SDS PAGE, a set of bands were present in the wild tepary, but not in the cultivated species (Figure 4). These bands had a molecular weight of approximately 35 kd and were within the range of the weight of proteins coded by other arcelin alleles. While the data suggest that G40199 may contain an arcelin allele, and it follows that this allele codes for a protein that is responsible for *A. obtectus* resistance, additional research is required to verify this hypothesis. Also, experience with arcelin in wild common bean accessions suggests that arcelins may not be the only factors involved in resistance to *A. obtectus*.

RESEARCH QUESTIONS AND CONCLUSIONS

With the bruchid resistance research, a number of questions remain to be answered. First, are the selected *arc1* containing lines effective at the farmer level? Because *arc1* confers resistance mainly to Z. *subfasciatus*, it will not prevent field infestations with *A. obtectus*. Resistance should be effective if *A. obtectus* is not present, and the gene may retard population buildup of *Z. subfasciatus* if both species are present. We also do not know if selection based solely on seed storage protein profile will be effective. There is a need to conduct parallel selections based on resistance reaction to determine if this strategy would be more effective in developing resistant lines. Can we transfer resistance from tepary bean into common bean? It should be possible with a concerted effort using embryo rescue.

Because the arcelin alleles all occur at one locus, it is impossible to combine two forms of arcelin in the same cultivar. As such, a multiline strategy might be effective, where a mixture of arcelin types could be grown in the same field.

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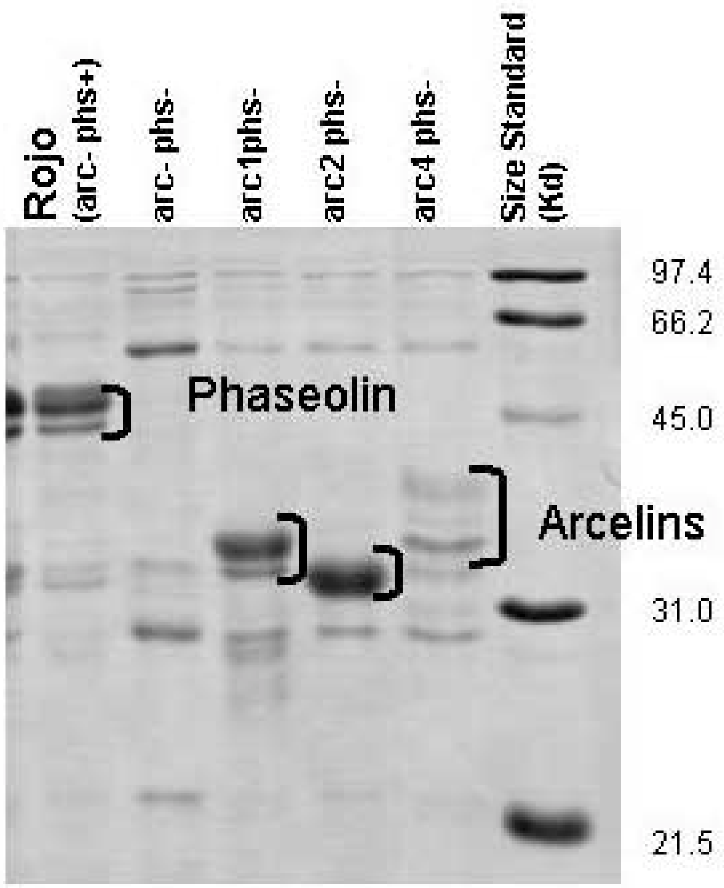

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Figure 1. SDS-PAGE of seed storage proteins from Rojo and four bean breeding Lines (*Phaseolus vulgaris*) with and without phaseolin and arcelin.

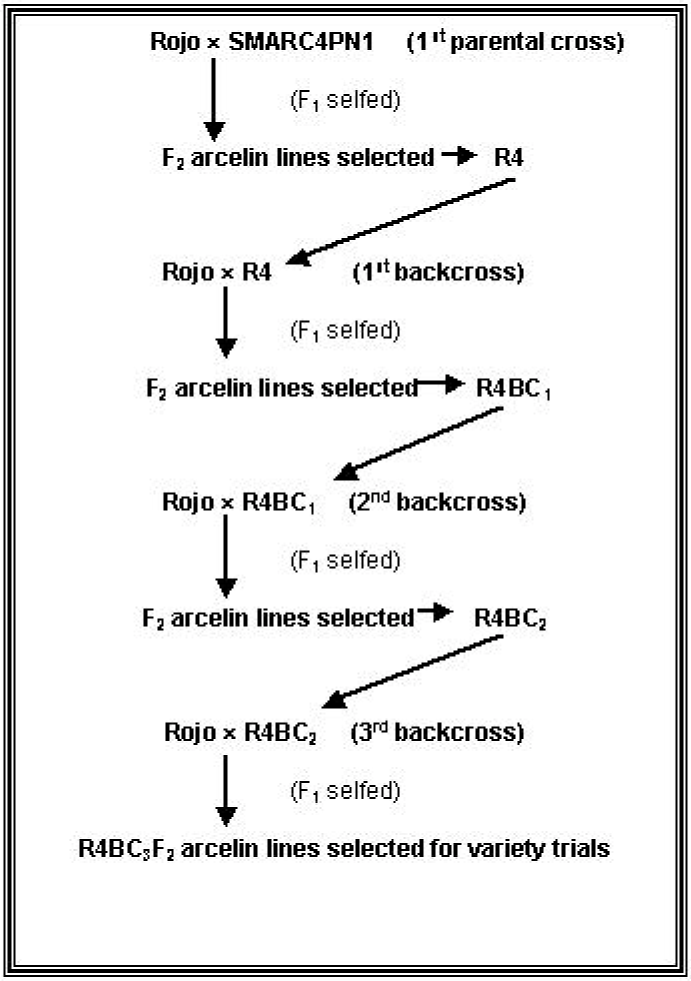

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Figure 2. Backcross procedure for transferring arcelin into the bean variety Rojo.

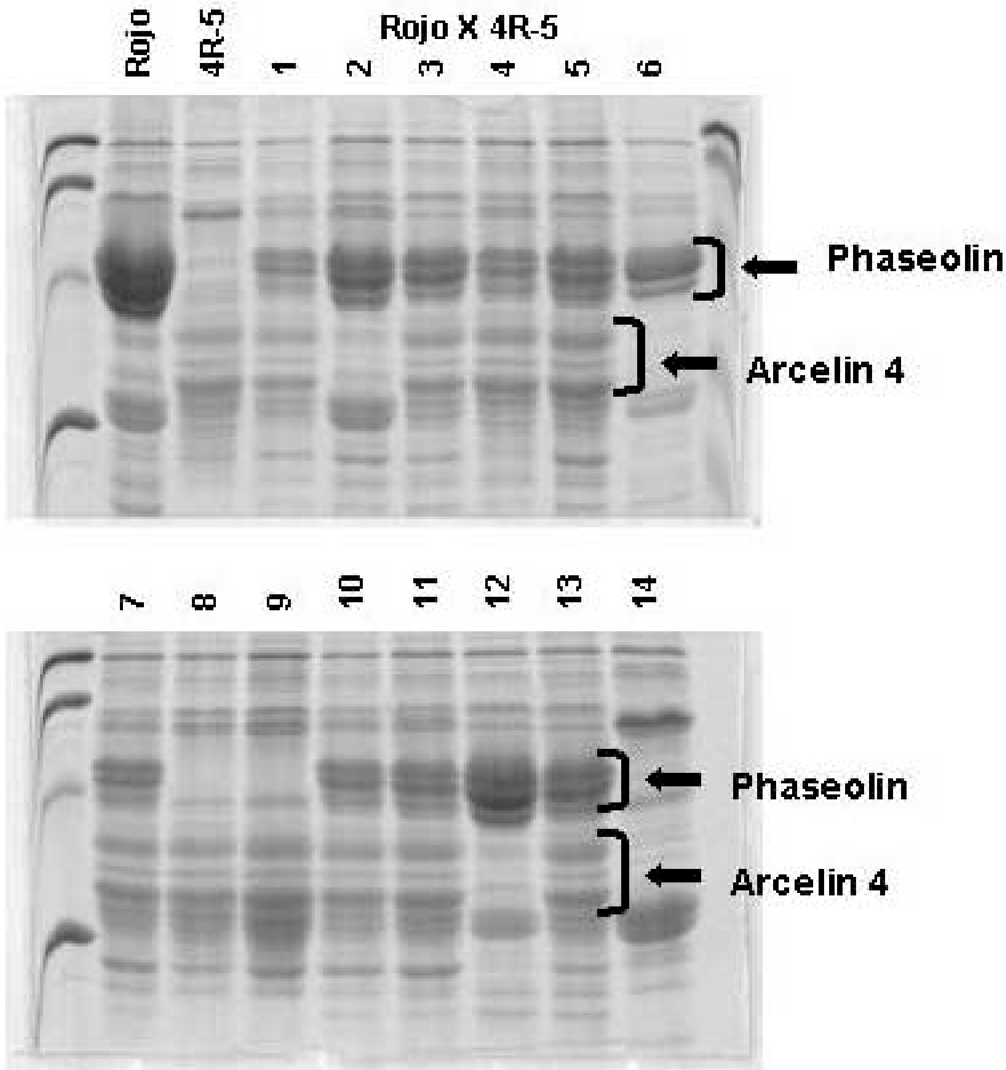

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Figure 3. SDS PAGE of seed storage proteins of the parents (Rojo and 4R-5) and fourteen progeny from the first backcross to assess transfer of phaseolin null and arcelin 4. 4R-5 is a product from the cross SMARC4PN1 X Rojo. Size markers in Kd from top to bottom: 97.4, 66.2, 45.0, 31.0, 21.5.

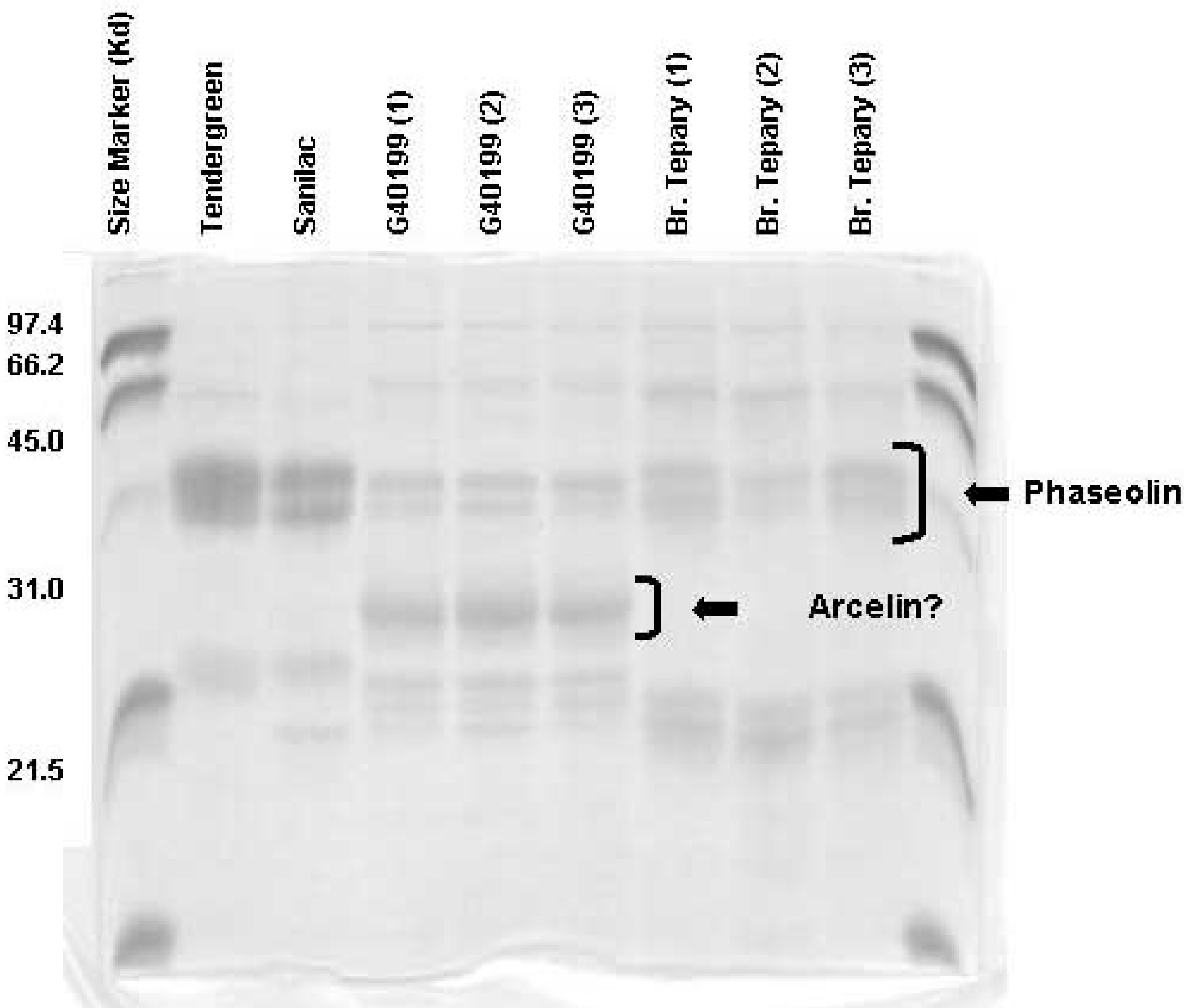

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Figure 4. SDS PAGE of seed storage proteins of common bean and tepary bean, showing the presence of an arcelin-like band in G40199 wild tepary bean. Brown (Br.) Tepary is a cultivated accession.

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