BREEDING BEANS RESISTANT TO BRUCHIDS

Rhea Harmsen[1], F.A. Bliss[1], and T.C. Osborn[2]

Depts. of Horticulture[1] and Agronomy[2], Univ. of Wisconsin, Madison, WI 53706

Although several thousand cultivated bean accessions have been screened for resistance to the Mexican bean weevil (Zabrotes subfasciatus) and the common bean weevil (Acanthoscelides obtectus), levels of resistance among cultivated materials have been too low to be of economic importance. Excellent sources of resistance have been found, however, among wild bean accessions. (CIAT, 1981).

A new seed protein was identified in a seed of a wild bean accession(PI 325690-3) and named arcelin. Although levels of the main protein fraction, phaseolin, were reduced when arcelin was present, total seed protein was not reduced, suggesting this new fraction compensated for the reduction in phaseolin. Presence of arcelin was dominant to absence (Romero Andreas et al., 1986). Three other distinct electrophoretic variants of this protein were identified in a wide survey of wild bean lines. Genetic studies revealed all four arcelin variants were codominant, and each was inherited as a single Mendelian unit (Osborn et al., 1986). The alleles have been designated Arc1, Arc2, Arc3, and Arc4. Examination of seeds of bruchid resistant wild lines invariably reveals the presence of arcelin, whereas in susceptible seeds it is absent.

Preliminary insect feeding trials were conducted on lines(from a cross of PI325690-3 to Sanilac) homozygous for the presence (Arc1/Arc1) and absence (arc/arc) of arcelin. Percentage of emerged adults of Z. subfaciatus was very low in the arcelin containing ($Arc1^+$) lines (2.3%) as contrasted with their arc^- counterparts (86%). Segregating lines had intermediate levels of emerged adults (36%) (Posso, personal communication). Only low resistance to A. obtectus existed in the Arc1 lines. Wild lines containing the Arc4 allele, however, had a striking level of resistance to A. obtectus (i.e. G12953, G12952, G12949).

Rat feeding trials of the $Arc1^+$ and arc^- lines showed that arcelin protein in cooked beans is not toxic to rats (Hosfield, personal communication).

To confirm the relationship of arcelin to bruchid resistance and to explore the implications of the diverse arcelin types on the different organisms, a recurrent backcrossing program was initiated to transfer all the arcelin types into the same adapted background. A white bean (Sanilac) and a black bean (Porrillo 70) were used as recurrent cultivated parents in two sets of crosses. The wild bean donors were G12882 (Arc1), G12866 (Arc2), G12922 (Arc3), and G12949 (Arc4).

Three backcrosses, followed by two cycles of selfing have been conducted (BC_3S_2 population). Lines developed in an identical manner have been obtained from each cross which are Arc/Arc and arc/arc. These two types of lines are designated "paired lines." A further backcross was conducted and these materials are currently being selfed to obtain another set of paired lines (BC_4S_2 population). The contrasts provided by these "near isogenic" paired lines allows the elimination of several confounding factors in determining the relationship of arcelin to resistance; including seed color, size and hardness of wild seed in comparison to susceptible cultivars, etc.

In the development of paired lines two methods were used to screen for the presence of arcelin and the steps at which screening was employed are detailed in

Table 1. The first method used was antibody screening using the Ouchterlony method (1949) in agarose gel diffusion plates. A small portion of each seed was scraped, the flour was suspended in phosphate buffered saline (PBS) pH 7.1 and an aliquot was placed in a well made in an agarose diffusion plate. Arcelin antibody was placed in the center well and the formation of a precipitate line halfway between the two wells indicated the presence of arcelin. Polyclonal antibody raised to arcelin-1 is cross-reactive for all arcelin types. Thus, this test is not specific for arcelin type. In cases where the type of arcelin must be confirmed or identified SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) is employed. (Lämmli, 1970).

Investigations in progress employing all sets of paired lines include: insect seed and pod feeding trials, evaluation of agronomic traits, cooking time trials, and rocket immuno-electrophoresis to determine arcelin protein quantity relative to the arcelin allele and to other seed protein fractions. Strategies for the deployment of the four arcelin alleles either in pure line or in multiline mixtures are being studied. Furthermore, artificial seeds using purified arcelin protein will be used in insect feeding trials.

The implications of the availability/existence of an arcelin single gene marker for bruchid resistance, coupled with this efficient, non-destructive method of screening single seeds, could be far reaching. It is a system which can be readily adapted to a large scale breeding program in the following steps: 1) Cross of a cultivated line to an arcelin donor. Adapted arcelin donors can be developed in each seed color and size class. 2) Recurrent backcrosses to the elite line, interspersed by individual seed screening using the antibody test. 3)Selfing to obtain homozygous arcelin containing lines. 4) In any further intercrossing (for improvement of other traits) the presence of arcelin can be traced through antibody screening each generation and selfing can be employed to obtain pure lines.

Table 1 - Screening protocol for development of paired lines.

Generation of screening	Material screened	Material selected/advanced
1. After backcrosses	single seeds	Arc^+ seeds, Arc/arc
2. After BC_nS_1	5 seeds per plant*	Segregating lines
3. After BC_nS_2	11 seeds per plant*	Homozygous lines - Arc^+ or arc^-

* Minimum numbers required to establish genetic identity with 95% certainty. (Hanson, 1959).

REFERENCES

CIAT, 1981. Bean Program Report. Centro Internacional de Agricultura Tropical, Cali, Colombia, p51.

Hanson, W.D. 1959. Minimum family sizes for the planning of genetic experiments. Agron. J. 51:711-715.

Lämmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680.

Osborn, T. C., T. Blake, P. Gepts and F.A. Bliss. 1986. Bean Arcelin 2. Genetic variation, inheritance and linkage relationships of a novel seed protein of Phaseolus vulgaris L. Theor. Appl. Genet. 71:847-855.

Ouchterlony, O. 1949. Antigen-Antibody Reactions in Gels. Acta Pathol. Microbiol. Scan., 26:507.

Romero Andreas, J., B.S. Yandell and F.A. Bliss. 1986. Bean Arcelin. 1. Inheritance of a novel seed protein of Phaseolus vulgaris L. and its effect on seed composition. Theor. Appl. Genet. 72:123-128.