Two New Molecular Markers Linked to *bc-3*

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Summary

The recessive *bc-3* gene provides resistance to all presently known strains of bean common mosaic virus (BCMV)[1]. We have used bulked segregant analysis[2] (BSA) on segregating F_2 populations to identify two RAPD markers linked to the *bc-3* gene. These markers have been used to identify an approximate map location for *bc-3*. Also, they may prove useful in speeding the introgression of the *bc-3* gene into cultivars of Andean origin. We speculate on the evolutionary origin of the *bc-3* gene and its implications for the application of molecular marker assisted selection (MAS) in bean breeding programs.

Procedure

We used five crosses between Malawian cultivars and *bc-3* donors (provided by CIAT) to develop populations segregating for the *bc-3* gene. The population design is as follows, in which the evolutionary origin of each cultivar is indicated as **M** (Mesoamerican), or **A** (Andean).

A. 1-1 (M) X MCM 3031 (M)
B. 6-5 (M) X MCR 2204 (A)
C. 21-5 (A) X MCR 2205 (A)
D. Namajengo (M) X MCM 3031 (M)
E. 11-1 (A) X MCR 2205 (A)

We collected both leaf samples and as many seed as possible from forty to sixty F_2 plants (depending on seed available) within each population. After inoculating F_3 families of at least 24 individuals with the NL3 strain of BCMV, we scored each plant for its disease reaction (necrotic, mosaic, or resistant). For F_3 families derived from homozygous susceptible or homozygous resistant F_2 individuals we extracted DNA from reserved F_2 leaf samples and prepared, for each population, contrasting equimolar bulks of DNA from susceptible and resistant individuals. We then screened random primers (purchased from Operon Technologies, Alameda CA) to seek differences in the banding patterns between the susceptible and resistant bulks within each population.

Results

Two primers (out of 175 screened so far) amplified polymorphisms between susceptible and resistant bulks. The markers identified were $OPC11^{350}/OPC11^{420}$ and $OPC20^{460}$, and they were both polymorphic in populations C and E. Both markers were then confirmed by verifying polymorphism in the parents and each individual comprising the susceptible and resistant bulks. Standard RAPD analysis of $OPC20^{460}$ shows that the marker is linked in *trans* to the recessive resistance allele of *bc-3*, and segregates as expected of a typical dominant RAPD marker. $OPC11^{350}/OPC11^{420}$, on the other hand, is somewhat anomalous. The marker appears to be codominant, but the 350 bp product is not as strongly amplified as the 420 bp product. Reliable amplification of these markers required overloading both the primer and Taq polymerase by 30% in the reaction cocktail. In many cases, the 420 bp product was amplified faintly when the 350 bp product was also present. $OPC11^{350}$, when considered alone, segregates as a standard dominant RAPD marker linked in *cis* to the recessive *bc-3* resistance gene. Separation and scoring of

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[1]Drijfhout, E., 1978. Genetic interaction between *Phaseolus vulgaris* and bean common mosaic virus with implications for strain identification and breeding for resistance. *Agricultural Research Reports* 872, Centre for Agricultural Publishing and Documentation, Waginengen, Netherlands

[2]Michelmore, R. W. , Paran, I., and Kesseli, R. V., 1991. Identification of markers linked to disease resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions using segregating populations. *PNAS* 88: 9828 - 9832.

the bands amplified by OPC11 requires the use of polyacrylamide gels, as agarose gels do not provide sufficient resolving power for products that are less strongly amplified.

Of the two markers identified by the Michigan State University bean group as being linked to *bc-3* ($OPS13^{660}$ and $OPAD19^{690}$)[3], only $OPS13^{660}$ showed polymorphism, and only in population A. These markers were identified through BSA on near-isogenic lines of navy bean cultivars.

Although *bc-3* is not segregating in the recombinant inbred population previously used for constructing the genetic map of common bean[4], the $OPC11^{350}$/ $OPC11^{420}$, $OPC20^{460}$, and $OPS13^{660}$ markers were polymorphic in the parents (BAT93 and Jalo EEP558). By scoring each of the recombinant inbred lines (F_7) of the population for their genotypes at $OPC11^{350}$, $OPC20^{460}$, and $OPS13^{660}$ we were able to estimate their general genomic location (via χ^2 analysis) as linked to D1018, D2050, and cdc-2 on linkage group D6.

We screened thirteen cultivars (8 Andean, 5 Middle American) for their marker genotypes to determine where the $OPC11^{350}$/ $OPC11^{420}$, $OPC20^{460}$, and $OPS13^{660}$ markers will be useful for MAS. For $OPC11^{350}$/ $OPC11^{420}$ and $OPC20^{460}$ all cultivars of Andean origin presently lacking *bc-3* were positive for the $OPC20^{460}$ and $OPC11^{420}$ markers and negative for the $OPC11^{350}$ marker. Thus the first two markers could be selected against in the F_3 or later generation, or the $OPC11^{350}$ marker could be selected for in the F_2 or later generation of crosses between parents homozygous for *bc-3*. Marker $OPS13^{660}$, which is linked in *trans* to *bc-3*, was found to be absent from all cultivars of Andean origin, as well as from Middle American cultivars Sutter Pink, Gloria, and Yolano (all belonging to race Durango). This marker was present only in cultivars BAT93, Black Turtle Soup, and Sal 778 (belonging to race Mesoamerica). Therefore, it appears that this marker may be useful in some cultivars of Mesoamerican origin presently lacking *bc-3*, but not all.

We have also obtained seeds of the original source of *bc-3* (PI 181954) courtesy of Richard Hannan (USDA/ARS, Western Regional Plant Introduction Station, Pullman WA). One dimensional SDS-PAGE seed protein analysis of this accession revealed the 'S' type phaseolin, as is found in most cultivated common beans of Middle American origin[5]. Based on this evidence, as well as the pattern of molecular marker diversity described above, it appears that the *bc-3* gene originated in beans of Middle American origin[6]. Further information is needed to determine among member(s) of which race in this gene pool. Consequently MAS for introgression of *bc-3* into standard cultivars is likely to be successful for all cultivars of Andean origin, as well as some of Middle American origin. However, identification of molecular markers linked to *bc-3* and polymorphic among cultivars of the Middle American gene pool may prove to be more difficult because the genetic distance among the parents is smaller than between Middle American and Andean cultivars.

Acknowledgments

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[3]J. D. Kelly, personal communication.

[4]Nodari, R. O., Tsai, S. M., Gilbertson, R. L., and Gepts, P., 1993. Towards an integrated linkage map o common bean 2. Development of an RFLP-based linkage map. *Theor. Appl. Gen.* 85: 513 - 520.

[5]Gepts, P., Osborn, T.C., Rashka, K., and Bliss, F.A. 1986. Phaseolin protein variability in wild forms and landraces of the common bean (*Phaseolus vulgaris*): evidence for multiple centers of domestication. *Econ. Bot.* 40:451-468.

[6]Singh, S.P., Gepts, P., and Debouck, D.G. 1991. Races of common bean (*Phaseolus vulgaris*, Fabaceae). *Econ. Bot.* 45: 379 - 396