CODOMINANT INTERPRETATION OF A DOMINANT SCAR MARKER LINKED WITH POTYVIRUS RESISTANCE IN COMMON BEAN

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A major disadvantage of many PCR based RAPD and SCAR markers is that they exhibit dominant inheritance, and thus cannot be used to discriminate between homozygous (AA) and heterozygous (Aa) genotypes. For marker-assisted selection purposes codominant markers provide greater efficiency than dominant markers (reviewed by Kelly and Miklas, 1998).

For conventional PCR twice as much template DNA of the target sequence (dominant DNA marker) is present in the PCR reaction for a homozygous (AA) versus heterozygous (Aa) individual; therefore, in principal twice as much product (dominant DNA marker) should be amplified for the homozygous genotype (AA). Unfortunately, this difference in the amount of dominant DNA marker amplified between AA versus Aa individuals by conventional PCR is undetectable by gel electrophoresis. An F_2 population segregating for the *bc-1*^{2} gene and linked dominant SCAR marker $SBD5_{1300}$ (Miklas et al., 2000) was used to investigate whether codominant interpretation of a dominant SCAR marker was plausible using quantitative PCR techniques.

The segregating population consisted of 59 F_2 plants derived from a cross between pinto bean breeding lines P94207-43 (*bc-1*^{2}*//bc-1*^{2}) and P94207-189 (*bc-1//bc-1*) that were near-isogenic except for resistance to BCMV (Miklas et al., 2000). Both parents possessed the *I* gene. P94207-43 was released as the pinto bean cultivar Kodiak (Kelly et al., 1999). DNA was extracted from the first trifoliolate leaf of all plant samples using the Fast-DNA kit (BIO 101, Inc., Carlsbad, CA) according to manufacturer's recommendations. DNA was quantified with a fluorometer (TD-700; Turner Designs, Inc., Sunnyvale, CA), and diluted to 20 ng/ul for use in quantitative PCR reactions. The 1329 bp DNA sequence corresponding to the $SBD5_{1300}$ SCAR marker was analyzed using Primer Express software (Applied Biosystems, Foster City, CA) to identify candidate sequences for PCR primers and probes.

Foward primer p43335F: 5′- d-TGTACTGTGCTACCACTGCTACATCTT-3′
Reverse primer p43424R: 5′-d-CAGAGCTCAGAATTGCAGCAA-3′.
Taqman probe p43T369C: 5′-ATGCTCCCTCACATTC ATTTAAGTTTGCTGCATAT-3′

PCR for each plant sample was performed in 50 ul reactions containing100 ng of purified genomic DNA, 900 nM forward primer p43335F, 900 nM reverse primer p43424R, 100 nM TaqMan™ probe p43T369C, 5 ul ddH_2O, and 25 ul of 2X TaqMan™ Universal PCR Master Mix (Applied Biosystems). Amplifications and detection of fluorescence were done using a GeneAmp 5700 Sequence Detection System (Applied Biosystems). All PCR reactions were performed using the manufacturer's suggested default cycling profile, which consists of an initial cycle of 2 min at 50° C, then a single cycle of 10 min at 95° C, followed by 40 cycles of 15 s at 95° C and 1 min at 60° C.

The relative amount of *bc-1*^{2} present in 100 ng of total genomic DNA for each plant sample was determined by plotting the C_T value for the PCR reaction on a standard curve plot generated using total genomic DNA of the homozygous dominant *(bc-1*^{2}*//bc-1*^{2}*)* parent P94207-43. Discrimination between plants that were homozygous dominant (*bc-1*^{2}*//bc-1*^{2}) or heterozygous (*bc-1*^{2}*//bc-1*) was based on

comparisons between the results for segregating F_2 plants with results for the reference sample of four comparative heterozygous F_1 plants.

A group mean (y) and standard deviation (s_y) was calculated for the four comparative heterozygous F_1 control plants based on the combined analysis of three PCR reactions for each plant. This group of comparative heterozygous F_1 controls fit a normal distribution, thus a 99% confidence interval for all heterozygotes was determined using the formula $y \pm 2.58s_y$. F_2 plants that fell within the confidence interval were classified as heterozygotes (*bc-1²//bc-1*). F_2 plants which fell outside the tail area to the right of the confidence interval were considered to be homozygous dominant (*bc-1²//bc-1²*). F_2 plants with no fluorescence were classified as homozygous susceptible (bc-1//bc-1).

Twenty F_3 progeny from each F_2 plant were inoculated with the NL-3 strain of BCMNV. Segregation or lack thereof for resistance and susceptibility to NL-3 strain within an F_3 family enabled genotypic classification of the 59 F_2 plants as either homozygous resistant (*bc-1²//bc-1²*), heterozygous (*bc-1² //bc-1*), or homozygous susceptible (*bc-1//bc-1*).

Quantitative PCR of the Taqman probe, specifically developed for the dominant SBD5 SCAR marker, correctly (100%) discriminated heterozygous *bc-1//bc-1²* plants from homozygous *bc-1²//bc-1²* plants in the F_2 generation as confirmed by F_3 progeny tests for reaction to NL-3 strain of BCMNV. The effective application of real time fluorescent PCR for assigning genotype to plants was demonstrated previously for the *Rhg 4* locus in soybean (Meksem et al., 2001), by a process known as allelic discrimination. However, allelic discrimination requires the availability of a codominant PCR marker.

Our results indicate that the method employed in this study for assigning plant genotype based on quantitative PCR may be broadly applicable to the genotyping of diploid plants for other loci of interest for which only dominant PCR linked markers are available. The application of the quantitative PCR assay described herein will result in more timely population improvement and reduce greenhouse and field space requirements dedicated to progeny testing for disease resistance, as plants that are homozygous for dominant marker-linked resistance genes can be identified as seedlings.

References

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