IDENTIFICATION OF AFLP MARKERS IN SOYBEAN LINKED TO RESISTANCE TO *MELOIDOGYNE JAVANICA* AND CONVERSION TO SEQUENCE CHARACTERIZED AMPLIFIED REGIONS (SCARS)

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ABSTRACT

Meloidogyne javanica is the most widely spread nematode pest on soybean in South Africa. Only a few registered cultivars have some resistance to this nematode and there is an urgent need for an efficient breeding programme for resistant cultivars of all maturity groups. However, breeding is hampered by laborious screening procedures for selection of resistant lines. The objective of this study was to develop an economically viable molecular marker system for application in selection procedures. Both RFLP and AFLP screening identified markers linked to gall index variation in a segregating population of 60 F_2 progeny from a cross between a resistant cultivar, Gazelle, and a highly susceptible variety, Prima. A codominant RFLP marker(B212) was linked significantly to resistance and explained 62% of the variation in gall index. Seven AFLP markers were linked significantly to the resistance trait, of which four were linked in repulsion phase and three in coupling phase. All seven AFLP markers mapped to LG-F on the public soybean molecular map. The major QTL for resistance mapped between markers E-ACC/M-CTC2 linked in coupling phase, 8212 and E-AAC/M-CAT1, linked in repulsion phase. These two AFLP markers bracketing the major resistance QTL were successfully converted to SCARs. Marker E-ACC/M-CTC2 was converted to a codominant SCAR marker SOJA6, which accounted for 41% of variation in gall index in the mapping population. Marker E-AAC/M-CAT1 was converted to a dominant SCAR marker (SOJA7) and explained 42% of gall index variation in the mapping population. These two markers mapped approximately 3.8 cM and 2.4 cM respectively from the resistance QTL. This study represents the first report of the development of PCR-based sequence specific markers linked to resistance to *M. javanica* in soybean.

Soybeans can be cultivated on a variety of soil types, but in South Africa it is limited to heavier soil types due to the high risk of nematode infection in lighter soil types. Only a few of the registered South African cultivars have some resistance to root-knot nematodes, but these cultivars are not representative of the wide variety of maturity groups needed. Root-knot nematodes can be controlled with nematicides, but these are expensive and harmful to the environment. It would be economically more viable to control nematodes with biological methods and there is thus an urgent need for more cultivars from other maturity groups with resistance to nematodes.

Meloidogyne javanica is widespread throughout South Africa and was found in 106 of 136 districts that were surveyed (KLEYNHANS, 1991). M. *incognita* (consisting of a variety of host races) showed a distribution in 67 out of the 136 agricultural districts investigated. *M. hapla (45* out of 136) and *M. arenaria* (32 out of 136) are less abundant and are mostly found on horticultural plants. The major root-knot nematodes causing severe loss of soybean yield are *M. incognita* race 2 and 4 and *M. javanica.*

LUZZI, *et al.* (1987) tested 2370 soybean genotypes for resistance to *M. incognita* (Mi), *M. arenaria* (Ma) and *M. javanica* (Mj) based on root galling and nematode reproduction. Sixty one genotypes were found to be resistant to Mi, 56 to Ma and 61 to Mj. Inheritance of resistance to *Meloidogyne javanica* was found to be quantitative, with moderate to high heritability (estimates ranging from 0.48 to 0.76) (LUZZI, *et al.,* 1995b). The authors proposed that the different parents might possess resistance genes at different loci or different alleles at the same loci. Resistance was not maternally inherited. TAMULONIS, et *al.* (1997b) identified RFLP markers linked to genes affecting resistance to *M. javanica.* Two QTL conditioning resistance to root galling of Mj were identified. The marker B212-1 accounted for 46% of the variation in gall number and mapped to linkage group F, and marker A725-2 accounted for 13% and mapped to LG-D1. Marker B212-1 was within a cluster of other disease resistance loci on LG-F (TAMULONIS, *et al.,*1997b).

A combination of the methods of bulked segregant analysis (BSA) (MICHELMORE, *at al.,* 1991) and selective genotyping in a stepwise fashion (MIKLAS, et al., 1996) was followed for the identification of the major QTL conditioning disease resistance in soybean. The mapping strategy included a three-step process: (1) Identification of AFLP corresponding between the two parents and resistant and susceptible bulks, (2) selective genotyping of individual lines within the bulks with informative primer combinations, (3)AFLPs that cosegregated with disease reaction were mapped in the larger population. The two bulk samples were constructed by combining the pooling strategies of Michelmore (MICHELMORE, *et al.*,1991) and Giovannoni (GIOVANNONI, *et al.,* 1991). The basic difference between these methods lies in the possible applications. Basing the pooling strategy on phenotype selects a single genetic point in a population segregating for the target trait. Selection and use of DNA pools based on existing marker data can target a defined genomic interval for filling in gaps in the map with more markers, or to isolate markers in intervals likely to contain genes of interest. The combination of both types of pooling, leads to pools highly enriched for the region of the genome containing the main QTL for the resistance trait. Both RFLP and AFLP techniques are laborious and expensive, and unsuitable for accommodating large numbers of progeny testing early in a breeding program. The breeding objective is to maximize the response per unit cost, and the marker-assisted selection should be superior to phenotypic selection. Converting a molecular marker to a PCR-based marker, could lower the cost and time of MAS substantially. The aim of this study was to develop a marker(s) detected by PCR amplification methods, which is much easier to use by laboratory technicians, relatively inexpensive to perform and time saving.