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Full Length Research Paper

Tagging of resistance gene(s) to rhizomania disease in sugar beet (*Beta vulgaris* L.)

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The rhizomania disease is one of the most important diseases in Iran and some other parts of the world which potentially could play a role in decreasing sugar yield in fields. One approach to combat with this disease is the use of resistance varieties. This varieties have been identified which are having resistance genes to rhizomania disease (i.e. Rz_1, Rz_2). In order to use these genes in breeding programs (MAS) tagging these genes with molecular markers is necessary. In our study, we used infected soil which was provided from infected fields then greenhouse test was done to identify resistance and susceptible plants. Extracted DNA from leaves of resistant and susceptible plants was bulked to provide two bulks for resistance and susceptible plants. Three-hundred RAPD primers were used in analysis of the two bulks and two F_2 populations. One population was obtained from a cross between Holly1-4 as resistance parent and an annual variety as susceptible plant. The second population was constructed by crossing between WB42 as resistance parent and L 261 as susceptible one. Finally genes were tagged using two RAPD primers and one of the markers is OP-09_{1150} which is 27 cM apart from Rz_1 gene in coupling phase. The second marker is OP-$AN9_{600}$ which is 13.7 cM apart from Rz_1 gene and in repulsion phase.

Key word: Sugar beet, rhizomania disease, resistance gene, RAPD marker.

INTRODUCTION

The Rhizomania disease is an important disease of sugar beet in the world and is reported from USA, European countries and many countries of Asian including Iran (Lennoforce et al., 2000; Wisler et al., 1997; Whitney, 1989; Izadpanah et al., 1996; Nielson and Nicolason., 2001). This disease leads to decrease yield sugar in field (Duffus et al, 1984, Johnsen, 1985). Rhizomania refers to the excessive hairy root proliferations that result from infection by the necrotic yellow vein virus (BNYVV, genus Benyvirus). This virus is transmitted by the soil-borne plasmodiophoride-like fungus, *Polymyxa betae* Keskin (1964) (Tamada and Richard, 1992). Source of resistance to rhizomania were found in Holly sugar beet company source (Lewellen, 1987). Resistance in Holly is simply inherited by a single dominant gene(Rz_1) (Lewellen et al., 1987; Scholten et al., 1996; Barzen et al., 1997; Readfearn and Asher, 1997; Asher and Kerr, 1996; Asher, 1998). Also resistance to BNYVV was observed in several wild beet (WB) accessions *Beta vulgaris* subsp. *maritima* originally collected from France, UK, Denmark and Italy (Whitney, 1989; Lewellen, 1995). For example WB42 is thought to have been originally collected in Denmark by Viggo Lund in the 1950 (Lewellen, 1995, 1997). Resistance in WB42 is due to another dominant gene (Rz_2) (Lewellen, 1995; Scholten et al., 1996, 1999; Francis et al., 1998, 1999).

MATERIALS AND METHODS

Studies on the inheritance of resistance to BNYVV were performed with the resistant sugar beet accession *Beta vulgaris* subsp. *vulgaris* Holly1-4, which is a selection from the Holly source (Lewellen et al., 1987) and the resistant wild beet accession *B. vulgaris* subsp. *maritima* WB42. Both accessions are diploid with 2n = 18. Plants of the resistant wild beet accession WB42 also were crossed in pairs with susceptible sugar beet germplasm 261 and annual beet accession. Resistant F_1 plants obtained after crosses

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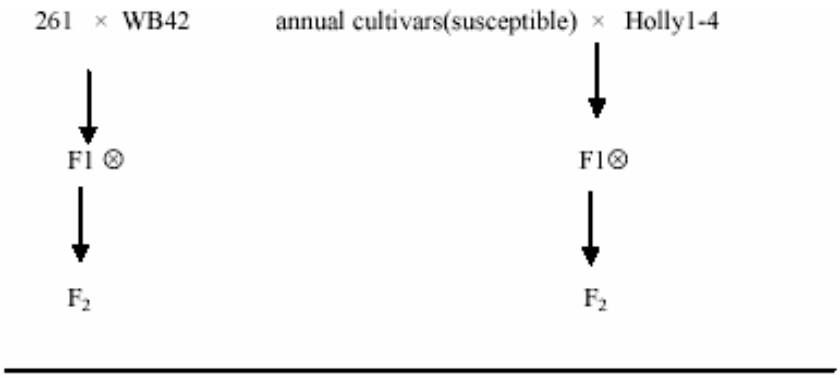

Figure 1. Crossed scheme used to generate the population studied.

of Holly1-4 with the susceptible sugar beet germplasm 261 and annual beet accession were selfed to product F_2 seeds (Figure 1).

Greenhouse test and ELISA

A greenhouse test for screening sugar beet for resistance gene to BNYVV, described by Paul et al. (1992) was used in the present study. Seed sown in autoclaved (121°C, 1.5 h) sand. Seedlings were transplanted at 4 leaf stage to uniform mixture of infested soil which was collected from Shiraz, Iran and contained the A type of BNYVV (Lennoforce et al., 2000). Regina germplasm was used in all of the tests as negative and positive controls. To produce negative control, plants seedling of *B. vulgaris* sugar beet cultivar Regina were transplanted into sand (not artificially infected plants as negative controls). To produce positive controls, seedlings of Regina were transplanted into a uniform mixture of infested soil and sand (3:7 V/V). Plants were watered twice a week with 30 ml of 0.2 diluted Hogland and Arnon (1950) solution (pH 7.0). Inoculation experiments were performed in the greenhouse at 25/17°C (day/night). Rootlets were analyzed for the virus by standard double antibody sandwich ELISA (DAS-ELISA) as described Clark and Adams (1977) using a commercial polyclonal antiserum and BNYVV infected *Nicotiana clevelandii* leaf (Bioreba AG, Switzerland). The 60 internal wells of microtiter plate (polystyrene Nunce. Flat bottom 442404) were used for ELISA, while the outer wells were filled with PBS-Tween 20 during all steps of the ELISA procedure. All samples were tested in duplicate and readings were performed with a Lab system Multiskan EX 355 at 405 nm. Plants were considered susceptible if their samples showed an absorbtion value more than twice of the negative controls.

DNA extraction

Genomic DNA extracted from frozen leaves from individual plants of F_2-A1-110 and F_2-93 populations following the procedure of Saghai-Maroof (1984). DNA concentrations were estimated by spectrophotometery. PCR was performed in a total volume of 25 µl containing 50 ng genomic DNA, 0.2 mM each of dATP, dCTP, dGTP, TTP, 25 ng primer (Operon kit), 2.5 µl 10x reaction buffer(100 mM Tris-Hcl, pH = 9; 500 mM KCl) and 1 unit Taq Polymerase (Smar Taq). DNA amplified was performed in a thermocycler (Biometra T3) in PCR reaction tubs. The thermal cycles used were: 1 cycle of 5 min at 94°C, followed by 40 cycle of 45 s at 94°C, 45 s at specific temperature and 80 s at 72°C, then finally 1 cycle of 10 min at 72°C for final extension. Amplified products were separated by gel electrophoresis using 1.2% agarose gel with TAE buffer and staining with ethidium bromide.

Bulk segregant analysis (BSA)

BSA (Michelmore et al., 1991) was performed for each segregation finally on bulks of DNA of 10 of the most resistant (with virus concentration up to $\log_{10}$ of 0.3 ng/ml virus) or of the most susceptible plants (with virus concentration at least 0.6 ng/ml virus). Primers which amplified a DNA fragment in only one of the bulks were confirmed on the same set of bulk, followed by PCR on ten individual resistant and ten susceptible plants. RAPD markers with the best linkage to resistance gene were evaluated further on an additional number of individual plants.

Mapping of the RAPD marker

RAPD markers were mapped by analysing the segregating families with the computer programming Mapmaker ver 3.0 (Lander, 1987). For each of marker at least 20 individual plants were analysed, distributed over resistant and susceptible classes. It was assumed that the 20 most resistant plants with a maximum $\log_{10}$ virus concentration of 0.3 ng/ml all contained the major gene for resistant, whereas the 20 most susceptible plants, with a minimum $\log_{10}$ virus concentration of 0.6 ng/ml all lacked the gene linkage was considered significant if the logarithm of odds (LOD) score was further than 3.0.

RESULTS AND DISCUSSION

Identification of RAPD marker linked to gene for resistance to BNYVV

To identify RAPD marker linked to genes for resistance to BNYVV, bulks DNA were composed of the most resistant and most susceptible plants of the segregating families of Holly1-4 and WB42. For each set of bulks, 300 Operon primers were screened. Between 10 - 20 primers amplified RAPD markers either in a resistant or in a susceptible bulk. These primers were examined further on individual plants. The primers Op-09 and Op-AN9 generated DNA fragments that were found to be linked to the resistant locus in F_2-A1-110 population, but no primers found for F_2-93. Therefore, investigation on F_2-93 population did not proceed further, while on F_2-A1-110 population, primers were examined on individual plants.

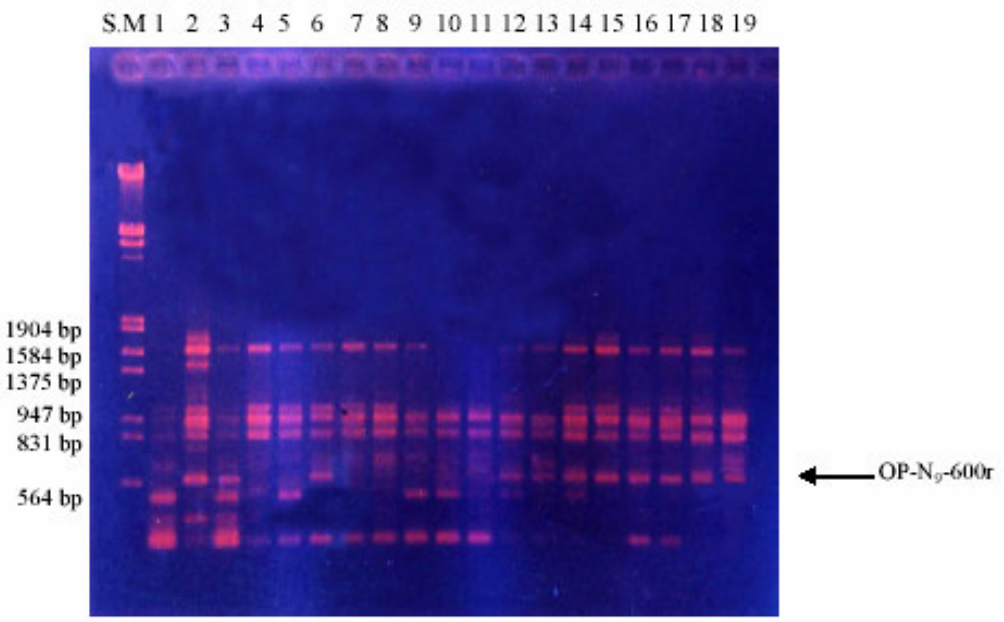

Figure 2. RAPD marker present in resistant (lan4-11) plants and absence in susceptible (lan12-19) plants of the segregant family F_2-A1-110. The arrow points to the segregating marker Op-09_{1150}.

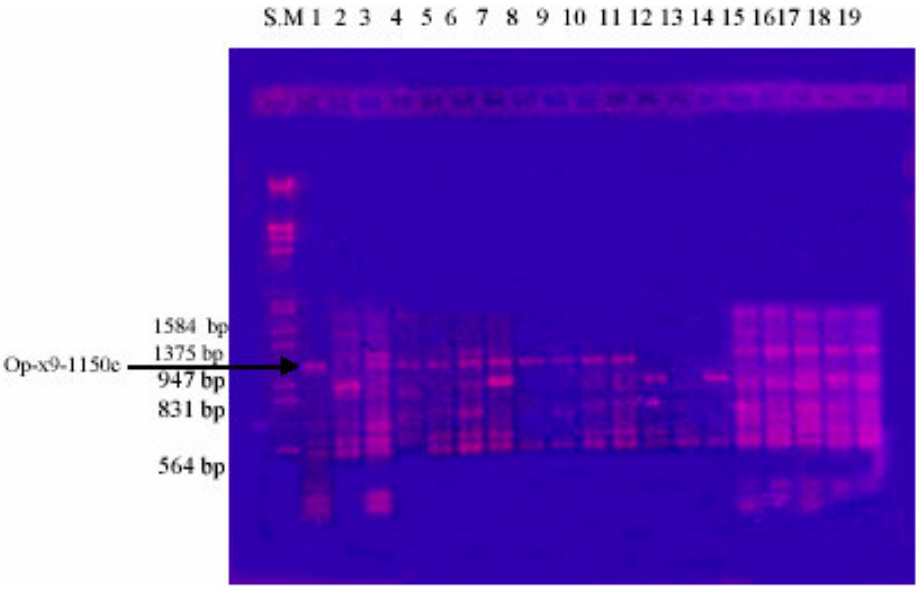

Figure 3. RAPD marker present in susceptible (lan12-19) plants and absence in resistance (lan4-11) plants of the segregant family F_2-A1-110. The arrow points to the segregating marker Op-$AN9_{600}$.

The primer Op-09 amplifies the RAPD marker Op-09_{1150} linked to resistance loci in coupling phases. The primers Op-AN9 amplifies Op-$AN9_{600}$ markers linked to resistance loci in repulsion phases.

Mapping of the RAPD markers

The presence of the RAPD markers Op-09_{1150} and Op-AN_{600} was analysed in the entire population of F_2-A1-110 (Figures 2 and 3). RAPD marker Op-09_{1150} was presented in most of the plants in resistance parts of the frequency distribution. RAPD markers linked to resistance were used for the construction of short rang map around the resistant locus in Holly1-4 accession using the computer programming Mapmaker (Lander, 1987). In the map was estimated with RAPD marker Op-09_{1150} located

on one side of the resistance locus within a distance of 27 cM in coupling phases and another RAPD marker Op-$AN9_{600}$ located within 13.7 cM in repulsion phases.

Conclusion

The RAPD marker Op-AN9 can be used as tool for identification and discriminates between dominant homozygous and heterozygous genes for resistance.

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