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VERIFY THE RESISTANCE TO RHIZOMANIA DISEASE IN SUGAR BEET (*BETA VULGARIS* L.)

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Abstract

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The Rhizomania disease is one of the destructive diseases in sugar beet that causes a great deal of damage to agriculture industry and causes the reduction in crops in Iran and all over the World. One of the effective solutions in challenging with virus is use of resistant cultivars in farms. The resistant cultivars have Rz_1 and Rz_2 genes which are resistant to virus. In this study a contaminated soil with Rhizomania virus (which had been brought from a known region with contaminated soil) used in a greenhouse test with two different populations. The first population was the result of cross between a Holly 1–4 resistant parent with an annual susceptible parent and the second population was the result of cross between 261 cultivar susceptible parent and resistant WB42 parent. The immunology test was done to distinguish between susceptible and resistant plants then inheritance of resistant gene(s) to disease and statistical analysis by the results of this test was verified.

Key word: ELISA test, Rhizomania disease, resistant gene, sugar beet
Abbreviations: ELISA – Enzyme-Linked Immunosorbent Assay; DAS-ELISA – Double-Antibody-Sandwich ELISA

Introduction

The Rhizomania infection was reported from many countries in Europe, American and Asian (Koenig et al., 1996; Wilson and Nicolson, 2001).This disease is one of the destructive commercial infections in sugar beet and causes a sever reduction in root function ability and sugar concentration in beet root (Wisler and Daffus, 2000; Johanson, 1983). In rhizomania disease abnormal lateral root creates which is the result of contamination roots with rhizomania virus (*beet necrotic yellow vein virus)* (BNYVV). This virus is grouped in *benyviruses* and can be transmitted with *Polymyxa betae* Keskin fungus. This fungus is grouped as plasmodiophoride (Richard and Tamada, 1992). The resistance to rhizomania disease first was seen in resistant substance to Cercospora in Po valley in Italy which consequently led to the production of Rizor hybrid. It should be mentioned that production of some resistant plants has been reported in Holly Sugar Company. The resistance in Holly source was simply controlled by a Rz_1 gene (Merdinoglu et al., 1995; Lewellen et al., 1987; Redfron and Asher, 1987; Barzen et al., 1997; Scholten et al., 1996, 1997). The resistance to BNYVV was reported in maritima group in Denmark, France, England and Italy (Whitney, 1987; Lewellen, 1995). For example WB42 can be referred from Denmark which was probability gathered by Viguland in 1950`s (Lewellen, 1995, 1997). The resistance in WB42 was dominant and it is controlled by a different gene that named Rz_2.

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Material and Method

Two plant samples were taken under investigation. One of these was a second generation of cross between resistant WB42 cultivar and 261susceptible cultivar. Another sample was second generation of cross between resistant Holly1-4 cultivar and annual susceptible cultivar (Figure 1). In addition of these populations, the parents of crosses (i.e. WB42 & Holly1-4) and a susceptible cultivar (Regina) and a resistance cultivar (Doretti) were used as control materials. also three pots that it's content was Regina cultivar and was cultivated in pure sand were used as negative control. These seeds were prepared by Amiri and his colleagues. These seeds first were cultivated in sterile sand under greenhouse condition in 17°C at night and 25°C during the day. After many weeks that plants were in two to four leave stage, they were ready to be taken to the main laboratory. The next phase of project was prepared to plants growth in contaminated conditions. A Mixture of contaminated soil with BNYVV (which was gathered in Shiraz) and sand were used and total samples were irrigated with Arnon and Hogland solution every week for two months. After two months the plants were ready to use in ELISA test.

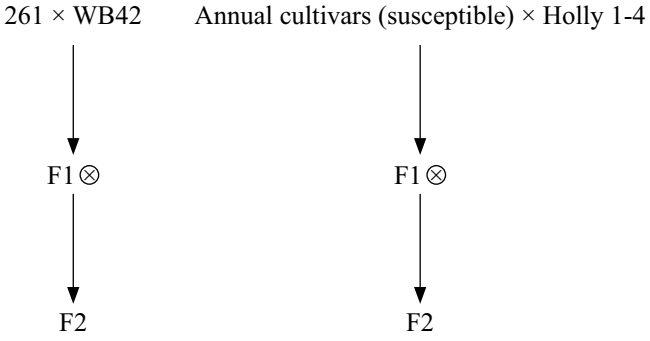

Fig. 1. Crossed scheme used to generate to population studied

ELISA test

The ELISA test was done to measure the mass amount of virus in roots. Antibody and conjugate antibody which were necessary for this test were brought from the Bioreba Company. The used plate was prepared from the Nank Company in Denmark. Then ELISA test was done by Double Antibody Sandwich method (DAS-ELISA) according to (Clark and Adams, 1977).

Then concentration of virus in root of plants was determined by ELISA Reader (Lab system Multiscan EX.355) at 405 nm length wave. Threshold to discrimination between susceptible and resistant plants was twice as much as the mean of absorbance healthy samples, (Amiri and colleagues). This number was calculated 0.19 for total samples. On this base, Table 1 was provided and similar groups were showed by similar alphabets. Then statistical analysis of results was done by SAS software and the hypothesis of the existence of one dominant gene was verified at $**\rho \leq 0.01$ and $*\rho \leq 0.05$ of probability by Chi-square test. (Table 2)

Results

Table 1 shows the mean of absorbance in ELISA test in second generation and control populations. According to this table the susceptible plants of F_2-A_1-110 and F_2-93 populations which were related to Holly and WB42 parents respectively, are categorized in a similar group and they didn't show any significant statistical differences. However the mean of the quantity of absorbance in ELISA test in re-

Table 1

DAS-ELISA mean of absorbance of F2 populations in rootlet of individual plants. (A 405 nm)

Population	Number of plants	Mean of absorbance in resistant plants	Number of resistant plants	Mean of absorbance in susceptible plant
Holly				
F2-A1-110	162	0.109603 c	85	0.7048403 b
WB42				
F2-93	186	0.1182662 c	49	0.6169795 b
Check				
Infected root (Regina)	89			0.8509239 b
Infected Leaf	30			4.255 a
Doretti	53	0.12367466 c		
Health root	89	0.095207407 c		

1 – The means of absorbance with two similar alphabets don't have any statistical difference.
2 – The resistant control (commercial variety from the Sweden Novartis seed company).

Table 2

Ratio resistant plants to susceptible plants and Chi-square test (x2-test)

Population	Total plants	Mean of absorbance resistant plant to susceptible plants (R:S)	Ratio of except	x2-test	Theory exist a dominant gene
Holly					
F2-A-110	162	85:77	03:01	42.66**	No accept
WB42					
F2-93	186	137:49	03:01	235:25**	No accept

sistant plants of F_2-93 group was slightly more than those in F_2-A_1-110 group. According to the amount of absorbance of ELISA test for healthy Regina root, they were similar to resistant plants and therefore were grouped in same category as the resistant Doretti cultivar. From the statistical view, the susceptible plants of F_2-A_1-110 and F_2-93 population and contaminated roots of Regina (positive control) were categorized in similar groups. The gathered results of this test are accorded with results of Amiri et al. (2003), Scholten et al. (1994) and Lewellen et al. (1995). According to Table 2 the hypothesis of one dominant resistant gene wasn't accepted at both $*\rho \leq 0.01$ and $**\rho \leq 0.05$ probability by Chi-square test.

Discussion

The ratio of resistant plants to susceptible plants in Table 2 doesn't accord with expectable ratio related to the hypothesis of one dominant gene (i.e. 3/4 resistant plants: 1/4 susceptible plants) but in the study of Scholten et al. (1996, 1997 and 1999) the existence of one gene with major effect had been confirmed.

But it was not unexpected result, because our goal of this study has been the verifying of molecular markers linked to resistant gene(s). Also because of the existence of some reports based on the escape of many samples from green house test in low condition of soil contamination, we had to use a soil with high level of contamination so that resistant plants are really identified from susceptible plants during the test. In some cases that the contamination in green house test is low, some susceptible cultivars might be mistakenly known as resistant cultivars. On the other hand, threshold of absorbance in this study was calculated 0.19 whereas in previous study was calculated 0.14 (Amiri et al., 2003), also because in study of Scholten et al. (1996) was used a pure race of virus, it was impossible to comparison of threshold in this study and our study. A ratio resistant plant to susceptible plants wasn't identical to what was expected and the mean of absorbance in this study was more in comparison with previous studies. It should be mentioned that in this study we used a mean two times more than the mean of healthy samples to discriminate susceptible and resistant Plants and it was calculated about 0.19. Results show that the resistance to Rhizomania disease in WB42 parent is more than Holly parent and this resistance to Rhizomania disease was observed in second generation of WB42 parent as well as.

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