Journal of Sugar Beet
http://jsb.sbsi.ir/
Journal of Sugar Beet 2012, 28(1)

Relative levels of *Beet necrotic yellow vein virus* in susceptible to resistant genotypes of sugar beets during growing season

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Mahmoudi SB, Ghanbari M, Amiri R, Darabi S, Kakuei nejad M, Aghaee zadeh M, Hasani M. Relative levels of *Beet necrotic yellow vein virus* in susceptible to resistant genotypes of sugar beets during growing season. J. Sugar Beet. 2012; 28(1): 1-6.

Received February 9, 2010; Accepted June 7, 2012

ABSTRACT

In order to study the trend of BNYVV concentrations during growing season, 6 cultivars including Dorohtea, Laetitia(as resistant cultivars), Zarghan (as a tolerant cultivar), Shirin (as a susceptible cultivar), F2-93 (an F2 population with 75 % Rz2 gene) and BC1-261-99 (a population with 25% Rz2 gene) were used. The cultivars were planted in split plot based on a randomized complete block design with 4 replications. Main plots were four different dates of sampling (2, 3 and 4 months after planting and harvesting time) for ELISA. In each sampling date, 12 plants were selected randomly from each plot for DAS-ELISA. The experiment was repeated for two years in a naturally rhizomania infested field. Mean of ELISA values was increased in the initial sampling dates and then decreased gradually up to the end of the season. Based on the trend of ELISA values, logical grouping of the genotypes was happened in the second and third sampling dates in the first and second years, respectively. The results showed that reaction of sugar beet genotypes to the disease could be identified 3-4 months after planting and that detecting infested fields in this period would be reliable.

Keywords: *Beta vulgaris*, ELISA, Rhizomania, sampling date

INTRODUCTION

Rhizomania is one of the significant diseases of sugar beet. Because of the severe reduction in sugar yield, durability in soil and being not easy to be challenged, the disease has turned out to be a restrictive factor in sugar beet cultivation and consequently sugar industry (Asher 1993). Rhizomania has been reported from all over the world and at present is the most destructive disease of sugar beet (Rush and Heidel 1995; Scholten and Lange, 2000). The disease was reported for the first time in Iran by Izadpanah et al. (1996) from Fars province followed by reports from the majority of sugar beet fields in the country (ToudeFallah et al. 2000).

The causal agent of Rhizomania disease is beet necrotic yellow vein virus and the vector is (Plasmodiophorid) *Polymyxabetae*Keskin.

Until present, different methods, including avoidance of cultivation in infected soils, use of agronomic techniques, chemical challenges and genetic resistances, have been employed to control the disease. Using resistant varieties is the best and, at the same time, the simplest approach to combat this disease. The primary efforts to select the resistant genotypes were based on the existing differences of the symptoms, such as yellowing or curling leaves, yellowing veins or the intensity of root rot at the harvesting time, in the varieties and the improved lines, which were cultivated in the field infected with the virus. Then, the root and sugar yield were evaluated so that

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the varieties with relative resistance to Rhizomania were distinguished by root and sugar yield figures (Scholten and Lange 2000). In 1990s, the various methods of evaluation of resistance in the field and greenhouse were improved on the basis of serological methods (Paul et al. 1993; Scholten 1997)

According to Wisler et al. (1992), it seems that, in field experiments, the levels of virus at the end of summer could not reflect the variety reactions. Therefore, in the field experiments, the condition of the main roots is the better criterion for the difference of varieties, because the levels of virus in the fibrous roots would decrease at the end of the season. They state that in order to recognize Rhizomania, soil samples should be collected at the end of growth season.

Totally it is apparent that in the U.S, breeders' general willing is to use field experiments accompanied with evaluating the disease symptoms, root yield and sugar yield to study the resistance to Rhizomania, (Wisler et al. 2003; Lewellen, 1995). While, in Europe, the selection of varieties is done by measuring the levels of virus through Elisa Test in sugar beet seedlings in controlled conditions (Scholten and Lang 2000; Scholten et al. 1996). As a whole, selection in the field conditions for the final steps of breeding programs, in which a few number of genotypes should be tested in the fields, is necessary (Asher 1989). Additionally, when the assessment of many genotypes is necessary and the agronomic characteristics should be taken into consideration, the selection under the field conditions is preferred (Lewellen and Biancardi, 1990). The results of the recent greenhouse studies in Germany represent the positive relation between the apparent sings of the root and root fresh weight, and the levels of virus and the resistance levels of varieties. Also, in the resistant varieties, the levels of virus were decreased from fourth week to twelfth week (Pferdmenges et al. 2009). The resistance to Rhizomania in sugar beet is monogenic and controlled by the Rz_1 or Rz_2 resistant gene (Scholten and Lange 2000).

Considering the fact that the resistance evaluation of many lines in greenhouse conditions, by standard testing (Amiri et al. 2003; Pferdmenges et al . 2009) is not easily possible, this study was done with the purpose of determining the optimum time for evaluation of resistance of sugar beet varieties and lines in the field conditions, through the study of the process of changes in levels of virus during the growth season, in varieties with different levels of resistance, so that the resistant lines, in the naturally infested conditions, could be distinguished with more trust and confidence.

MATERIALS AND METHODS

Plant Materials

The plant materials included 6 varieties, Dorothea, Laetitia (resistant varieties), Zarghan (Tolerant variety), Shirin (sensitive variety), F_2-93 (F_2 population, carrying 75% resistant gene Rz_2) and BC_1-261-99 (first back-cross population carrying 25% resistant gene Rz_2) (Amiri et al. 2003).

Experimental Design and Sampling

The experiment was done in a split plot on the basis of randomized complete block design at 4 sampling dates and 6 genotypes, with 4 blocks and 12 samples per genotype, in a naturally BNYVV-infested field at the Agricultural Research Station in Zarghan, Fars province. The sampling dates and genotypes are considered as the main-plot and sub-plot factors, respectively. The sampling dates were 2, 3, 4 and 6 months after cultivation. The experiment was done in May 3, 2005 and May 19, 2006. The agronomical operations were conducted according to the same usual carefulness as the research tests were done. In each sampling date, 12 samples were randomly taken from the middle of each plot and used for the Elisa Test.

ELISATest

The measurement of virus levels in the plant roots, through ELISA Test was done by using the method of Double Antibody Sandwich Enzyme – linked Imunosorbent Assay (Clark and Adams 1977). Antiserums and leaves extracts of *N.clevelandii* infested with BNYVV were supplied by Bioreba Company (from Switzerland) as a positive control.

As the data related to quantities of ELISA absorption were not normal, before data analysis they were normalized by logarithmic transformation. The average quantities of ELISA absorption for 12 randomized samples per plot were used for variance analysis. After analysis and comparison of treatments over the transformed data, the genotypes mean was converted into original scale. The analysis of the split plot was conducted by using SAS software. F test was done by using mathematic expected mean of squares assuming

Table 1. Mean of squares of the combined analysis of the project in the first and second years of the experiment (2005-2006)

Source of variation	Degrees of freedom	Sum of squares	Means of square
year	1	8.7156	8.7156**
Repelication in year (E_1)	6	0.5339	0.08898**
Sampling date	3	0.1792	0.0597ns
Sampling date × year	3	0.1247	0.04158**
(E_2)	18	1.1445	0.008028ns
Genotype	5	3.4024	0.68047ns
Genotype × sampling date	15	0.1852	0.01235ns
Genotype × year	5	2.2430	0.44860**
Genotype × year × sampling date	15	0.1966	0.01311*
E_3	120	0.8046	0.06705ns

*, ** and ns are significant at levels of 5%, 1% and insignificant, respectively. C.V. = 9.23%

year and block as effects and genotype and sampling date as fixed effects. The comparison of interactions of genotype × year and sampling date × year was done by using MSTATC software. In the comparing of two means, considering the combined analysis result, error 3 and the pooling of 2 and 3 errors were recognized as the experimental error.

RESULTS

On the basis of the combined analysis results, no significant difference, in sampling date and genotype, was observed (Table 1).

The interactions of sampling date × year and genotype × year and also genotype × year × sampling date were significant. The quantities of ELISA absorption in the different sampling dates had significant differences, so that, in the first and second years, the highest quantities of ELISA absorption were observed in the second and third samplings, respectively (Table 2).

Table 2. Grouping of average quantity of Elisa absorption for the interaction of sampling date × year (2005-2006)

year	Sampling stage	Duncan grouping	Elisa absorption
First year	1	D	0.2276
	2	C	0.2844
	3	Cd	0.2661
	4	Cd	0.2480
Second year	1	B	0.6347
	2	B	0.6788
	3	A	0.9448
	4	B	0.7498

*Means with the same letter in each column, on the basis of Duncan test, have no significant differences at 5% level

The Table 3 shows the results of the comparison of genotypes means in the first year of experiment. At the first sampling date (2 months after sowing) statistically significant differences were not observed among the genotypes in quantities of ELISA absorption. At the second sampling date (3 months after sowing), the differences among genotypes were decreased in such a way that overall 6 genotypes were located in 2 groups, named a and b. At the third sampling, only population F_2 (75% resistant) showed a significant difference with Shirin genotype. Finally at fourth sampling, with the relatively decreased quantities of ELISA absorption in genotype, compared with the second third samplings (except for Dorothea in which the quantity of absorption was a little increased), no differentiation was observed among the genotypes. At this date, the completely susceptible Shirin did not show any significant difference with the resistant genotype Dorothea. The Figure 1 represents that, at the first sampling, the differences of genotypes were negligible, then differences were maximized at the second

Table 3. Grouping of average quantity of Elisa absorption for sugar beet genotype in different sampling dates in the first year (2005)

Average quantities of Elisa absorption					
genotype	First sampling	Second sampling	Third sampling	Fourth sampling	Average
Dorothea	0.21863a	0.2206 b	0.22068 ab	0.25761 ab	0.23289 c
Laetitia	0.22203a	0.22842 b	0.23034 ab	0.20966 b	0.22299 c
F_2-93	0.21294a	0.27149 ab	0.20689 b	0.22973 b	0.23759 bc
Zarghan	0.22591a	0.28265 ab	0.29751 ab	0.20966 b	0.25346 bc
BC_1- 261-99	0.21591a	0.35604 a	0.30413 ab	0.24988 b	0.28345 ab
Shirin	0.23061a	0.33625 a	0.32715 a	0.32222 a	0.30886 a
Infested leaf (*Nicotiana clevelandii*)					1.92568
Uninfected root					0.21911
Infection Threshold					0.31047

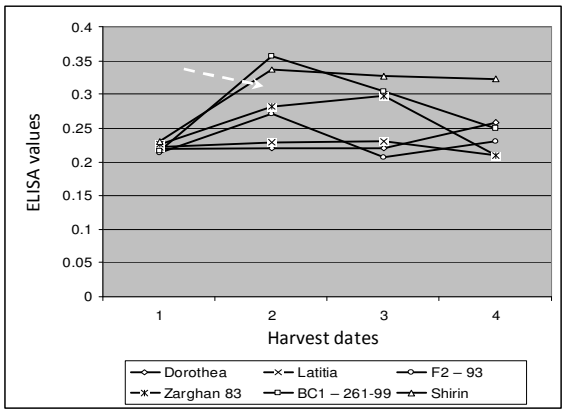

Fig. 1. Variations of ELISA values of sugar beet genotypes at four sampling dates in the first year (2005)

sampling, and gradually, toward the test sampling, the differences of genotypes were reduced in quantities of ELISA absorption. Meanwhile, at the third and fourth samplings, the quantities of ELISA absorption of some genotypes did not match with their levels of resistance. As seen in Table 2, each year, there were different quantities of ELISA absorption among the different sampling dates,. As Table 2 shows, the nature of interaction of sampling × year is a type of quantity change in average, so it is not high.

In the second year, the quantities of ELISA absorption in the different sampling dates (Table 2) represented the relatively intensive infestation in growth in the experimental field, so that, in the susceptible variety Shirin, the quantities of ELISA absorption in different sampling dates (Table 5) did not differ from Control, which is approximately 3 times more than the threshold of infestation (Table 2). As seen in Table 4, in the second year, there was a significant difference between the Rhizomania-resistant Dorothea and Laetitia and the other genotypes. Meanwhile, in the first year,

Table.4. Grouping of average quantity of Elisa absorption for the genotype × year interaction in the combined analysis of the experiment for 2 years (2005-2006)

Year	genotype	Quantity of Elisa absorption	Duncan grouping
First year	Dorothea	0.2265	F
	Laetitia	0.21948	F
	F_2-93	0.21799	F
	Zarghan	0.23210	F
	BC_1- 261-99	0.2494	Ef
	Shirin	0.27427	De
Second year	Dorothea	0.320289	D
	Laetitia	0.29616	D
	F_2-93	0.64063	C
	Zarghan	0.93911	B
	BC_1- 261-99	1.16434	A
	Shirin	1.14265	A

*Means with the same letter in each column, on the basis of Duncan test, have no significant differences at 5% level

the differences among the genotypes were low, so that the two Rhizomania-resistant varieties and populations of F_2-93 and BC_1-201-99 and Zarghan- did not show significant differences.

The reactions of the genotypes in the different sampling dates in the second year of experiment are summarized in Table 5. The results show that, at the first sampling, the genotypes were classified in 4 groups and in the next samplings they were in 3 groups. In the third sampling, the logical reaction of the genotypes to the disease has been represented. More details of the grouping can be found in the Figure 2.

DISCUSSION

The quantities of ELISA absorption, which reflect the levels of virus in plant, in the first and second years of experiments, primarily tended to increase and then until the time of harvest it was

Table 5. Grouping of average quantity of Elisa absorption for sugar beet genotypes in different sampling dates in the second year (2006)

Quantities of Elisa absorption					
Genotype	First sampling	Second sampling	Third sampling	Fourth sampling	Average
Dorothea	0.5903 c	0.4894 c	0.5053 c	0.3639 c	0.4872 c
Laetitia	0.4555 d	0.4358 c	0.5381 c	0.4680 c	0.4736 c
F_2-93	0.7833 b	0.7517 b	0.9363 b	0.8050 b	0.8191 b
Zarghan	0.9049 a	0.9473 a	1.017 b	0.9839 a	0.9633 a
BC_1- 261-99	0.9243 a	0.9872 a	1.164 a	1.073 a	1.037 a
Shirin	0.9414 a	0.9473 a	1.185 a	1.019 a	1.02 a
Infested leaf (*Nicotiana clevelandii*)					1.5750
Uninfected root					0.2148
Infection Threshold					0.4296

*Means with the same letter in each column, on the basis of Duncan test, have no significant differences at 5% level.

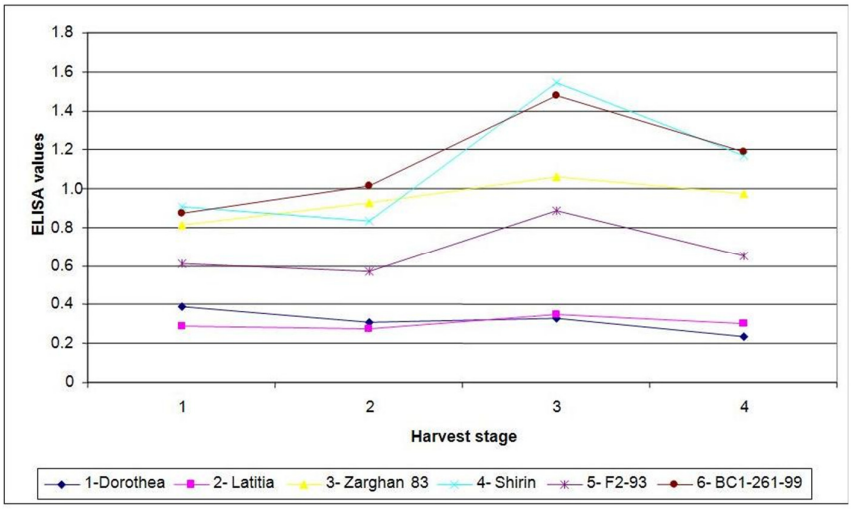

Fig. 2. ELISA values of studied genotypes at four sampling dates in the second year (2006)

decreased gradually (Figures 1 and 2). The alternations, in the first year, from the first to the second sampling date happened and the range was not considerable (Figure 1), but it increased at the second year until the third sampling date (4 months after sowing) and enhanced after that until the harvesting time. The range of ELISA absorption quantities in the second year was higher than the first year. The range of ELISA abortion quantities, in different sampling dates, among the susceptible and resistant genotypes shows (Table 3) that the infestation intensity in the field in the first year is low whereas it is high in the second year. Also, Table 5shows that, at the first sampling date, genotypes have significant differences with each other in the quantities of ELISA absorption and were divided into 4 groups, while in the first year, this was not significant (Table 3) and all the genotypes are classified in one group. Considering that the vector of Rhizomania exists in soil, and in the soil-borne diseases the spreading is observed in form of stains on leaves and no uniformity could be seen in the fields, this causes the high difference in the infestation intensity of disease between the two years. In Wisler et al. (1999) study, in which 3 sampling dates 72, 105, 170 days after sowing, respectively, were conducted , the first sampling date (approximately 72 days after sowing) had the highest level of quantities of ELISA absorption until the third sampling date. In their study, the sowing date was April 30.

In the first year, the best grouping of genotypes was observed at the second sampling date, so that the resistant genotype and susceptible genotype and the BC_1 population were located in two different groups (respectively a and b) and two resistant genotypes of Zarghan 83 and F_2 population were put between the two groups. In this year, at the third and fourth sampling dates the quantities of ELISA absorption of the different genotypes were gradually decreased and the grouping, obtained from the quantities of ELISA absorption, did not reflect the real grouping of the genotypes. These results match the results of Wisler et al. (1999) and Lami (1992), considering the fact that the levels of virus do not show the varieties reactions. In the second year, because of the intensive infestation, in all sampling dates, the genotypes are kept apart from each other, but in the third sampling date the separation was logical based on the resistance of varieties.

The sowing date, in the second year, was 20 days later than the first year and probably this was one of the reasons of differences in the results of the first and second years. In the first year, the best separation of genotypes was observed in the second sampling date.

Scholten and Lange (2000) have stated that in the field experiments the taproots are the better criterion for the separation of varieties, because the levels of virus in the secondary roots would be decreased during the growing season. This outcome is also matched with the obtained results of the study. Sugar beet is not a suitable systemic host for BNYVV (Dubois et al. 1994) and usually the virus is not conveyed from fibrous roots to the taproot in higher quantities (Giunchedi and Poggi-Pollini 1988). Therefore, the evaluation of the taproots might not include viruses to such an extent to reveal the genotypes reactions.

It is noticed in the Figures 1 and 2 that the alternations in the levels of virus in the resistant

varieties (Dorothea and Laetitia), at the different sampling dates, were less than that in susceptible varieties. On the other hand, in the case of high infestation in the fields, the varieties with high resistance could be selected at the beginning of growing period on the basis of sampling and the levels of virus (ELISA Test). The results match the other researchers' studies (Wisler et al, 1999). Recently, the results of greenhouse studies have shown that 3 months after sowing in the infested soil is an appropriate time for evaluation of the real reactions of varieties to the different genotypes of the viruses (Pterdmenges et al. 2009).

According to the results of this study and other researchers' findings (Wisler et al. 1999), if the goal is to compare the resistance of the commercial sugar beet varieties or hybrids, root yield and sugar yield are good criteria for the differentiation of the varieties, but if the purpose is to identify the genotypes and the resistant materials, it could be done through 3-4 samplings after sowing and ELISA test, and there is no need to wait until harvest.

ACKNOWLEDGEMENT

Herewith we extend our thanks and appreciation to Eng. Mohsen Bazrafshan for his contribution in analyzing the test results of this study.

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