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Short Communication

Confirmation of some SCAR molecular markers linked to *Rhizomania* resistance gene (Rz_1) in sugar beet

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Abstract

For the involvement of *Rhizomania* resistance genes in breeding programs, tagging these genes by molecular markers is necessary. In this study, some breeding populations and commercial varieties of sugar beet originated from Holly source were used for the validation of three repulsion SCAR molecular markers correlated with ELISA and field resistance. Comparison between ELISA and field resistance and molecular markers showed that repulsion marker ZN5 had 96% and 98% agreement with ELISA and field resistance, respectively with 95% and 100% presence in susceptible and resistant varieties, respectively. Therefore, this marker can be used for screening of homozygous lines resistant to *rhizomania* originated from Rz_1 gene source.

Key words: Agreement, breeding, disease, SCAR, selection

Rhizomania is the most destructive viral disease of sugar beet worldwide. One of the most effective resistance sources was found in Holly Sugar Company resource. The Holly resistance is governed by a dominant gene called Rz_1 (Scholten et al. 1996; Biancardi et al. 2002). In recent years, many attempts have been made to identify molecular markers linked to the rhizomania resistance genes. Pelsy and Merdinoglu (1996) used BSA (bulk segregant analysis) to identify RAPD (Random Amplified Polymorphic DNA) markers linked to the rhizomania resistance gene from Holly (Rz_1) source. Scholten et al. (1997) used BSA method for identification of RAPD markers linked to the BNYVV resistance gene in segregating families of four resistant accessions including Holly-1-4, R128, R104, and WB42. Scholten et al. (1999) suggested the name Rz_2 for WB42 gene(s). Amiri et al. (2003) reported that the resistance in WB42 source is governed by a resistance gene (Rz_2) located at a distance of 35 cM from the Rz_1 gene derived from the Holly source. Through using AFLP (Amplified Fragment Length Polymorphism) markers and BSA method, Grimmer et al. (2007) developed a linkage map comprising 233 markers harbouring 497.2 cM with 2.1 cM marker distance. Norouzi et al. (2011) reported the presence (>80%) of a coupling-phase SCAR (Sequence Characterized Amplified Region) marker called PN1 in most commercial cultivars. Stevanato et al. (2012) applied the BSA method in a F_2 segregating population and identified three SNP markers linked to rhizomania resistance gene (Rz_1).

The aim of this study was to confirm the linkage of three selected SCAR markers and Rz_1 through the comparison of markers with ELISA and field resistance data and also to determine the per cent of markers presence in susceptible and resistant sugar beet populations by marker assisted selection.

Different full-sib (S1) and half-sib families and susceptible and resistant varieties of sugar beet derived from Holly resistance source were used (Tables 1 and 2).

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Sixteen full-sib (S1) families along with the resistant and susceptible controls (Table 1) were planted in four replications (each replication was one plot consisting of a row with 3m length) in rhizomania-infested soil in Zarghan station in Fars province, Iran.

Table 1. ELISA values and genotypes grouping for disease weighted mean in the field

Genotype	ELISA OD* mean	Disease weighted mean	Phenotype in the field
Resistant	2.47	0.13	S1-88119
	2.5	0.119	S1-88239
	2.75	0.109	Resistant check
Semi resistant	3.13	0.139	S1-88229
	3.16	0.08	S1-88032
	3.29	0.114	S1-88136
	3.45	0.129	S1-88127
	3.54	0.123	S1-88125
	4.16	0.186	S1-88162
	4.25	0.126	S1-88027
	4.41	0.178	S1-88161
	4.5	0.179	S1-88178
Semi susceptible	4.75	0.136	S1-88120
	4.75	0.217	S1-88196
Susceptible	6.04	0.245	S1-88034
	6.29	0.379	Susceptible check
	6.54	0.441	S1-88173
	7.66	0.358	S1-88264

*OD= Optical Density

The assessment of disease infection in the field was based on a 1 to 9 scale for root appearance, bearded root and vascular tissue color (Luterbacher et al. 2005). For each genotype, the beet roots were scored on the basis of a severity scale ranging from 1 (completely healthy root) to 9 (the tap root is completely bearded, replaced by secondary and tertiary roots, due to rhizomania). Disease weighted means were calculated for each plot based on the severity scale as follows: [(number of roots × 1) + … + (number of roots × 9)] ÷ total number of roots harvested. Essentially, it is a weighted mean of all roots in the genotype of interest. Based on disease weighted mean, each genotype was grouped as resistant (less than 3), semi-resistant (between 3-4.5), semi-susceptible (between 4.5-6), and susceptible (more than 6). In September, the samples of the leaves and roots from each plot were randomly prepared and transferred to –80^{o}C freezer and kept till ELISA and molecular analyses.

Roots were analyzed for the virus by standard DAS-ELISA (double-antibody sandwich ELISA) as described by Clark and Adams (1977) using a commercial polyclonal antiserum (*Bioreba AG*, Reinach, Switzerland). Mean of optical density (OD) of healthy plants in each ELISA plate ($\bar{X}$) and standard deviation (Sd) of healthy plants were calculated. Two limits ($2_{\bar{X}}$ and $\bar{X}$+3Sd) were used for resistance evaluation of the plants. The samples were considered as infested and susceptible if OD>$2\bar{X}$, infested and semi resistant if OD between $\bar{X}$+3Sd and $2\bar{X}$, and healthy and resistant if OD<$\bar{X}$+3Sd.

DNA was extracted from fresh or frozen leaves according to Dellaporta et al. (1983). Quality and quantity of genomic DNA were estimated using 0.8% agarose gel electrophoresis. The molecular markers have been already developed by cloning of the RAPD markers linked to rhizomania resistance genes (Rz_1) and converting to SCAR specific markers (Unpublished data). Three repulsion SCAR markers named ZN3, ZN4 and ZN5 were used with primers of 18-21 nucleotides. PCR reactions and agarose gel electrophoresis were performed by the laboratory

Table 2. The confirmation results of the repulsion SCAR molecular markers linked to rhizomania resistance gene (Rz_1) in sugar beet

Row	Marker code	No. of tested populations	Identified populations %	ELISA and marker agreement %	Field resistance and marker agreement %	Presence % in susceptible varieties	Presence % in resistant varieties	Error of the marker
1	ZN3	19	100	90	92	95	80	0.08
2	ZN4	18	78	88	93	75	62	0.07
3	ZN5	50	100	96	98	95	100	0.02

protocol.

Per cent agreement of disease score and ELISA data with molecular data was calculated by dividing the number of samples matching with molecular data to total number of tested plants. Also, repulsion molecular markers error was calculated as per the following equation:

The error for repulsion marker = percent of susceptible plants without marker band.

The S1 populations had different disease weighted mean and ELISA value in infested soil (Table 1). Agreement between per cent of the SCAR markers to ELISA and field resistance are very important for confirmation of the molecular markers in breeding populations. Also, the presence per cent of the SCAR markers in susceptible and resistant varieties are considered for selecting one molecular marker. For the validation of developed markers and their reproducibility, ELISA data obtained from field resistance evaluation of several full sib families, and both susceptible and resistant commercial varieties were used. The results of each of the three SCAR markers used in present study were explained in Table 2 and Figs. 1 to 3.

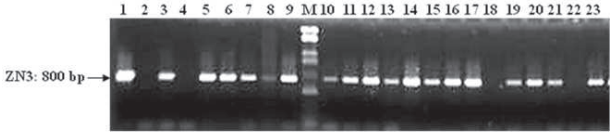

Fig. 1. Banding pattern of ZN3 (first repulsion marker) in S1-88127. Lanes 2 and 4 are resistant plants without the marker. Lanes 1, 3, 5 to 9 are susceptible plants with the marker, Lanes 10 to 17, 19 to 21 and 23 are resistant plants with the marker, Lanes 18 and 22 are susceptible plants without the marker, M: DNA size marker (Lambda DNA restricted by *Eco*RI+*Hin*dIII)

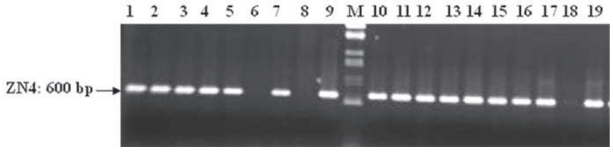

Fig. 2. Banding pattern of ZN4 (second repulsion marker) in S1-88125. Lanes 6 and 8 are resistant plants without the marker, Lanes 1 to 5, 7 and9 are susceptible plants with the marker, Lanes 10 to 17 and 19 are resistant plants with the marker, Lane 18 is susceptible plant without the marker, M: DNA size marker (Lambda DNA restricted by *Eco*RI+*Hin*dIII)

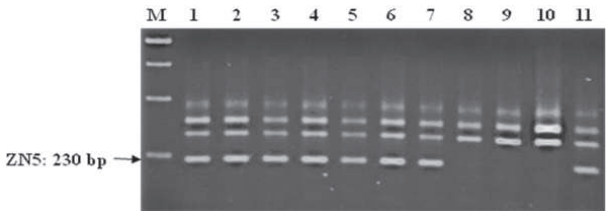

Fig. 3. Banding pattern of ZN5 (third repulsion marker) in S1-88027. Lanes 8 and 9 are resistant plants without the marker, Lanes 1 and2 are susceptible plants with the marker, Lanes 3 to 7 and 11 are resistant plants with the marker, Lane 10 is susceptible plant without the marker, M: DNA size marker (GeneRuler 1kb DNA ladder)

As RAPD markers are dominant markers, they cannot be used directly in marker-assisted selection. Hence, SCAR markers have been developed from the RAPD markers (Norouzi et al. 2015, 2016). The repulsion markers are linked to susceptible allele and therefore, they were amplified in most of the susceptible varieties and resistant heterozygote commercial varieties (Table 2). In present study, all of the SCAR markers developed from the related RAPD markers showed dominance. It means, for detection of each three putative genotypes, the primers of one coupling SCAR marker and one repulsion SCAR marker must be applied in a duplex PCR simultaneously.

It seems that by simultaneous use of the two SCAR markers {(coupling phase ZN1, (Norouzi et al. 2015), and one repulsion-phase; ZN3, ZN4 or ZN5 in the present study)} in a duplex PCR reaction, it is possible to identify the three genotypes of the Rz_1 gene, select the rhizomania resistant pollinator lines and populations, decrease the time and cost of breeding programs, increase the precision of the single plant selection and hence increase the selection efficiency.

Authors' contribution

Conceptualization of research (PN); Designing of the experiments (SBM, SD, MK); Contribution of experimental materials (SBM, SD); Execution of field/ lab experiments and data collection (SD, MK, PN); Analysis of data and interpretation (PN, SD, MK); Preparation of manuscript (PN, SBM, MK).

Declaration

The authors declare no conflict of interest.

References

Amiri R., Moghaddam M., Mesbah M., Sadeghian S. Y., Ghannadha M. R. and Izadpanah K. 2003. The inheritance of resistance to beet necrotic yellow vein virus (BNYVV) in *B. vulgaris* subsp. *maritima,* accession WB42: Statistical comparisons with Holly-1-4. Euphytica, **32**: 363-373.

Biancardi E., Lewellen R. T., De Biaggi M., Erichsen A. W. and Stevanato P. 2002. The origin of rhizomania resistance in sugar beet. Euphytica, **127**: 383-397.

Clark M. F. and Adams A. N. 1977. Characteristics of the micro plate method of enzyme linked immunosorbent assay for the detection of plant viruses. J. Genet. Virology, **34**: 475-483.

Dellaporta S. L., Wood J. and Hicks J. B. 1983. A plant DNA minipreparation version II. Plant Mol. Biol. Reporter, **1**: 19-21.

Grimmer M. K., Trybush S., Hanley S., Francis S. A., Karp A. and Asher M. J. C. 2007. An anchored linkage map for sugar beet based on AFLP, SNP and RAPD markers and QTL mapping of a new source of resistance to Beet necrotic yellow vein virus. Theor. Appl. Genet., **114**: 1151-1160.

Luterbacher M. C., Asher M. J. C., Beyer W., Mandolino G., Scholten O. E., Frese L., Biancardi E., Stevanato P., Mechelke W. and Slyvchenko O. 2005. Sources of resistance to diseases of sugar beet in related *Beta* germplasm: II. Soil-borne diseases. Euphytica, **141**: 49-63.

Norouzi P., Mahmoudi S. B., Aghaiezadeh M., Kakueinezhad M., Orazizadeh M. R., Vahedi S. and Fathi M. R. 2011. Repeatability of Some Molecular Markers Linked to Rhizomania Resistance Gene (Rz_1) in Sugar Beet (*Beta vulgaris* L.) Genotypes. J. Crop Breed., **3**(8): 30-42 (In Persian, Abstract in English).

Norouzi P., Sabzehzari M. and Zeinaly H. 2015. Efficiency of some molecular markers linked to rhizomania resistance gene (Rz_1) for marker assisted selection in sugar beet. J. Crop Sci. Biotech., **18**: 319-323.

Norouzi P. 2016. To develop a SCAR marker linked to rhizomania resistance gene in sugar beet. Novin Genet., **10**: 549-556 (In Persian, Abstract in English).

Pelsy F. and Merdinoglu D. 1996. Identification and mapping of random amplified polymorphic DNA markers linked to a rhizomania resistance gene in sugar beet (*Beta vulgaris* L.) by bulked segregant analysis. Plant Breed., 371-377.

Scholten O. E., Jansen R. C., Keizer L. C. P., De Bock T. S. M and Lang W. 1996. Major genes for resistance to beet necrotic yellow vein virus (BNYVV) in *Beta vulgaris*. Euphytica, **91**: 331-339.

Scholten O. E., Klein-Lankhosrst R. M., Esselink D. G., De Bock T. S. M. and Lange W. 1997. Identification and mapping of random amplified polymorphic DNA (RAPD) markers linked to resistance against beet necrotic yellow vein virus (BNYVV) in *Beta* accessions. Theor. Appl. Genet., **94**: 123-130.

Scholten O. E., De Bock T. S. M., Klein-Lankhorst R. M. and Lange W. 1999. Inheritance of resistance to beet necrotic yellow vein virus in *Beta vulgaris*, conferred by a second gene for resistance. Theor. Appl. Genet., **99**: 740-746.

Stevanato P., Trebbi D., Norouzi P., Broccanello C. and Saccomani M. 2012. Identification of SNP markers linked to the Rz_1 gene in sugar beet. Inter. Sugar J., **114**: 715-718.