LENNEFORS, BRITT-LOUISE[1*], EUGENE I. SAVENKOV[2], JAN BENSEFELT[1], ELISABETH WREMERTH-WEICH[1], PETRA VAN ROGGEN[1], STIG TUVESSON[1], LISETTE LAURIN[1], JARI P. T. VALKONEN[3] and JAN GIELEN[4], [1]Syngenta Seeds AB, Box 302, SE-261 23 Landskrona, Sweden, [2]Department of Plant Biology and Forest Genetics, Swedish University of Agricultural Sciences, P.O. Box 7080, SE-750 07 Uppsala, Sweden, [3]Department of Applied Biology, P.O. Box 27, FIN-00014 University of Helsinki, Finland and [4]Syngenta Seeds, 12 chemin de l'Hobit, 31790 Saint-Sauveur, France. **Transgenic rhizomania resistant hybrids of sugar beets, providing strong resistance against different strains of BNYVV.**

ABSTRACT

Rhizomania, one of the most important sugar beet diseases, is caused by *Beet necrotic yellow vein virus* (BNYVV). In fields infested by rhizomania it is essential to grow rhizomania resistant varieties to maintain high yields of sugar beet. Rhizomania is consistently spreading to new areas and fields. It is suggested that some strains of BNYVV are more pathogenic than others *e.g* the P-type of BNYVV and isolates with the amino acids valine (V) and leucine (L) in position 67 and 68, respectively, of P25 on RNA3 of BNYVV. There are reports on break down of the resistance in varieties based on "Holly", which is until now the most widely used resistance source against rhizomania.

Transgenic sugar beets carrying an inverted repeat of a 0.4 kb fragment derived from the replicase gene of BNYVV were produced, and it was shown that the resistance mechanism was based on gene-silencing. The transgenic resistance to rhizomania was evaluated in comparison to conventional sources of resistance to rhizomania in green-house and field tests. In the green-house studies soil samples from USA, Europe and Asia were used. The field trials were performed in heavily rhizomania infested fields in Sweden. The results of the different trials are presented.

Objectives

The objectives were to evaluate the level of resistance to BNYVV in transgenic sugar beet hybrids carrying an inverted repeat of a 0.4 kb fragment derived from the replicase gene of BNYVV. Comparison was made to the resistance level achieved in hybrids based on conventional sources of resistance to rhizomania. The evaluation was done in field and green-house trials.

Procedures

Soils from USA, Germany (BNYVV B-type), France (BNYVV P-type), Spain (BNYVV A-type) and Iran (BNYVV A-type) were used in the green-house tests. The American soil samples were collected in Imperial Valley in California and Willmar, Crookston, Clara City in Minnesota. The obtained amino acid sequences in position 67-70 of P25 at RNA3 of the BNYVV isolates were VCHG from Willmar and AHHG from Crookston and Clara City. These three soils contained BNYVV as well as *Beet soil borne mosaic virus* (BSBMV) and *Beet soil borne virus* (BSBV). The soil samples from Imperial Valley, Willmar, Crookston, Spain and France were collected in fields where rhizomania symptoms in diploid rhizomania resistant hybrids based on "Holly" were observed.

In the green-house tests one week old plants were transplanted into tubes containing rhizomania infested soil diluted with sterile sand in ration 1:1. The plants were grown in

green-house with +20-22°C. After 4 weeks the plants were harvested and the sap of the cleaned individual roots was extracted and diluted with buffer in ration 1:20 (w/v). The concentration of BNYVV in the sap samples was determined by enzyme linked immunosorbent assay (ELISA). The statistical significance of the $\log_{10}$ transformed values of the BNYVV titres (ng ml^{-1}) measured in the different sap samples was evaluated by analysis of variance (ANOVA).

The field trials were performed in fields heavily infested with BNYVV B-type. The sugar beet roots were harvested 5 months after sowing and 5 cm of the very end of the main root tips was collected. Sap was extracted from the epidermic slices and diluted. The samples were evaluated with ELISA and ANOVA as described above.

Conclusions

In the green-house and field trials the plants of the transgenic rhizomania resistant hybrids always contained significantly less BNYVV than plants of hybrids based on "Holly" (*Rz1*). The transgenic hybrids were also significantly more resistant than hybrids carrying resistance genes from both "Holly" and C48 (*Rz1* + *Rz2* + *Rz3*) when grown in the soils from Willmar, Crookston, Imperial Valley, Spain and France. The transgenic resistance was not influenced by BSBMV. This indicates that the transgenic rhizomania resistance presented in this study has a potential to be of high importance in the future, especially at locations where the conventional varieties become highly infected by BNYVV. The transgenic resistance is the strongest resistance against rhizomania that we so far have observed.

References

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