INTERACTIONS BETWEEN MAJOR GENES, AND INFLUENCE OF THE GENETIC BACKGROUND IN THE EXPRESSION OF RHIZOMANIA RESISTANCE.

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ABSTRACT

Interactions between different major genes of resistance to rhizomania were studied in BNYVV bioassays.

The virus content (ELISA value) and the gene dose effect were analysed.

Genes of different origin differ in their gene action; moreover, expression of resistance is also strongly dependent on the genetic background.

Some of these differences are measurable as differences in yield components under conditions of strong natural infection.

The implications for practical breeding and hybrid choice will be discussed.

Key words: Beet Necrotic Yellow Vein Virus, Rhizomania resistance, major genes.

ABREGE

Interactions entre differents gènes majeurs de résistance à la rhizomanie, et influence du « fond génétique » dans l'expression de cette resistance.

Des interactions entre différents gènes "majeurs" de résistance à la rhizomanie ont été étudiées par le biais d'essais d'inoculation artificielle en laboratoire.

Les titres en virus ont été mesurés (valeur ELISA) et l'effet "doses – gènes" a été investigué.

Des gènes d'origines différentes agissent selon des mécanismes distincts; par ailleurs l'expression de la résistance est influencée par le "fond génétique".

Certaines de ces différences sont mesurables au champ (composantes du rendement) dans des conditions d'infection élevée.

Les implications qui en découlent pour la sélection ainsi que les recommandations variétales possibles seront discutées.

Mots clés: virus des nervures jaunes et nécrotiques de la betterave, résistance à la rhizomanie, gènes majeurs.

KURZFASSUNG

Interaktionen zwischen Hauptgenen, und Einfluss des genetischen hintergrundes auf die ausprägung der Rhizomania Resistenz.

Interaktionen zwischen unterschiedlichen Hauptgenen wurden anhand BNYVV bioassays untersucht.

Dabei wurde der Virustiter (ELISA-Wert) gemessen und der Gen-Dosis-Effekt untersucht.

Gene unterschiedlicher Herkunft unterscheiden sich in ihrer Genwirkung; darüberhinaus ist die Ausprägung der Resistenz ebenso stark vom genetischen Hintergrund abhängig.

Unter starken natürlichen Befallsbedingugen sind einige dieser Unterschiede messbar, anhand ihrer unterschiedlichen Ertragskomponenten.

Auswirkungen auf die praktische Züchtung und Hybriderkennung werden diskutiert.

Stichworte: Beet Necrotic Yellow Vein Virus, Rhizomaniaresistenz, Hauptgene

INTRODUCTION

Major genes condition resistance to rhizomania, one of the most important diseases of the sugar beet worldwide.

The first successful resistant hybrids were released in the eighties in Europe, and were based on a major gene found in the variety "Rizor" (De Biaggi, 1987). This gene is still used in breeding programmes.

The resistance gene present in the varieties that are the most widely used today was discovered in a breeding line belonging to the Holly Sugar company (USA) (Lewellen et al., 1987).

Other major genes of rhizomania resistance derived from wild beet accessions have been studied (Scholten et al., 1999) and introduced in sugar beet lines (Lewellen & Whitney, 1993; Lewellen, 1995). Asher et al. (1997) reported resistance to the vector in a *B.maritima* population.

Varieties containing either the "Rizor" or the "Holly" gene, mostly heterozygously present, allowed the sugar industry to survive the rapid spread of the disease seen in most sugar beet areas during the last 10 years.

More recently, however, severe rhizomania symptoms and lower yield performances observed in 2001 in Pithiviers (France) on "Holly resistance" based hybrids are considered as a potential threat to the growers (Richard-Molard, 2002; Harju & Richard-Molard, 2002).

Virus isolates found in this area belong to the very aggressive BNYVV strain group P, also present in China, Japan, Kazakhstan (Miyanishi et al., 1999; Koenig & Lennefors, 2000) and discovered more recently in East Anglia (UK) (Harju & Richard-Molard, 2002).

This paper summarises a set of experiments on the relationship between different BNYVV isolates and the host resistance / susceptibility of lines and hybrids with different major gene combinations. The influence of the genetic background of resistant hybrids has also been investigated.

The experiments were carried out both in the lab (rhizomania bioassays) and in the field (yield trials in diseased conditions).

1.- MATERIAL AND METHODS

1.1.- GENOTYPES

Fourteen female lines (11 susceptible (S1-S11) & 3 resistant (R1-R3)), covering a broad genetic diversity, were testcrossed with a pollinator homozygous for the "Holly" gene.

Each resistant female line is homozygous for one major gene:

- R1: a gene from a proprietary *Beta maritima* accession introgressed by 2 successive (marker assisted) backcrosses into a susceptible elite line.
- R2: line with the "Rizor" gene.
- R3: line with the "Holly" gene.

The resistant females were also crossed (testcrosses) with 4 pollinators: 2 susceptible lines (diploid or tetraploid), and 2 resistant diploid lines (homozygous for the "Rizor" or the "Holly" gene).

1.2.- BIOASSAYS

Contaminated soils from Illkofen (Germany), Oulches-la-vallée and Pithiviers (France) were collected and used for the biological test. These soils contain high and comparable levels of *Polymyxa betae* Keskin which is infested with BNYVV pathotype B (Illkofen and Oulches-la-vallée) or P (Pithiviers).

Hexagonal tubes were filled with a prepared soil-sand mixture and grouped by 7 in a plastic pot. For each of the genotypes, one-week-old seedlings were transplanted to these pots.

The experimental design was a Randomizsed Complete Block Design (RCBD) with 4 blocks, each containing one pot for each genotype.

After a 4 week incubation at 19°C, 10.000 lux and 70% relative humidity, the roots of the plantlets were gently washed, dried, weighed and freeze-dried. Crude extracts were prepared by dissolving the ground samples in a PBS-Tween buffer. Following extraction, the viral content of the supernatant was determined by a triple enzyme-linked immunosorbent assay (TAS-ELISA) using antibodies raised against epitopes of the BNYVV viral coat protein.

1.3.- YIELD TRIALS

Field data were obtained from 10 m^2 trial plots laid out in Randomised Complete Block Design. Test fields were located in:

- France: Alsace (BNYVV strain group A & B), Champagne (group B).
- Germany: Altach (group B).
- Spain: Daimiel (Ciudad Real), Pobladura and Toro (group A).

The harvest was performed using mobile tarehouses.

2.- RESULTS

2.1.- LINES IN BIOASSAY: COMPARISON OF 3 MAJOR GENES IN HOMOZYGOUS STAGE

No major difference is observed between the susceptible lines tested; they react similarly in the 3 soil conditions. This is not the case for the resistant lines, especially for the lines with the "Holly" or "Rizor" gene, showing a significantly higher virus content with the Oulches and Pithiviers isolates.

Table 1: BNYVV content(1) in the lateral roots of lines tested in bioassays.

Tableau 1: Concentration en BNYVV(1) dans les racines latérales de lignées testées en biotests.

Tabelle 1: BNYVV-Gehalt(1) in Seitenwurzeln bei Linien, getestet mithilfe von Bioassays.

Line		Origin of soil		
Type	Resistance gene	Illkofen	Oulches	Pithiviers
R1	"B. maritima"	0.10 (± 0.09)	0.11 (± 0.10)	0.20 (± 0.12)
R2	"Rizor"	0.20 (± 0.17)	0.94 (± 0.44)	0.96 (± 0.50)
R3	"Holly"	0.37 (± 0.22)	0.95 (± 0.56)	0.84 (± 0.51)
S1	-	1.34 (± 0.68)	1.20 (± 0.73)	1.29 (± 0.18)
S5	-	1.68 (± 0.38)	1.77 (± 0.17)	1.15 (± 0.31)
S8	-	1.58 (± 0.35)	1.45 (± 0.55)	1.26 (± 0.17)

(1) Virus content of the roots is represented by the absorbance expressed in O.D. (optical density): mean (± standard deviation).

The line with the "*B. maritima*" gene is strongly resistant to all 3 isolates. Although the Oulches and Illkofen isolates belong to the same strain group B, the Oulches soil exhibits a higher aggressiveness, similar to that observed with the "Pithiviers" samples.

2.2.- DIPLOID HYBRIDS IN BIOASSAY: HOW MAJOR GENES INTERACT

The genotype combinations containing major genes typically have higher virus content in Pithiviers than in Illkofen soil (table 2 & figure 1). This trend is similar to that observed with lines homozygous for single genes (table 1).

The Elisa values were higher in the hybrids heterozygous (ie. in combination with the susceptible pollinator) for each of the 3 major genes.

The genotypes with the "*B. maritima*" gene, when in combination with one of the two other genes, exhibited the highest levels of resistance of all hybrid combinations tested in the very aggressive P group isolate.

This kind of differentiation between major genes was not observed in B soil conditions.

Table 2: *BNYVV content(1) in the lateral roots of hybrids tested in bioassays.*

Tableau 2: *Concentration en BNYVV(1) dans les racines latérales d'hybrides testés en biotests.*

Tabelle 2: *BNYVV-Gehalt(1) in Seitenwurzeln von Hybriden, getestet mithilfe von Bioassays.*

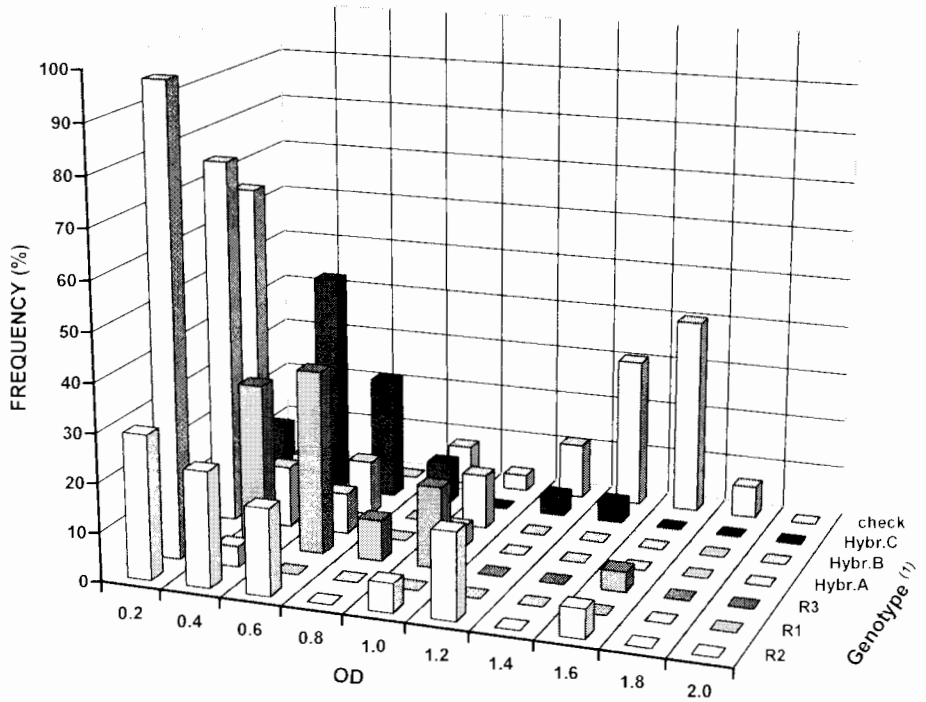
Diploid Female line	Resistance gene	Origin of soil	Diploid male line		
			Susceptible	"Rizor gene"	"Holly gene"
R1	"B. maritima"	Illkofen	0.33 (± 0.27)	0.16 (± 0.06)	0.20 (± 0.12)
		Pithiviers	1.11 (± 0.40)	0.37 (± 0.29)	0.28 (± 0.15)
R2	"Rizor"	Illkofen	0.35 (± 0.18)	0.31 (± 0.14)	0.25 (± 0.14)
		Pithiviers	1.05 (± 0.35)	1.09 (± 0.51)	0.75 (± 0.41)
R3	"Holly"	Illkofen	0.38 (± 0.17)	0.34 (± 0.20)	0.37 (± 0.12)
		Pithiviers	1.11 (± 0.41)	0.94 (± 0.30)	0.75 (± 0.39)
S9	-	Illkofen	1.11 (± 0.14)	0.59 (± 0.22)	0.58 (± 0.15)
		Pithiviers	1.61 (± 0.12)	1.14 (± 0.44)	1.05 (± 0.44)

(1) **Virus content of the roots is represented by the absorbance expressed in O.D. (optical density): mean (± standard deviation).**

Figure 1: Frequency distribution of BNYVV content(1) in lateral roots from resistant lines and hybrids (see table 2) tested in bioassay with Pithiviers soil

Figure 1: Histogramme de fréquences de la concentration en virus(1) des racines latérales de lignées et d'hybrides (Tableau 2) testés en biotests (sol de Pithiviers).

Diagramm 1: Häufigkeitsverteilung des BNYVV-Gehalts(1) in Seitenwurzeln von getesteten Linien und Hybriden (Tabelle 2) anhand von Bioassays in Pithivierboden.



(1) Virus content of the roots is represented by the absorbance expressed in O.D.

(2) Genotypes:
R1 = resistant line homozygous for "B.maritima" gene.
R2 = resistant line homozygous for "Rizor" gene.
R3 = resistant line homozygous for "Holly" gene.
hybr.A = cross between female homozygous for "B.maritima" gene & male homozygous for "Rizor" gene.
hybr.B = cross between female homozygous for "B.maritima" gene & male homozygous for "Holly" gene.
hybr.C = cross between female homozygous for "Rizor" gene & male homozygous for "Holly" gene.
Check = susceptible variety "Cadyx".

2.3.- DIPLOID HYBRIDS IN BIOASSAY: THE ROLE OF THE GENETIC BACKGROUND

Resistant hybrids (different females crossed with the same resistant male), heterozygous for the "Holly" gene, expressed a broad range of resistance levels (table 3). Their virus content ranged from low (values similar to those observed for double resistant hybrids), to very high values, sometimes close to those measured for the susceptible control (e.g. combinations with susceptible lines n° 6 to 11).

As seen earlier (table 1 & 2), all hybrids were infected more strongly with the inoculum of Oulches and Pithiviers than with the soil of Illkofen.

Table 3: BNYVV content(1) in lateral roots of hybrids(2) tested in bioassays.

Tableau 3: Concentration en BNYVV(1) dans les racines latérales d'hybrides (2) testés en biotests.

Tabelle 3: BNYVV-Gehalt(1) in Seitenwurzeln von Hybriden(2), getestet mithilfe von Bioassays.

Female line		Origin of soil		
Type	Resistance gene	Illkofen	Oulches	Pithiviers
R1	"B.maritima"	0.14 (± 0.12)	0.40 (± 0.25)	0.55 (± 0.39)
R2	"Rizor"	0.19 (± 0.14)	1.05 (± 0.36)	1.13 ± 0.47
R3	"Holly"	0.34 (± 0.25)	0.95 (± 0.56)	1.03 (± 0.48)
S1	-	0.12 (± 0.09)	0.68 (± 0.56)	0.65 (± 0.45)
S2	-	0.24 (± 0.21)	0.64 (± 0.47)	0.63 (± 0.34)
S3	-	0.28 (± 0.19)	0.80 (± 0.44)	n.t.
S4	-	0.33 (± 0.23)	0.98 (± 0.39)	n.t.
S5	-	0.43 (± 0.35)	0.54 (± 0.42)	0.93 (± 0.42)
S6	-	0.45 (± 0.41)	1.12 (± 0.48)	n.t.
S7	-	0.58 (± 0.39)	1.15 (± 0.38)	n.t.
S8	-	0.59 (± 0.42)	1.22 (± 0.38)	1.15 (± 0.34)
S9	-	0.67 (± 0.35)	n.t.	1.05 (± 0.44)
S10	-	0.72 (± 0.44)	1.18 (± 0.46)	n.t.
S11	-	0.78 (± 0.41)	1.15 (± 0.33)	1.28 (± 0.30)
Check (3)		1.48 (± 0.31)	1.61 (± 0.30)	1.69 (± 0.14)

(1) Virus content of the roots is represented by the absorbance expressed in O.D. (optical density): mean (± standard deviation).
(2) The hybrids tested in this bioassay were made with a resistant pollinator homozygous for the "Holly gene".
(3) Check: susceptible variety "Cadyx".
(4) n.t. = Not tested.

2.4.- EVOLUTION OF RESISTANCE EXPRESSION IN A BACKCROSS PROGRAMME

After 2 successive backcrosses of a "*B. maritima*" gene into a highly susceptible line the resistance level was partly reduced; this reduction was greater in the heterozygote than in homozygote progenies (table 4). This demonstrates the importance of the background of the susceptible line for disease expression.

Table 4 : BNYVV content[1] in lateral roots from B. maritima derived progeny[2] tested in bioassays (soil sample from Illkofen).

Tableau 4 : Concentration en BNYVV[1] dans les racines latérales de lignées dérivées de B. maritima[2] testées en biotests (sol d'Illkofen).

Tabelle 4 : BNYVV-Gehalt[1] in den Seitenwurzeln von Nachkommen[2] von B. Maritima, getestet mithilfe von Bioassays (Bodenprobe von Illkofen)

Generation	Number of plants tested	Genotypes [3]		
		Homozygote Resistant	heterozygote	Homozygote Susceptible
F1S1	263	0.02	0.10	1.00
BC1S1	324	0.04	0.22	1.10
BC2S1	218	0.20	0.70	1.20

(1) Virus content of the roots is represented by the absorbance expressed in O.D.
(2) Successive backcross steps of resistant line R1 with susceptible line S9.
(3) The genotypes were characterised and selected for the presence of major gene alleles with molecular markers.

2.5.- RESISTANCE OF DIPLOID AND TRIPLOID HYBRIDS BASED ON RESISTANT FEMALES AND SUSCEPTIBLE MALES

Table 5 : BNYVV content[1] in lateral roots from hybrids tested in bioassays.

Tableau 5 : Concentration en BNYVV[1] dans les racines latérales d'hybrides testés en biotests.

Tabelle 5 : BNYVV-Gehalt[1] in Seitenwurzeln von Hybriden, getestet mithilfe von Bioassays.

Female line		Ploidy of the susceptible male line	
Type	Resistance gene	2n	4n
S9	-	1.57 (± 0.12)	1.54 (± 0.15)
R1	"B.maritima"	0.40 (± 0.27)	1.15 (± 0.33)
R2	"Rizor"	0.44 (± 0.34)	1.13 (± 0.36)
R3	"Holly"	0.47(± 0.32)	1.12 (± 0.28)

(1) Virus content of the roots is represented by the absorbance expressed in O.D. (optical density): mean (± standard deviation).

In this bioassay (soil sample from Illkofen), a significant gene dose effect was observed for each major gene, with the diploid showing lower absorbances than corresponding triploid hybrids.

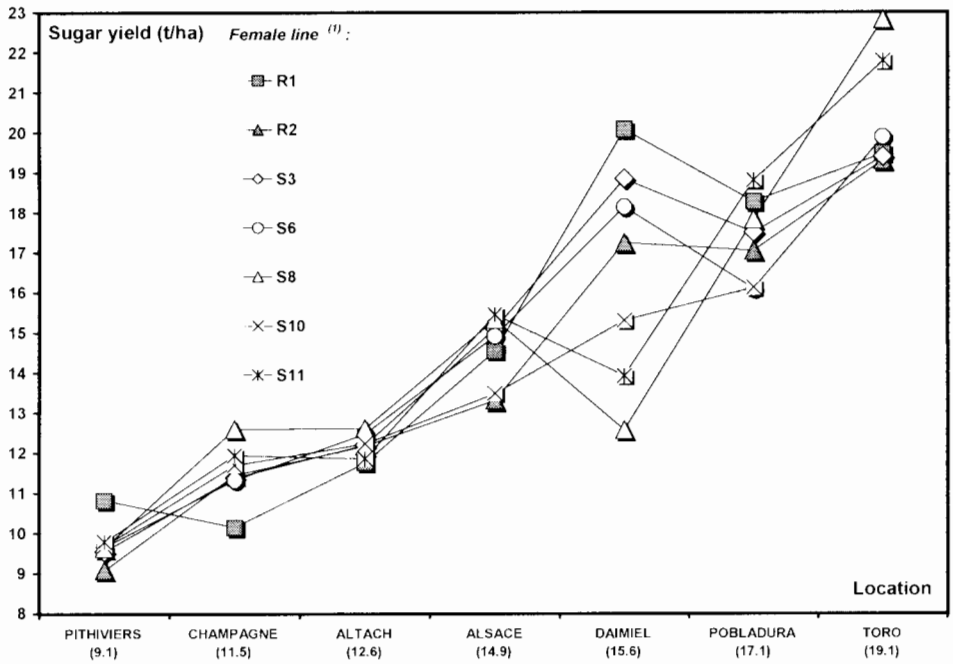
2.6.- SUGAR YIELD IN RHIZOMANIA INFESTED FIELDS

Strong genotype–environment interactions were observed amongst resistant hybrids based on the "Holly" gene (figure 2), especially in the centre of Spain (Ciudad Real area).

Figure 2 : Sugar yield of hybrids(1) across European rhizomania contaminated fields.

Figure 2 : Performances (rendement en sucre) d'hybrides(1) testés dans un réseau d'essais européens infestés par la rhizomanie.

Diagramm 2 :Zuckerertrag von Hybriden(1) in verschiedenen, mit Rhizomania befallenen Feldern in Europa.



(1) All hybrids tested in these field trials were made with a diploid pollinator homozygous for the "Holly gene".

The sugar yield ranking of these hybrids was specifically altered in this location.

High yielding hybrids, performing at the top level in most heavily diseased locations (e.g. combination with susceptible line S8) were broken down in this highly contaminated area; the strongest alteration was observed for root yield, although sugar content was also severely affected in some cases.

The "double resistant" hybrid made with the "*B. maritima*" gene outperformed the other hybrids in this region; it was also the best performing in Pithiviers.

The best single resistant, "Holly"-based, hybrid combinations, made with

susceptible females S3 and S6 were at the same sugar yield level as the best double BNYVV resistant hybrid ("*B. maritima*" & "Holly" genes).

These observations are consistent with the BNYVV content measured in bioassay (table 3).

3.- DISCUSSION

The efficiency of 3 major genes of BNYVV resistance was tested in different genetic backgrounds and at two ploidy levels. A broad range of resistance expression was observed as measured in lateral roots by the Elisa test after viral bioassays.

Cross comparison between the data suggests that these 3 major genes show some similar properties, including a higher resistance when homozygous than when heterozygous, and a lower resistance at the triploid than at the diploid level. This is consistent with the observations of Wisler et al., (1999) about a close association between the dosage of the "Holly" gene and the levels of BNYVV in lateral roots harvested in the field. Furthermore incomplete dominance involving major genes seems to be common in plant pathosystems involving viruses (Fraser, 1990).

At line level the "*B.maritima*" gene seems to act differently from "Holly" or "Rizor" genes in the presence of more aggressive isolates of BNYVV, suggesting a different mode of action.

No specific interaction (as defined by Vanderplank, 1988) was observed between the major genes and BNYVV strain group B and P. Nevertheless in some soils (including soil with pathotype P) higher resistance levels were observed with double resistant hybrids (especially combinations with "B. maritima" and "Holly" genes). These same genotypes performed better in field trials with soil of pathotype P or in field trials with a high level of infection (e.g. centre of Spain). This effect was repeatedly observed in our private trials; but in less aggressive rhizomania areas, the yield potential was less competitive.

Independently, Richard-Molard (2002), and Harju & Richard-Molard, (2002) also reported the much better performances in Pithiviers of a similar type of double BNYVV resistance ("Holly" x "C48" genes) relative to any of the single resistant hybrid (based on the "Holly" gene) tested in these extremely severe conditions.

Although we observed that the genetic background interacts with major genes both in backcross programmes and in hybrid testing, some susceptible lines strongly "support" the expression of major genes, giving a level quite similar to that of double resistances. This might give a false impression of dominance of these major genes, which is in fact induced by interactions with minor genes ("resistance enhancers").

We have to be very cautious when we compare major genes of resistance; the possible interference of the genetic background may be confusing unless comparisons are made in truly isogenic backgrounds. These studies are in progress. Successful long-term resistance breeding programmes have to take these enhancing genes into account.

Do we have to consider aggressive isolates of BNYVV as a threat for sugar beet growers? A higher aggressiveness was reported previously with the P strain group (Heijbroek, et al., 1999), and linked to the presence of a fifth genomic RNA (Tamada et al., 1996).

The high level of aggressiveness, observed in this study with soil from Oulches (group B) indicates that some variation may be observed for this trait amongst isolates lacking the RNA 5. It is clear that the sugar beet plant pathosystem is regulated with partial resistances on the host level and with aggressiveness on the pathogen level. So we are far from the vulnerability described in the gene by gene interaction system (Flor, 1942), which is common in most polycyclic diseases (e.g. rust, downy and powdery mildew). In this case any new resistance gene is sooner or later "broken down" by a new gene of virulence, which can spread very fast, leading in extreme cases to the so called "Vertifolia effect" (Vanderplank, 1968; Robinson, 1976; Rapilly, 1998). It was referred as "interactive" resistance by Vanderplank (1978); antithetic to the concept of additive resistance observed in this study.

The very aggressive rhizomania strain group P seems to have been in Pithiviers for decades, possibly introduced from Asia on mulberry trees (silk industry) during the 18-19th centuries (De Bruyne, personal communication). The Pithiviers area was also known for growing saffron, a plant also originating from Asia and the P pathotype could have been imported with those plants (Harju & Richard-Molard, 2002). To date, this strain has failed to spread to other sugar beet areas in France.

A large-scale survey will be carried out in Europe in 2003. It is organised by the Pest and Diseases group of IIRB, involving several countries, institutes, research teams and breeding companies. The objective is to improve our knowledge about the variability and aggressiveness of BNYVV isolates (Molard, 2002; Harju & Molard, 2002).

We can not exclude that changes in climatic factors like the increase of rainfall in some regions, as well as particular influence of other organisms may alter the level of inoculum in soils and increase crop damage. It is nevertheless difficult to estimate the level of virus needed in the root tissues before significant yield losses are observed in resistant hybrids.

CONCLUSION

After growth under comparable and standardised conditions in soils from rhizomania infected fields, sugar beet genotypes do differ for BNYVV content in the rootlets of the seedlings. Genotypes known to limit yield losses in infected fields have typically lower virus levels.

For the sources of resistance that are in current use in commercial varieties (hybrids made by crossing a resistant line to susceptible one), the resistance is effectively dominant in diploids; where the susceptible partner is tetraploid, however, a gene dosage effect can often be seen.

For the "Holly" gene, the expression and degree of dominance of the resistance , measured as BNYVV content in a bioassay, was shown to be dependent on the line used as the susceptible partner in the hybrid. Similar

effects of the background were seen for root yield and sugar content when measured on hybrids in fields chosen for strong disease pressure. The effect of the background was also illustrated during introgression of a major gene from a sea beet into a sugar beet. These findings emphasise that generalisations on the mode of action, the degree of expression or the breaking of a resistance should be handled with care.

The virus content of a sugar beet genotype is influenced by the origin of soil and isolates used in the bioassay. We have used very aggressive soil/isolate combinations to study the interactions between major genes. Under these conditions, the combination of "*Beta maritima*" gene with either a "Holly" or a "Rizor" major gene was superior to any other combination tested. We conclude that the major genes have different and apparently complementary modes of action. The effect of enhanced resistance did not significantly influence the yield parameters of hybrids in field trials done on a number of representative sites in Europe. On a few sites, however, genotype inversions are observed and the rank of hybrids more closely parallels their levels of resistance on virus multiplication in strong bioassays. These sites are known to differentiate strongly between materials of different origin and/or to contain the P type of resistance.

The presence of different, enhancing or complementary resistance mechanisms towards BNYVV in the sugar beet germplasm is promising as a tool to cope with future evolution in disease pressure.

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