Mapping Leaf Spot Disease Resistance Genes in Sugar Beet

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SUMMARY

Leaf spot disease is one of the most important foliar diseases of sugar beet (*Beta vulgaris* L.) caused by the fungal agent *Cercospora beticola* Sacc. The disease is currently controlled by both the application of fungicide and the use of resistant cultivars. Disease resistance is inherited as a complex trait with medium to low heritability. As a result, the molecular mapping and identification of disease resistance genes is hard to achieve. Different approaches for genetic mapping will be described and results will be discussed.

Based on a F_2 AFLP marker mapping, single plants with known quantitative trait loci (QTLs) for resistance were selfed (F_3), mass selected, selfed again (F_4) and in parallel crossed to a male sterile line (MS x F_3). F_4 lines and F_3 hybrids were scored for disease severity in replicated observation trials. Results will be presented with respect to (1) the achievement of a dissection of the complex inherited disease resistance trait into simple segregating disease resistance genes, (2) a preliminary conclusion on the efficacy of the QTL based selection to leaf spot disease resistance, and (3) discussion of alternative approaches to facilitate marker assisted breeding for *Cercospora* leaf spot resistance.

ZUSAMMENFASSUNG

Cercospora-Blattflecken stellen die bedeutendste Blattkrankheit an Zuckerrüben (*Beta vulgaris* L.) dar. Sie werden verursacht durch den Pilz *Cercospora beticola* Sacc. Die Krankheit wird gegenwärtig durch die Anwendung von Fungiziden und die Verwendung von resistenten Sorten kontrolliert. Die Krankheitsresistenz wird als komplexes Merkmal mit mittlerer bis geringer Heritabilität vererbt. Folglich ist eine molekulare Kartierung und Identifizierung der Krankheitsresistenzgene schwer zu erreichen. Verschiedene Ansätze zur genetischen Kartierung werden vorgestellt und die Ergebnisse diskutiert.

Ausgehend von einer F_2 AFLP Markerkartierung wurden einzelne Pflanzen mit bekannten quantitativen Merkmalsloci (QTLs) geselbstet (F_3), über Massenauslese weiter selektiert, erneut geselbstet (F_4) und parallel mit einer männlich sterilen Linie gekreuzt (MS x F_3). F_4 Linien und F_3 Hybriden wurden auf ihre Krankheitsanfälligkeit in wiederholten Beobachtungsversuchen bonitiert. Es werden Ergebnisse vorgestellt in Bezug auf (1) den Erfolg dieses komplex vererbte Merkmal Krankheitsresistenz in mehrere einfach vererbte Krankheitsresistenzgene aufzulösen, (2) eine vorläufige Schlussfolgerung zur Effizienz der QTL basierten Selektion der Blattflecken-Krankheitsresistenz und (3) eine Diskussion alternativer Ansätze zur Ermöglichung einer

markergestützten Züchtung der Cercospora-Blattfleckenresistenz.

ABRÉGÉ

La Cercosporiose est une des maladies foliaires les plus importantes de la betterave sucrière (*Beta vulgaris* L.), causée par le champignon *Cercospora beticola* Sacc. La maladie est contrôlée par les traitements fongicides et l'utilisation de variétés résistantes. La résistance est transmise génétiquement de manière complexe avec une héritabilité moyenne à faible. La cartographie moléculaire et l'identification de gènes de résistance est donc très difficile. Des méthodes différentes de cartographie génétique sont décrites et les résultats en sont discutés.

Sur la base d'une cartographie de marqueur F_2 AFLP, des plantes individuelles, possédant des QTLs repérés pour la résistance, ont été autofécondées (F_3), sélectionnées massalement, autofécondées à nouveau (F_4) et croisées en parallèle à une lignée mâle stérile (MS x F_3). L'intensité de la maladie des lignées F_4 et hybrides de F_3 a été évaluée dans des essais reproduits. Des résultats seront présentés concernant (1) la dissection d'un caractère complexe relatif à la résistance à une maladie en une série de gènes uniques et à ségrégation simple, (2) une conclusion provisoire sur l'efficacité de la sélection pour la résistance contre la Cercosporiose sur la base der QTLs, (3) une discussion sur des stratégies alternatives pour simplifier la sélection par marqueurs moléculaires pour la résistance contre la Cercosporiose.

INTRODUCTION

Leaf spot disease is one of the most important foliar diseases of sugar beet (*Beta vulgaris* L.) caused by the fungal agent *Cercospora beticola* Sacc. The disease is controlled both by the application of fungicide and the use of resistant cultivars. Host plant resistance is currently agriculturally preferred because of the absence of environmental pollutants and ease of implementation. The economic superiority of resistant varieties is proportionally related to yield performance under both diseased and non-disease conditions. Resistance breeding efficiency is therefore highly crucial. However, disease resistance is inherited as a complex trait with medium to low heritability. High environmental variability influencing correct trait assessment and production cost for progeny testing preventing fast breeding success (ASHER et al. 2000). Molecular breeding can circumvent these disadvantageous factors with *marker-assisted-selection*, MAS. The prerequisite for MAS include the need for molecular mapping and the identification of disease resistance genes. Different approaches for genetic mapping and validation will be described and results will be discussed.

TOWARD QTL VALIDATION

Based on a F_2 AFLP (*amplification fragment length polymorphism*) marker mapping (SETIAWAN et al. 2000), single plants with known quantitative trait loci (QTLs) for resistance were selfed (F_3), mass selected, selfed again (F_4) and in

parallel crossed to a male sterile line (MS x F_3). F_4 lines and F_3 hybrids were scored for disease severity in replicated observation trials. Results will be presented with respect to (1) the achievement of a dissection of the complex inherited disease resistance trait into simple pattern of segregating disease resistance genes, (2) a preliminary conclusion on the efficacy of the QTL based selection to leaf spot disease resistance, and (3) discussion of alternative approaches to facilitate marker assisted breeding for *Cercospora* leaf spot resistance.

MATERIAL

SETIAWAN *et al.* (2000) detected 8 significant QTLs by applying leaf disk assays and field observation trials explaining up to 25 % of the phenotypic variance each. Following the rationale of having each QTL segregating, while the other QTLs are fixed for the respective susceptibility alleles, 23 F_2 genotypes were selected based only on QTL marker information. From these 5 F_3 families could be harvested in sufficient amounts for the following studies. Within these F_3 families a phenotypic selection of each the 3 most susceptible and the 3 most resistant single F_3 plants was performed in field observation trials under artificial inoculation (mass selection). Again, subsequent generations were produced and 16 F_4 lines and 18 MSF_3 test hybrids could be harvested in sufficient amounts. Disease observation trials were performed in Italy and Germany with the achieved F_4 lines and MSF_3 test hybrids. Additionally, one yield performance trial with the test hybrids was performed in Germany.

A GLIMPSE INTO INHERITANCE OF LEAF SPOT RESISTANCE

None of the F_2 genotypes selected was configured with the ideal allele configuration due to the limited number of available genotypes and the high number of possible allele combinations when dealing with up to 8 loci – 3^8 = 6561 genotypic combinations. Tab. 1 presents the 8 QTL allele configurations of the 5 F_2 genotypes of which F_4 lines and MSF_3 test hybrids were assayed later in the field.

Several disease severity measurements are possible and were also applied. F_2's and advanced material were unfortunately tested under different conditions and were scored in different ways due to seed limitations and changes in practice applied over the years of the experiment. On the other side, by careful interpretation of the different trait evaluations, we were able to reach conclusions concerning the feasibility and limitation of each method applied and could additionally uncover conformity. It becomes obvious from Tab. 2 that the most susceptible and resistant accessions are always the same irregardless of the method (artificial *vs.* natural infestation) and material (*per se vs.* test hybrid) under study. However, extreme differences could also be pointed out by scoring all single plants in a field observation trial with the F_3 lines which clearly showed substantial segregation. Especially in early generations limitations of plot scoring methods were obvious (data not shown).

Tab. 1: *Allele configurations of 5 F_2 genotypes. The 8 QTLs are given in the first column, followed by the suffix "Id" for QTL identified in leaf disk assays and "fs" for QTL identified in a field observation trial. All QTL were significant and the most important QTLs are typed in bold. A: homozygous for the susceptible parent; B: homozygous for the resistant parent; H: heterozygous.*
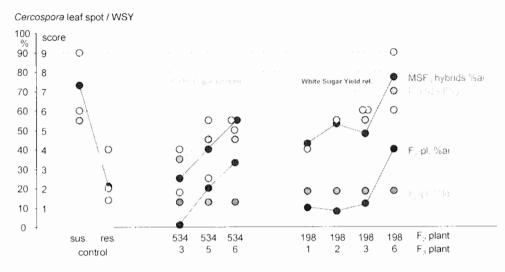
QTL/Genotype	198	376	383	534	651
1_ld	A	H	H	H	A
2_fs	H	B	B	B	H
3_ld	A	B	H	B	H
4_ld	A	A	A	H	B
5_fs	A	A	A	H	H
6_fs	A	H	B	A	B
7_fs	H	A	H	A	H
8_fs/ld	A	B	B	B	B

Tab. 2: *Disease severity of 5 F_2 genotypes and the corresponding F_3 lines. Phenotypic scores were achieved with F_2 single plant scored for % diseased leaf area in leaf disk assays, F_2 half sib families scored from 1-9 in a field trial in Italy, F_3 families scored for % diseased leaf area and according a 1-9 scale in an artificially inoculated field trial in Germany and the same F_3 families scored from 1-9 in a field trial in Italy.*

Genotype/Line	198	376	383	534	651
F_2 % ld	18	10	13	13	14
F_2-HSF fs	n.t.	7.5	6.5	n.t.	7.5
F_3 % art. inoc. Germany	21	8	9	12	12
F_3 fs art. inoc. Germany	6	2	2	2	3
F_3 fs Italy	9	4	5	6	4

A compacted summary of the achieved data is given in Fig. 1 for an exemplary part of the material. F_2 genotype 198 was rated highly susceptible and segregating for only 2 QTL approximating our QTL marker selection goal closest. Selection of distinct susceptible lines was achieved and could be proved with subsequent populations. White sugar yield (WSY) is remarkably stable if nor superior in the more resistant material. This is a first hint that disease resistance and yield performance are not always negatively related for example by epistatic effects which could not genetically be separated from each other. This is also supported by the results of the derived material from F_2 plant 534. In this case the most promising results were achieved by successful resistance selections confirmed in subsequent generations and stable yield performance of the final test hybrids.

Fig. 1: Disease severities and white sugar yield (WSY) are shown for a selected part of the material in this study. For the material cf. Material, Tab. 1 and 2. Please note that various traits are drawn at the same y – scale. Genotypes denoted at the x – scale are the trait selected F₃ single plants.

OUTLOOK

The ultimate goal of the study is to identify and validate agronomically important QTLs for *Cercospora* leaf spot resistance for application in MAS. Purely based on marker selected F_2 plants QTL segregating material was processed to recombinant inbred lines (RILs) and assayed for disease severity and yield performance. Finally, RILs will be studied for their QTL allele configuration to prove correlation between estimated QTL effects and disease severity observed in the field.

ACKNOWLEDGEMENT

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