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Originalarbeit

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Resistance to anthracnose in narrow-leafed lupin (*Lupinus angustifolius* L.): sources of resistance and development of molecular markers

Widerstandsfähigkeit gegen die Anthraknose bei der Blauen Lupine: Resistenzquellen und Entwicklung molekularer Marker

Abstract

Breeding lines and genebank accessions were screened for novel anthracnose resistances by use of a reliable and standardized resistance test under greenhouse conditions. While all the German cultivars tested proved to be susceptible, two breeding lines were identified which displayed strong resistance to *Colletotrichum lupini*. One of these was subsequently tested in the field and strong resistance could be confirmed. F2 populations for the mapping of the potentially novel resistances were started to be set up. For mapping purposes, 150 gene-based molecular markers provided by M. Nelson (University of Western Australia, Perth) are being used. Sequence information from the *Medicago truncatula* and *Lotus japonicus* genomes is also addressed to develop additional molecular markers in narrow-leafed lupin.

Key words: *Lupinus angustifolius*, resistance, molecular markers, *Colletotrichum lupini*

Zusammenfassung

Zuchtlinien und Genbankakzessionen der Blauen Lupine wurden unter Anwendung eines zuverlässigen und standardisierten Resistenztests unter Gewächshausbedingungen auf Anthraknose-Resistenz evaluiert. Während alle getesteten deutschen Blaulupinensorten sich als anfällig erwiesen, konnten zwei Zuchtlinien mit hoher Widerstandsfähigkeit gegenüber *Colletotrichum lupini* identifiziert werden. Eine dieser Linien wurde bislang im Feldversuch getestet, wo sich ihre hohe Resistenz bestätigte. Es wurde mit dem Aufbau von F2-Kartierungspopulationen für die genetische Analyse dieser möglicherweise neuen Anthraknose-Resistenz begonnen. Für die Kartierungsarbeiten stehen vor allem 150 genbasierte molekulare Marker der University of Western Australia zur Verfügung. Darüber hinaus werden die Genomdaten der leguminosen Modellgenome von *Medicago truncatula* und *Lotus japonicus* für die Entwicklung zusätzlicher molekularer Marker in der Blauen Lupine genutzt.

Stichwörter: *Lupinus angustifolius*, Resistenz, molekulare Marker, *Colletotrichum lupini*

Introduction

One of the most important lupin diseases is anthracnose, caused by the fungus *Colletotrichum lupini*. The disease is present in most parts of the world where lupin is cultivated (Yang et al., 2004) For the time being, fungicide application and seed treatment are used to prevent *Colletotrichum* infection or spreading of the disease. Thus, resistance to anthracnose has to be improved in narrow-leafed lupin and is one of the major objectives in lupin breeding

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(YANG et al., 2004; ECKARDT et al., 2004). To date, the high-yielding resistant cv. 'Mandelup' as well as the highly resistant cv. 'Tanjil' have been used for breeding strategies in Australia to improve anthracnose resistance (YANG et al., 2004; YANG et al., 2008). Resistance of cv. 'Tanjil' is inherited by a dominant gene which was designated *Lanr1* (YANG et al., 2004) and which can be tracked in breeding programmes by use of a closley linked co-dominant molecular marker (YOU et al., 2005). We have started efforts to screen genetic resources of narrow-leafed lupin for novel resistances which are effective under the growing conditions met in Germany's agriculture, and to genetically analyse these resistances including the mapping relative to molecular markers.

Materials and Methods

Plant materials

Screening for novel resistances. A total of 13 cultivars ('Arabella', 'Bolivio', 'Bora', 'Bordako', 'Boregine', 'Boruta', 'Borweta', 'Haagena', 'Haags Blaue', 'Mandelup', 'Polonez', 'Tanjil', 'Vitabor'), 15 breeding lines as well as 26 gene bank accessions were available for resistance screening in the greenhouse. The breeding lines Bo7212 and Bo3533, the German cv. 'Arabella' and the Australian cv. 'Tanjil' were included in a field test for anthracnose infestation in 2007.

Mapping populations. Breeding lines Bo7212 and Metel1 were used as pollen parents in crosses with cvs. 'Arabella', 'Haagena', and 'Haags Blaue'.

The parent (83A:476, P27255) of the *L. angustifolius* mapping population used by BOERSMA et al. (2005) and NELSON et al. (2006) was kindly provided by B. WOLKO (Institute of Plant Genetics, Polish Academy of Sciences).

Anthracnose strains. Plants were inoculated in the greenhouse with strain BBA70358 of *C. lupini* var. *setosum*. Plants were inoculated in the field with a mixture of five different strains of *C. lupini var. setosum* (BBA70400, BBA70397, BBA70358, BBA70385, BBA71238). The strains were kindly provided by H. I. NIRENBERG at the former Federal Biological Research Centre for Agriculture and Forestry.

Methods

Resistance tests

Greenhouse resistance tests were performed according to YANG et al. (2004). Plants were inoculated by spraying with a conidial suspension (10^5 conidia per ml). The inoculated plants were incubated in the dark for 16 h. Disease was recorded 10-14 days after inoculation in a climate chamber. Plants with superficial scars were assessed as beeing resistant. Plants displaying collapsed spikes and/or lesions bearing pink conidial masses were regarded as susceptible.

For field testing a randomized block design with two replications was used. Field testing was done in 2007 at the two locations of Bocksee and Groß Lüsewitz. For inoculation under field conditions, 5 infection rows per block were used. Each infection row comprised 15 seeds of cv. 'Arabella' contaminated with conidia and sown when the test plants were at the 2-5 leaf stage. The seeds for infection rows had been prepared by immersing in a conidial suspension of 10^5 conidia per ml for 4 h and subsequent drying overnight. Scoring was performed three times, i.e., at the 6-8-leaf stage, at flowering time and at the early-pod stage.

Molecular markers

Sequence information of PCR markers from *Lupinus angustifolius* was kindly provided by M. NELSON, Univ. of Western Australia, Perth.

Medicago truncatula primers were used as recommended in the *mtgenome* database (http://mtgenome.ucdavis.edu/index.html).

By using the NCBI database (http://www.ncbi.nlm.nih.gov/sites/) EST sequences from *Lotus japonicus* were transferred to the SSRIT software application (http://www.gramene.org/gramene/searches/ssrtool) for searching SSR motives.

Primers for ESTs from *Lotus japonicus* were designed using the software package Prime3 (ROZEN and SKALETSKY, 2000).

For PCR of STS and SSR markers from *Lupinus angustifolius, Lotus japonicus* or *Medicago truncatula*, 50-100 ng of genomic DNA was used in a solution containing 1 x reaction buffer (Qiagen), 200µM dNTPs, 5 pmol primers and 0.5 U of *Taq* DNA polymerase (Qiagen). PCR products were separated on 2.5% agarose gels followed by ethidium bromide staining or 10% page followed by silver staining (BUDOWLE et al., 1991).

Results and Discussion

Search for potential resistance sources

In the resistance test under greenhouse conditions, each of the 12-15 plants tested from 11 European cultivars proved to be susceptible to anthracnose. In contrast, the Australian cvs. 'Tanjil' (Fig. 1) and 'Mandelup' were highly resistant. Of the 15 breeding lines tested, two breeding lines (Bo7212, Metel1) were found to be resistant while 12 entries were classified as susceptible. Breeding line Bo3533 displayed an intermediate reaction, with lesions considerably smaller than those observed with the susceptible entries.

Resistance testing under field conditions gave slightly higher infestation rates with the Groß Lüsewitz (G.L.) location as compared to the location of Bocksee (Fig. 2). This may be due to the more humid conditions at the near-coastal location of G.L. in 2007. Despite these small differences the reaction patterns of entries were identical at both locations (Fig. 2). Breeding line Bo7212 was significantly less affected as compared to the remaining entries. Hence, the strong resistance of Bo7212 observed

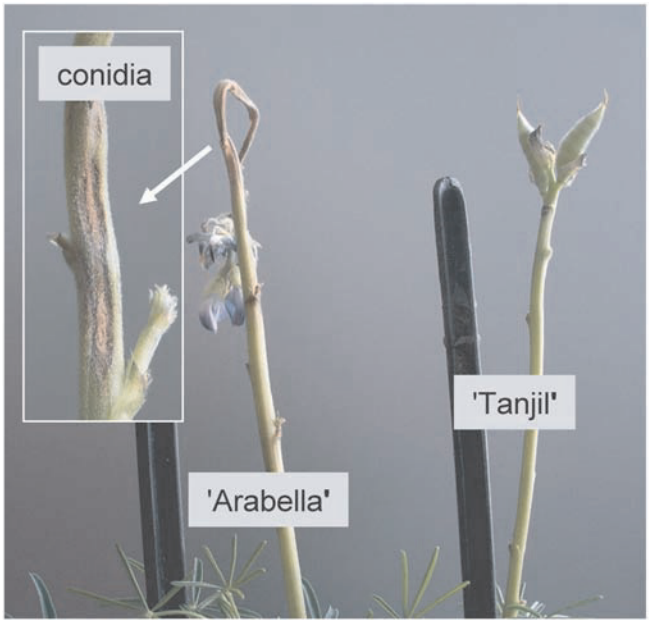

Fig. 1. Greenhouse resistance test 10 days after inoculation with a conidial suspension. Susceptible plant of cv. 'Arabella' (left) and resistant plant of cv. 'Tanjil' (right).

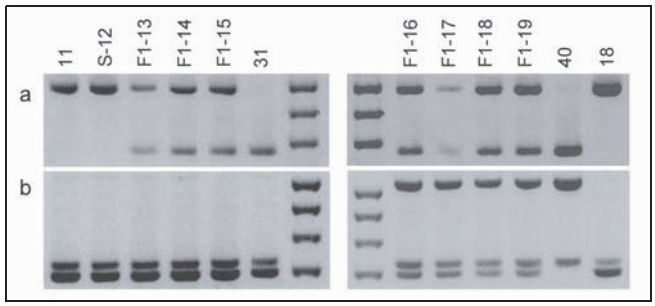

Fig. 3. Checking hybridity of F1 progeny by use of CAPS markers. **a**, marker *Lup228* (*Bst*NI); **b**, marker *Lup343* (Taq$^{\alpha}$I). Left-hand panel: parents cv. 'Arabella' (11) and Metel1 (31) and their offspring; right-hand panel: parents cv. 'Arabella' (18) and Bo7212 (40) and their offspring.

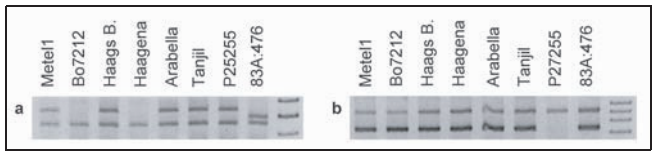

Fig. 4. Screening of SSR markers *VIII003F04* obtained from *Medicago truncatula* (a) and *LJM69* from *Lotus japonicus* (b).

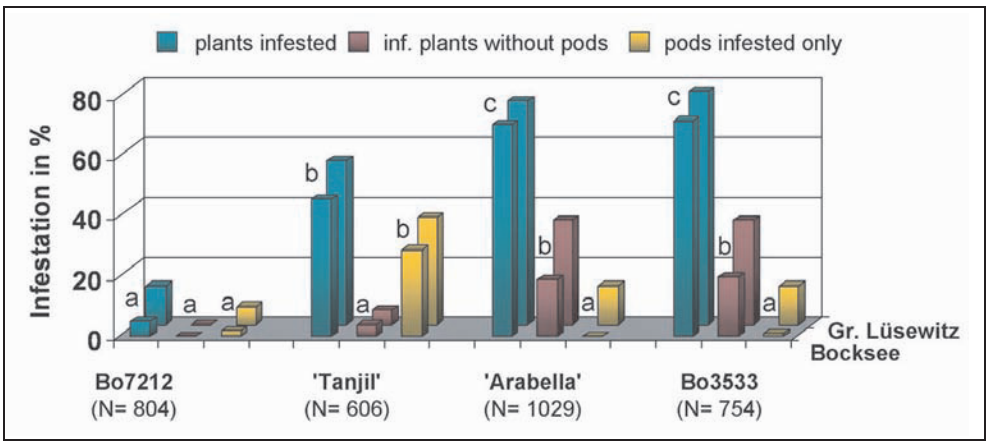

Fig. 2. Field testing for infestation with anthracnose at two locations in 2007. Letters a-c refer to significant differences according to the LSD-test (α = 0.05) test.

under controlled conditions in the greenhouse could be confirmed under variable field conditions. While cv. 'Arabella' as well as breeding line Bo3533 became highly diseased, the Australian cv. 'Tanjil' displayed a somewhat intermediate reaction with a significantly lower percentage of diseased plants than 'Arabella' and Bo3533 but significantly higher infestation as compared to Bo7212. It is notable that 'Tanjil' displayed high infection of the pods (Fig. 2). The quite dissimilar reactions of Bo7212 and cv. 'Tanjil' as shown in Fig. 2 may be due to the presence of different resistance genes. Further elaboration via molecular-marker analysis, though, will be needed to draw final conclusions on this question.

Mapping Populations

Crosses between susceptible cultivars ('Arabella', 'Haags Blaue', 'Haagena') and the two novel resistance resources, Bo7212 and Metel1, were performed in December 2007 and January 2008. Hand pollinations of the seed parents yielded up to five pods per plant with 1-5 seeds per pod. Plants grown from these seeds were checked for hybridity using molecular markers. One seed from each pod was checked with 6 polymorphic markers. With the exception of one pod (S-12) which turned out to be selfed offspring of the seed parent of cv. 'Arabella', all the other pods contained seeds displaying both parental marker alleles, i.e., one from either cv. 'Arabella', 'Haagena', or 'Haags Blaue' as the female parent and the other one coming from Bo7212 or Metel1, respectively, as the male parent (Fig. 3a). The F1 hybrids were selfed to give rise to F2 progeny part of which will be pooled to mapping populations.

Molecular markers

A screening for marker polymorphisms was started by using three different marker resources, namely, (i) the STS and CAPS markers derived from the genetic map of *Lupinus angustifolius* (NELSON et al., 2006), (ii) SSR markers from *Medicago truncatula* (*mt*genome database) and (iii) SSR markers derived from the genome of *Lotus japonicus* (NCBI database). CAPS marker *Lup228* (*Bst*NI) which had been mapped before on linkage group LG8 (NELSON et al., 2006) proved to be polymorphic between cv. 'Arabella'

and Metel1 (Fig. 3a). CAPS marker *Lup343* (Taq$^{\alpha}$I) was polymorphic between cv. 'Arabella' and Bo7212 (Fig. 3b). Both markers are co-dominant and useful for linkage analysis with the corresponding mapping families.

In an initial screening of SSR markers derived from *Medicago truncatula*, a subset of markers turned out to work in *L. angustifolius*. As an example, *VIII003F04* represents a marker which was polymorphic among resistant and susceptible crossing parents and which may be useful in our F2 mapping populations in spite of its dominant inheritance (Fig. 4a).

A screening for ESTs bearing SSR motives was started by using the EST resource from *Lotus japonicus* (NCBI database). In a first approach 3500 sequences were analysed using the SSRIT software and resulted in 512 (14.6%) sequences containing SSR motives with ≥5 repeats. One hundred primer pairs were designed and used for marker development. Whereas the parents of the mapping population used by BOERSMA et al. (2005) and NELSON et al. (2006) were polymorphic for a subset of these markers (Fig. 4b) we did not yet observe polymorphism with regard to the parents chosen for our F2 mapping populations. It would be premature at the present state of our marker survey to draw conclusions as to the overall extent of marker polymorphism in our mapping populations. Using the parents of the Australian mapping population might be an option to circumvent lack of marker polymorphism when mapping anthracnose resistance genes of Bo7212 and Metel1, however at the expense of applicability of markers in relevant German breeding materials.

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