DEVELOPMENT OF INNOVATIVE BREEDING STRATEGIES FOR INCREASING YIELD AND YIELD STABILITY AND PROMOTING GROWTH OF NARROW-LEAFED LUPIN (*LUPINUS ANGUSTIFOLIUS*)

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ABSTRACT

A multi-lateral approach is being used to improve yield, quality and resistance of narrow-leafed lupin in Germany. Yield and protein content is being increased via development of tetraploids. To this end, various colchicine concentrations and application techniques were screened to obtain vital tetraploid plants. To increase the crop area of narrow-leafed lupins in Germany, the pH-tolerance has to be improved. This approach involves the investigation of different root-growth parameters. Plants grown under calcareous and alkaline soil conditions were characterised through quantification of stunted roots and shoots. The fostering effect of different rhizobial strains was also evaluated and plants inoculated with 'HiStick' showed less growth repression.

Stability of lupin seed production will be improved by the development of disease-resistant lupin cultivars. Wild accessions and cultivated varieties of *L. angustifolius* were tested to select novel resistance resources to soil-borne diseases. Six accessions showed tolerance to *Sclerotinia sclerotiorum*. Some cultivars were less susceptible to *Fusarium oxysporum.* Three breeding lines were tolerant to *Thilaviopsis basicola* and one cultivar showed tolerance to *Rhizoctonia solani*. Pathogen-specific primers were developed for PCR-based diagnosis of isolated pathogens, which also worked in plants infected by multiple pathogens.

KEYWORDS

tetraploidy, pH-tolerance, soil-borne diseases

INTRODUCTION

In Germany, narrow-leafed lupins (NLL) are mainly grown in the north-eastern part of the country. NLL are very intolerant to alkaline soils. This might be due to the fixation of minerals such as P, Fe, Cu, Zn, Mn (Egle *et al.* 1999) and the occurrence of bicarbonate (HCO_3^-) and Ca in such soils. Therefore, the cultivation is mainly restricted to the aforementioned regions where sandy, moderately acid soils occur (Peiter *et al.* 2000).

Other threats to NLL crops are fungal diseases which affect the root or the base of the plant such as *Fusarium spp., Sclerotinia sclerotiorum, Thielaviopsis basicola* and *Rhizoctonia solani*. All of these biotic stresses strongly reduce seed yield (Infantino *et al.* 2006). Diverse germplasm collections and knowledge of geographic distribution are the basis of a successful screening for disease resistance (Infantino *et al.* 2006, Campbell *et al.* 1994).

An old approach to improve yield characteristics is polyploidisation. Since the late 1930s colchicine has been used as a mitotic inhibitor to create polyploid plants. Several researchers have tried to achieve polyploidy in different species using different methods. *Lupinus luteus* (Troll *et al.* 1963) and *Glycine max* (Gobs-Sonnenschein 1941) were two legume subjects of their studies. In contrast to other species, NLL is rather recalcitrant to colchicine treatment (Schumann 1960). However, colchicine appeared to be effective in other leguminous species, such as *Vicia faba* (Prashant *et al.* 2004) and clover (Evans 1955).

In the following sections, approaches aimed at increasing the potential of *L. angustifolius* in sustainable agriculture are described and discussed.

MATERIALS AND METHODS

ROOT GROWTH IN MODERATELY ALKALINE SOIL (PH 7.2) AFTER SEED TREATMENT WITH DIFFERENT RHIZOBIAL PRODUCTS

Transparent acrylic glass (PMMA) pipes with a diameter of 60 mm and a length of 1m and viewing boxes with measures of 0.15 m x 1.25 m x 1 m with a transparent, robust PMMA pane at the front were filled with soil and leant on a frame at an angle of 63°. The soil type was a loamy sand, with a pH of 7.2 and a $CaCO_3$ content of 1.1%. Three different rhizobial products ('Rizobin' [*Bradyrhizobium japonicum*], 'HiStick' and 'Radicin' [*Bradyrhizobium lupini*]) were tested on the indeterminate growth habit cultivar 'Boregine' using 16 replicates per treatment which were completely randomised on a single plant basis. The

IN J.A. Palta and J.B. Berger (eds). 2008. 'Lupins for Health and Wealth' Proceedings of the 12th International Lupin Conference, 14-18 Sept. 2008, Fremantle, Western Australia. International Lupin Association, Canterbury, New Zealand. ISBN 0-86476-153-8.

length of the roots and shoots were measured every second and third day respectively. The dry matter of the plants was determined by oven-drying them at 80°C and weighing afterwards.

POLYPLOIDISATION OF BREEDING LINES OF *LUPINUS ANGUSTIFOLIUS*

Plantlets or seeds of a range of NLL breeding lines were treated with colchicine solutions ranging from 0.005% to 0.2% for 7 h to 48 h. Mixtures of alkaloids such as colchicine-nicotine solutions were also tested. To all solutions dimethyl sulfoxide (DMSO) was added. To each mg of colchicine, 10 µl of DMSO were added. Plantlet application was varied as outlined below.

i) Seed treatment

Seeds were pre-soaked in either normal tap water or distilled water for 15 h or 24 h and then put into the colchicine solutions. Another method was to submerge the seeds in the solution without pre-soaking (Table 1). After taking the seeds out, they were rinsed with water and planted in trays or normal pots.

ii) Treatment of plantlets

The plantlets were treated following the method of Esser (1953) in order to prevent damage to the roots. Six seeds were put around a Petri dish in a pot filled with soil. Plantlets were bent over the rim of the Petri dish and submerged into the colchicine solution. A smaller dish placed on top kept them below solution level.

Table 1. Cultivars and treatments for polyploidisation.

Cultivar	Treatment	Colchicine concentr. (%)	Nicotine concentr. (%)	Duration	Seeds per treatment
Mandelup	Seed treatment 15 hr pre-soaked	0.008, 0.01	0.05	8, 24 h	30
Haagena, Borlu	Seed treatment not pre-soaked	0.02, 0.025, 0.03, 0.04	0.01	8, 15, 20 h	16
Haags Blaue	Seed treatment not pre-soaked	0.125	0.01	6, 10, 14, 20 h	16
Borlu, Tanjil, Bora	Esser's method	0.03, 0.04, 0.05, 0.06, 0.075, 0.08, 0.01, 0.015		15, 20, 25, 30, 48 h	6

Table 2. Scoring system for symptoms of *Sclerotinia sclerotiorum* (Ss), *Thielaviopsis basicola* (Tb), *Fusarium oxysporum* (Fo) and *Rhizoctonia solani* (Rs) on *L. angustifolius.*

Score	Description
1	Healthy plant.
3	Slight growth depression (all), light chlorosis (Tb, Fo), slight discolouration of root vessels (Fo), start of eyespot (Rs), 25% of tissue.
5	Stronger growth depression (all), stronger chlorosis (Tb), partial chlorosis (Fo), stronger discolouration of root vessels (Fo), clearly visible eyespot (Rs), 50% of tissue.
7	Growth depression, decay and dead parts (Ss, Tb, Rs), wilt (Fo), strong discolouration of root vessels (Fo), eyespot almost girdling the stem (Rs), 75% of tissue.
9	No germination or decay at seeds (Ss), plant dead (Tb, Fo, Rs), discolouration in necrotic tissue (Fo), stem-girdling eyespot, rotten seeds (Rs), 100% of tissue.

ROOT DISEASES AND RESISTANCE TESTS

Infected plants were taken from different sites to characterise, isolate and multiply the fungi. Wild lupin accessions were multiplied to provide enough seeds for resistance tests. The seeds were first disinfected in a 1.5% sodium hypochlorite solution for 5 minutes and the soil was steam-treated before the tests. Each inoculum (oat-wheat-mixture) of *Sclerotinia sclerotiorum* (2.9 g l^{-1} of soil), *Thielaviopsis basicola* (1.7 g l^{-1} of soil) or *Rhizoctonia solani* (1.7 g l^{-1} of soil) was mixed with soil for the respective trial. 24 seeds per fungus and test were planted afterwards. As for *Fusarium spp.* inoculation, the roots of the plants were shortened by 5 mm at the 2 leaf stage (BBCH 21) and submerged into the conidia suspension (1 x 10^6 mL^{-1}) for 15 minutes. Thereafter the plants were grown in pots and disease symptoms scored from 1 to 9 (Table 2). *S. sclerotiorum* symptoms were scored 8 days after inoculation (DAI), symptoms of *T. basicola* 14 DAI on the roots and 21 DAI on the shoots, of *F. oxysporum* 4 weeks and of *R. solani* 18 DAI. Scores were then transformed into a disease index (DI%) ranging from 0 to 100% in order to improve the infection scale (Feiler, 1998):

$$\text{Disease index (\%)} = \underline{\Sigma (n_1 + (n_3 * 3) + (n_5 * 5) + (n_7 * 7)...) * 100} / N * 9$$

n_1 – n_9 = Number of plants in the respective infestation group (1 to 9), N = Total number of inoculated plants.

RESULTS AND DISCUSSION

3.1 Root growth in moderately alkaline soil (pH 7.2) after seed treatment with different rhizobial products.

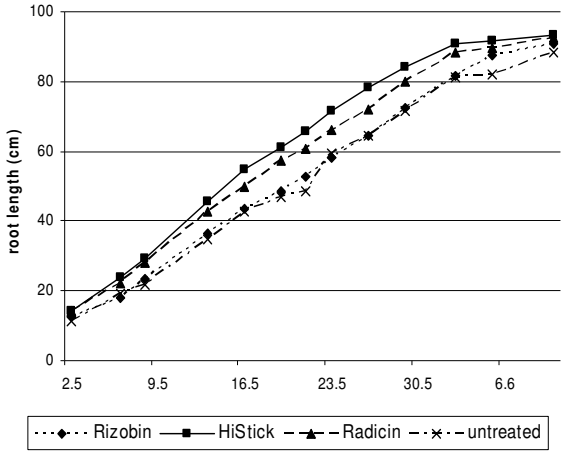

Fig. 1. Development of root length over time.

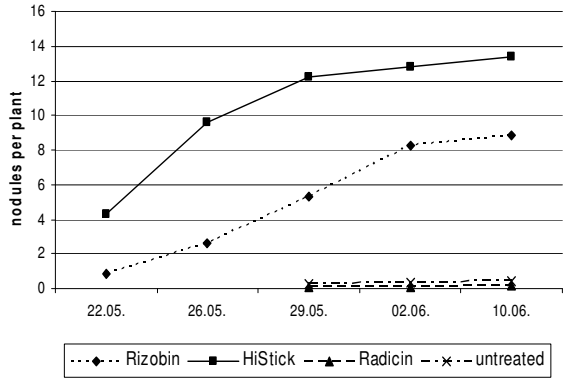

Fig. 2. Development of nodulation over time.

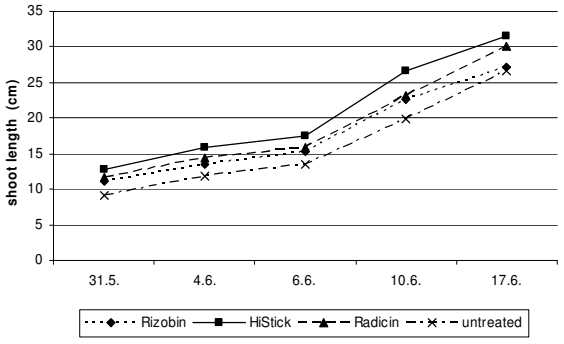

Fig. 3. Development of shoot length (cm) over time. Four different treatments: Rizobin (*Bradyrhizobium japonicum*), HiStick, Radicin (*Bradyrhizobium lupini*), untreated. Cultivar: Boregine (n = 16).

Cv. 'Boregine' showed considerable differences in growth of both roots and shoots (Figs 1 and 3). Plant roots treated with 'HiStick' grew faster than those of untreated and 'Rizobin'-treated plants (Fig. 1). Roots of 'Radicin'-treated plants showed a little slower development than 'HiStick'. 'HiStick' roots reached the final depth (bottom of the case or pipe) three to four days earlier than 'Rizobin' roots and 10 days earlier than roots of untreated plants. 'HiStick'-treated plants also developed the highest number of nodules (13.3) in the range of the upper 10 to 30 cm of the root, followed by Rizobin (9.0), while untreated and 'Radicin'-treated plants developed almost no nodules (Fig. 2). Untreated plants showed the expected reduced growth rate at higher pH as described by Peiter *et al.* (2001) and Tang *et al.* (1993).

Shoot length of the 'HiStick' treatment suggests that the product boosts plant growth on alkaline soils (Fig. 3). 'HiStick' plants were taller than the other treatments until the peak of anthesis (10 June). Dry matter production followed a similar trend in the early stages of growth, however the plants are not yet ready for harvest.

POLYPLOIDISATION OF BREEDING LINES OF *LUPINUS ANGUSTIFOLIUS*

Seed treatments of different solutions, alkaloid mixtures and different breeding lines were tested (Table 1). No polyploid plants have been produced. Different reactions between the cultivars could not be observed. Solutions of 0,01% to 0,02% Colchicine for 8 h to 24 h caused symptoms such as slow emergence and very short and thick hypocotyls. The first pair of leaves protruded very slowly and remained very small and thick and eventually died. The effect of the additional alkaloid nicotine was obvious. These symptoms were caused by a lower colchicine-concentrated solution + 0.01-0.05% nicotine. Not all plants treated with this method died. Some had chimeric tissue and the diploid cells, with the faster growth rate, overgrew the tissue of which we think it was polyploid. Before the trials, Esser's method (1953) appeared very promising as the roots are not affected by colchicine. Treating plantlets with 0.1% colchicine for 25 h (Table 1) led to thicker stems immediately above the cotyledons, after the first pair of leaves died off. After a period of stasis, new leaves were produced and the plant developed a normal, diploid shoot which was confirmed by flow-cytometric analyses. No polyploid plants have been produced so far.

ROOT DISEASES AND RESISTANCE TESTS

Sclerotinia sclerotiorum

The first disease symptoms of the plantlets were visible approximately 8 DAS when the cotyledons had unfurled. 'Tallerack' was most prone to *S. sclerotiorum* (88.9%) while 'Belara' (65.0%), 'Boregine' (60.7%), 'Tanjil' (61.4%) and the wild accession 'PI 289163' (60.5%) showed intermediate-high susceptibility. In contrast, 'PI-283633' (29.7%), 'PI-385078' (26.1%), 'PI-308616' (13.2%), 'BO-9027' (23.3%), 'BGE-023639' (35.2%) and 'BO-7212' (17.6%) were less susceptible.

Table 3. Disease Indices (DI%) of German cultivars derived from resistance tests, number of trials in brackets.

Cultivars	*Sclerotinia sclerotiorum*	*Fusarium oxysporum*	*Thielaviopsis basicola*	*Rhizoctonia solani*
Borlu	43 (4)	68 (1)	49	36 (1)
Bora	47 (2)	50 (1)		
Boruta	56 (2)	48 (2)		
Boregine	61 (6)			46 (1)
Boltensia	54 (4)	37 (1)		
Vitabor	50 (2)	50 (1)	68 (1)	48 (1)
Probor	40 (5)	29 (2)	63 (2)	51 (1)
Haags Blaue	43 (1)		27 (1)	
Sonet		45 (1)		
Idefix			65	

Thielaviopsis basicola

The first symptoms were visible on the roots 14 days after inoculation, and on the shoots after 21 days. The highest susceptibility to *Thielaviopsis basicola* was seen on 'Graf' (79.4%), 'Idefix' (64.5%) and 'Vitabor' (68.4%). By contrast, the wild accessions 'PI-274814' (27.2%) and 'PI-383249' (32.1%) and 'Haags Blaue' (26.7%) were less susceptible.

Fusarium oxysporum

Four weeks after inoculation the first symptoms were noticed on the plants. The highest susceptibility to *F. oxysporum* was observed on 'Borlu' (67.5%) and 'Bora' (50.0%), whereas 'Rose' (25.0%), 'Probor' (29.3%) and 'Tanjil' (32.9%) were less prone to *Fusarium oxysporum.*

Rhizoctonia solani

The first symptoms were visible on the roots 18 days after inoculation when 4 leaves had emerged. The highest susceptibility to *Rhizoctonia solani* was shown by 'Probor' (51.0%) and 'Vitabor' (48.0%). 'Borlu' (35.8%) showed fewer symptoms and is therefore less vulnerable to the pathogen.

CONCLUSIONS

The performance of narrow-leafed lupins grown on moderately alkaline soils can be improved by treating the seeds with 'HiStick' before sowing. The roots as well as the shoots develop faster than without treatment. As for polyploidisation, a lot of seeds and plantlets have to be colchicinised to increase the likelihood of a positive outcome. The relatively high number of chromosomes could be the reason why doubling the genome of NLL is more difficult than in other species.

Plants grown in the field are usually infected by multiple pathogens and are therefore more difficult to screen for diseases. The advantage of these resistance tests is the unambiguous classification of symptoms. Primers, developed at the Institute for Land Use in Rostock, help to identify single pathogens on multi-infected plants. These tests can be integrated into further breeding programs but, in point of fact, the unclear differentiation between tolerant and resistant cultivars is unsatisfying.

REFERENCES

Campbell, C.L. and D.M. Benson. 1994. Epidemiology and Management of Root Diseases. Springer-Verlag, Heidelberg.

Egle, K., W. Römer, J. Gerke and H. Keller. 1999. The Influence of P Nutrition on Organic Acids Exudation of Roots in Three Lupin Species. Proceedings of the 9th International Lupin Conference, Klink/Müritz.

Esser, K. 1953. Eine Eintauchmethode zur Colchicinbehandlung. Der Züchter 23(4/5): 148.

Evans, A.M. 1955. The Production and Identification of Polyploids in Red Clover, White Clover and Lucerne. New Phytologist 54(2): 149.

Feiler, U. 1998. Auftreten und pathologische Bedeutung wurzel- und stängelbesiedelnder Pilze bei Lupinus luteus unter verschiedenen Standortbedingungen. Thesis. Verlag für Wissenschaft und Forschung.

Gobs-Sonnenschein, C. 1943. Die experimentelle Erzeugung polyploider Sojabohnen mit Alkaloidgemischen in Verbindung mit Kreuzungen polyploider Rassen. Der Züchter 15(3): 62.

Infantino, A., M. Kharrat, L. Riccioni, C.J. Coyne, K.E. McPhee and N.J. Grünwald. 2006. Screening techniques and sources of resistance to root diseases in cool season food legumes. Euphytica 147: 201.

Peiter, E., F. Yan and S. Schubert. 2000. Are Mineral Nutrients a Critical Factor for Lime Intolerance of Lupins? Journal of Plant Nutrition 23(5): 617.

Peiter, E., F. Yan and S. Schubert. 2001. Lime-induced growth depression in Lupinus species: Are soil pH and bicarbonate involved? Journal of Plant Nutrition and Soil Science 164: 165.

Prashant J. and C.V. Rakesh. 2004. High Frequency Production of Colchicine Induced Autotetraploids in Faba Bean (Vicia faba L.). Cytologia 69: 141.

Tang, C., A.D. Robson, N.E. Longnecker and H. Greenway. 1993. Physiological responses of lupin roots to high pH. Plant and Soil 155/156: 509.