Innovative Breeding Strategies for Increasing Yield and Promoting Growth of Narrow-leafed Lupin

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Introduction

1. Root growth with different rhizobial products
2. Polyploidisation of breeding lines
3. Root diseases and Resistance tests

Root growth in moderately alkaline soil with different rhizobial products

Intent:

- Improving tolerance to alkaline and calcareous soil conditions

  → expand producing area to regions with such soils

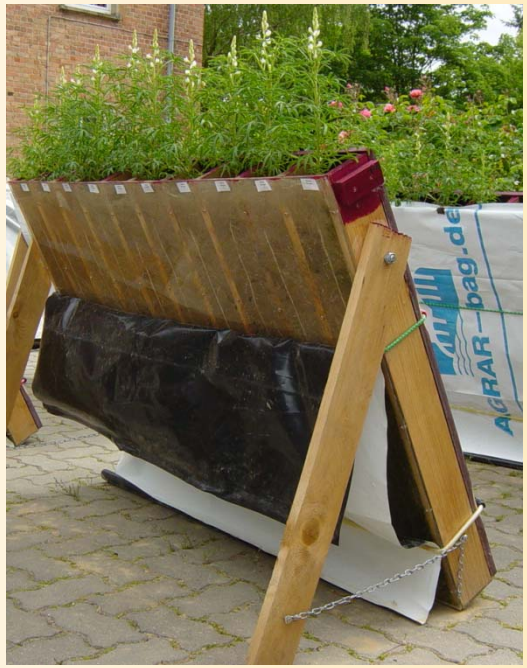
- analysis of root growth, nodulation, development of shoot

Root growth with different rhizobial products

Material and Methods:

- Narrow-leafed lupin cv.´Boregine` (indeterminate growth habit)

- viewing boxes 0.15m x 1.25m x 1m with a transparent, robust PMMA pane at the front



Root growth with different rhizobial products

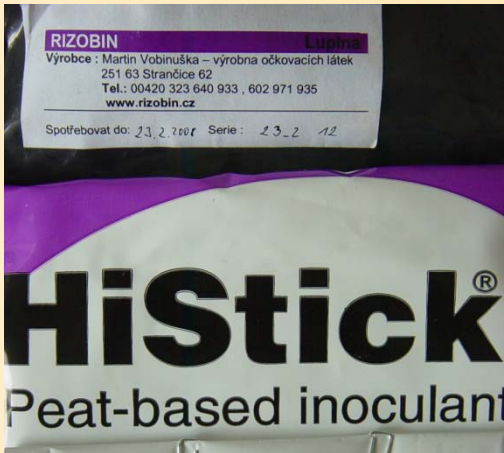
Material and Method:

- Loamy sand:
  pH: 7.2, $CaCO_3$: 1.1%

- Three different rhizobial products:
  - "Rizobin" [*Bradyrhizobium japonicum*],
  - "HiStick",
  - "Radicin" [*Bradyrhizobium lupini*]

- 16 replicates per treatment

- completely randomised
  on a single plant basis.

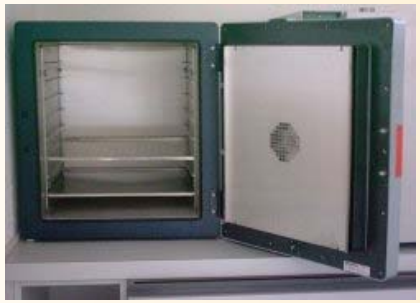


Root growth with different rhizobial products

Material and Method:

- Length of the roots and shoots

- Dry matter: 80°C and weighing afterwards



Root growth with different rhizobial products

Results:

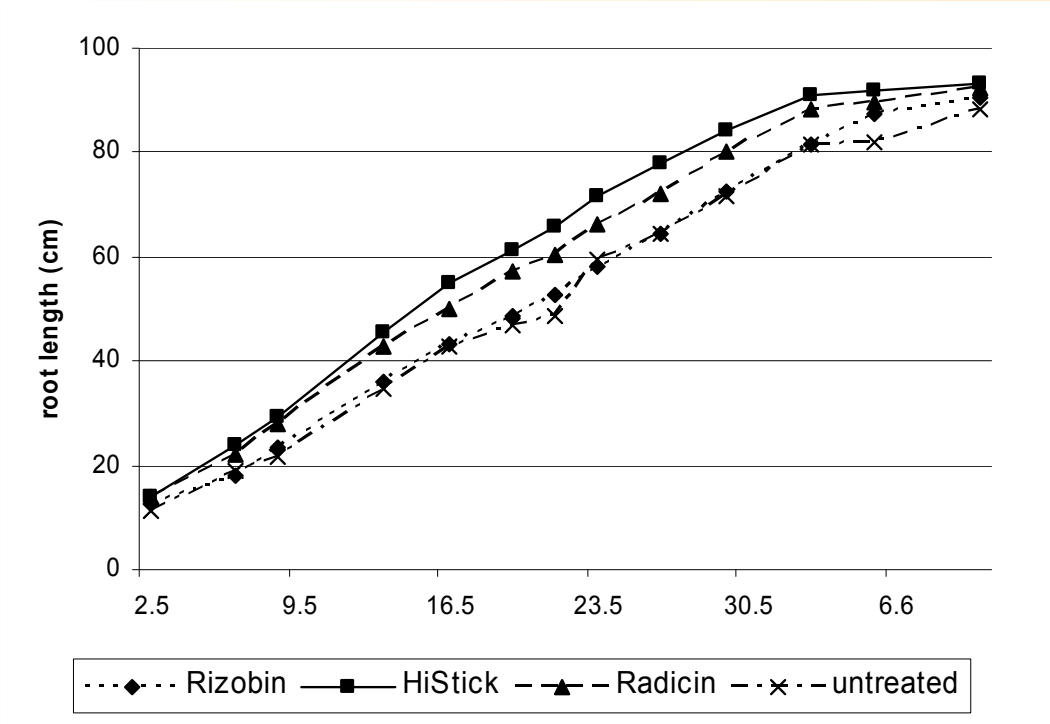


Fig 1: Development of root length over time, sowing date: 21th April, 4 different treatments: Rizobin [*Bradyrhizobium japonicum*], HiStick, Radicin [*Bradyrhizobium lupini*], untreated. Cv: Boregine (n=16), soil pH: 7.2, $CaCO_3$ 1.1%.

Root growth with different rhizobial products

Results:

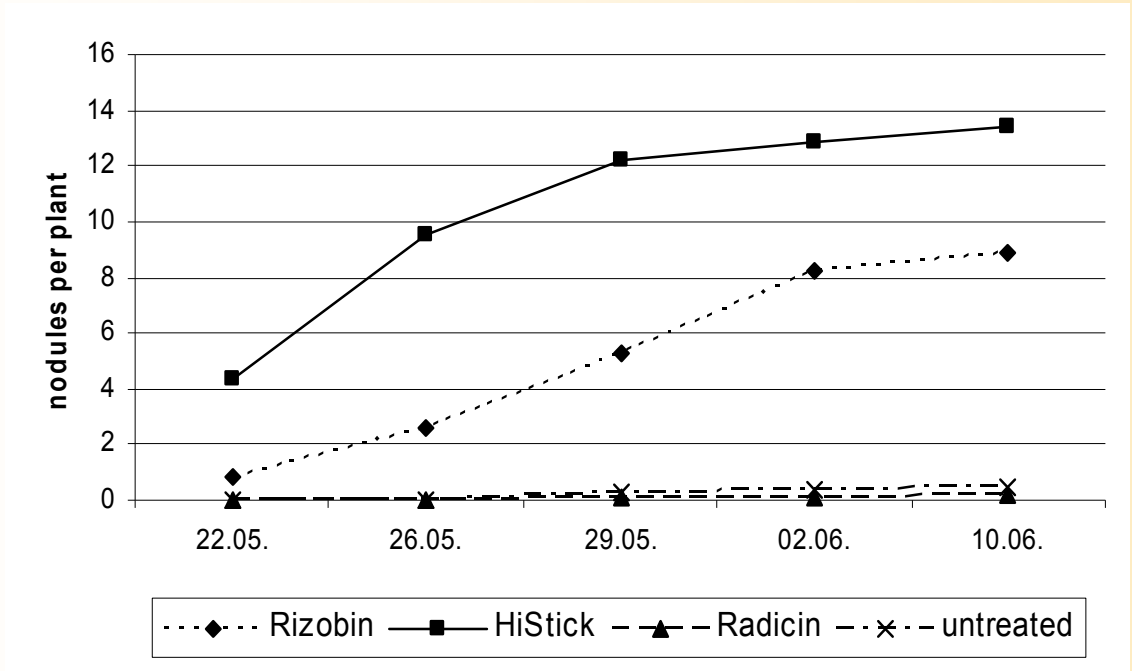


Fig 2: Development of nodulation over time, 4 different treatments: Rizobin [*Bradyrhizobium japonicum*], HiStick, Radicin [*Bradyrhizobium lupini*], untreated. Cv: Boregine (n=16), soil pH: 7.2, $CaCO_3$ 1.1%.

Root growth with different rhizobial products

Results:

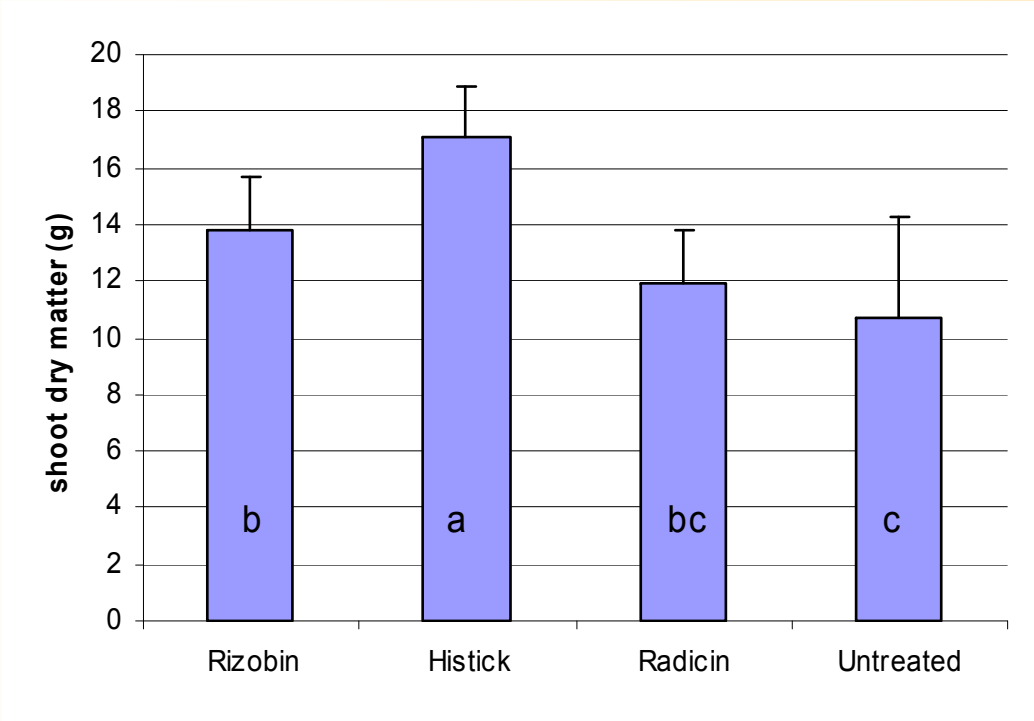


Fig 3: Shoot dry matter, 4 different treatments:
Rizobin [*Bradyrhizobium japonicum*], HiStick, Radicin [*Bradyrhizobium lupini*], untreated. Cv: Boregine, soil pH: 7.2, $CaCO_3$ 1.1%.
(different letters indicate statistical significance (LSD, p≤0.05, n=16))

Root growth with different rhizobial products

Tab. 1: Yield (g), number of seeds and TKW (g) per plant.
Rizobin [*Bradyrhizobium japonicum*], HiStick, Radicin [*Bradyrhizobium lupini*], untreated. Cv: Boregine, soil pH: 7.2, $CaCO_3$ 1.1%.
(different letters indicate statistical significance (LSD, p≤0.05, n=16)

	"Yield" (g)	Number of seeds	TKW (g)
Rizobin	5.8 b	47 b	126 abc
HiStick	**8.7 a**	**62 a**	**140 a**
Radicin	5.0 bc	43 bc	116 bc
Untreated	4.1 c	36 c	111 c

Overview

1. Root growth with different rhizobial products
2. Polyploidisation of breeding lines
3. Root diseases and Resistance tests

Polyploidisation of breeding lines

<u>intent and expectations</u>:

- **Doubling of genome**

  Thereby improved yield, quality and resistence traits

Polyploidisation of breeding lines

Method:

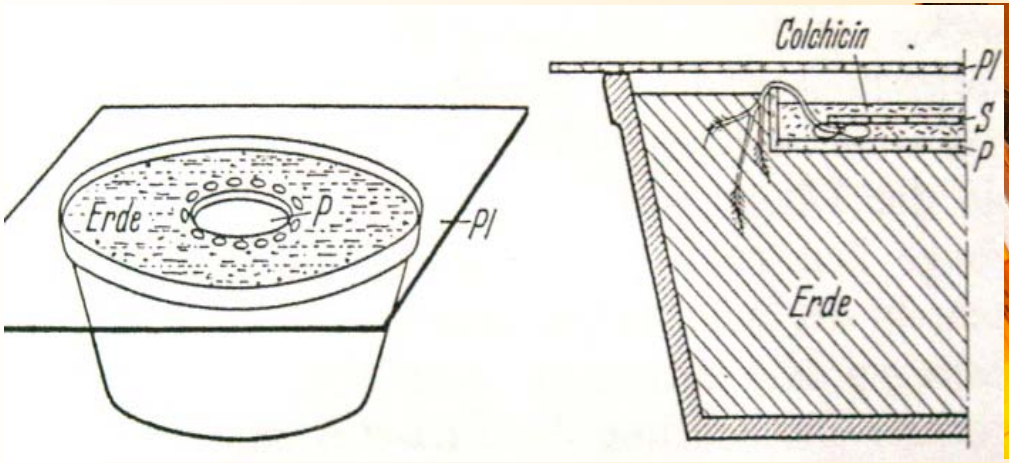
- Seed treatment
  - Seeds submerged in Colchicine solutions with specific concentrations for a certain period of time
  - mixtures of alkaloids tried for re-vitalising purposes

    Colchicine + Nicotine

Polyploidisation of breeding lines

Method:

- **Plantlet treatment:**
  - **Paper: Esser(1953): „Eine Eintauchmethode zur Colchizinbehandlung“**
    „A method of submerging for Treatment with Colchicine “



Polyploidisation of breeding lines

Material and Methods:

Tab. 2: Cultivars and treatments for polyploidisation

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

Polyploidisation of breeding lines

Results:

- **Flow-cytometric analyses of leaf tissue**

  → at this stage, no tetraploid plants

  but single leaves have tetraploid character:

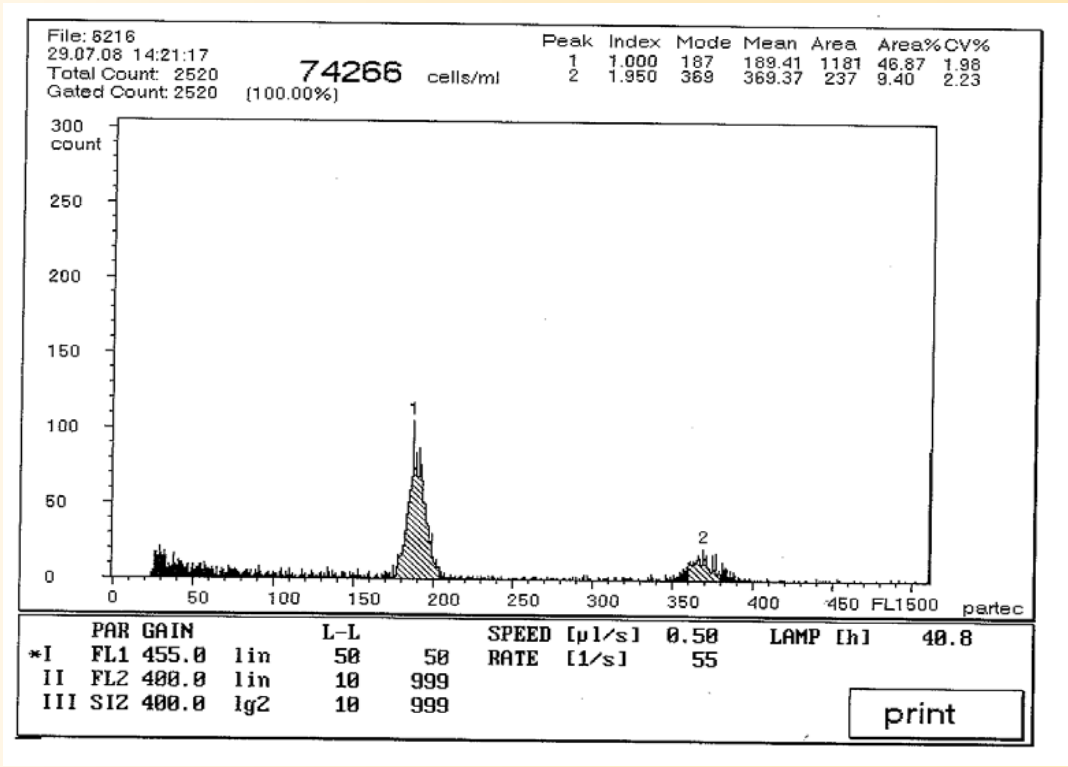


Fig 4: Results of flow-cytometric analysis

Polyploidisation of breeding lines

Fig. 5: different methods

Overview

1. Root growth with different rhizobial products
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Root diseases and Resistance tests

- Fungal diseases:

  Fusarium spp.,

  Sclerotinia sclerotiorum,

  Thielaviopsis basicola and

  Rhizoctonia solani.

- Diverse germplasm collections,

  Geographic distribution

  → successful screening for disease resistance

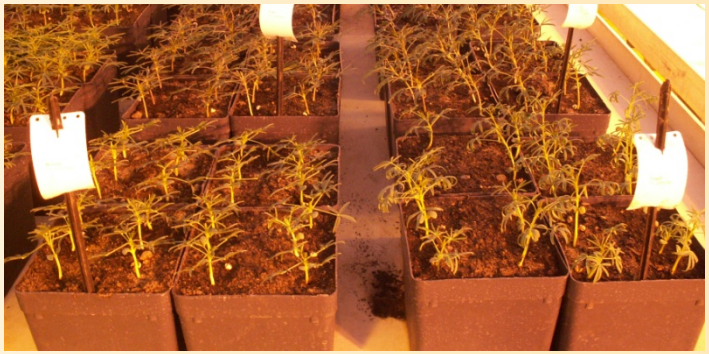
Root diseases and Resistance tests

Material and Methods:

- Seeds disinfected (1.5% sodium hypochlorite, 5 min)
- Soil was steam-treated
- Inoculum (oat-wheat-mixture) of

  Sclerotinia sclerotiorum (2.9g l^{-1} of soil),
  Thielaviopsis basicola (1.7g l^{-1} of soil),
  Rhizoctonia solani (1.7g l^{-1} of soil)

  mixed with soil for the respective trial
- 24 seeds/ test



Root diseases and Resistance tests

Material and Methods:

Fusarium oxysporum inoculation:

- Roots shortened by 5mm at the 2 leaf stage (BBCH 21)
- Conidia suspension ($1x10^6$ ml^{-1}) for 15 min

Disease symptoms scored from 1 to 9

Root diseases and Resistance tests

Material and Methods:

Tab. 3: 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

- Scores transformed into a disease index from 0 to 100%

Root diseases and Resistance tests

Results:

Sclerotinia sclerotiorum

First disease symptoms 8 DAI when cotyledons had unfurled.

High Susceptibility: **‘Tallerack’ (88.9%)**

Intermediate-high susceptibility: **’Belara’ (65.0%), ’Tanjil’ (61.4%), ’Boregine’ (60.7%), ’PI 289163’ (60.5%)**

Intermediate - low susceptibility: **’BGE-023639’ (35.2%), ’PI-385078’ (26.1%), ’PI-283633’ (29.7%), ’BO-9027’ (23.3%), ’BO-7212’ (17.6%), ’PI-308616’**

Root diseases and Resistance tests

Results:

Thielaviopsis basicola

First symptoms on the roots 14 DAI,
on the shoots 21 DAI

High susceptibility: **‘Graf’ (79.4%),**
’Idefix’ (64.5%),
’Vitabor’ (68.4%)

Intermediate – low susceptibility: **’PI-383249’ (32.1%),**
’PI-274814’ (27.2%),
’Haags Blaue’ (26.7%)

Thielaviopsis black root rot

Root diseases and Resistance tests

Results:

Fusarium oxysporum

First symptoms on the plants 4 weeks after inoculation

High susceptibility: ’Borlu’ (67.5%),
’Bora’ (50.0%),

Intermediate – low susceptibility: ’Tanjil’ (32.9%),
’Probor’ (29.3%),
’Rose’ (25.0%)

Root diseases and Resistance tests

Results:

Rhizoctonia solani

First symptoms on the roots 18 DAI when 4 leaves had emerged

High susceptibility: **'Probor' (51.0%),**
'Vitabor' (48.0%)

Intermediate – low susceptibility: **'Borlu' (35.8%)**

Hypocotyl rot

Summary

Root growth with different rhizobial products

- “HiStick” treatment boosts plant growth on moderately alkaline soils
  → faster growth of root and shoot
  → higher yielding parameters: TKW, number of pods and seeds

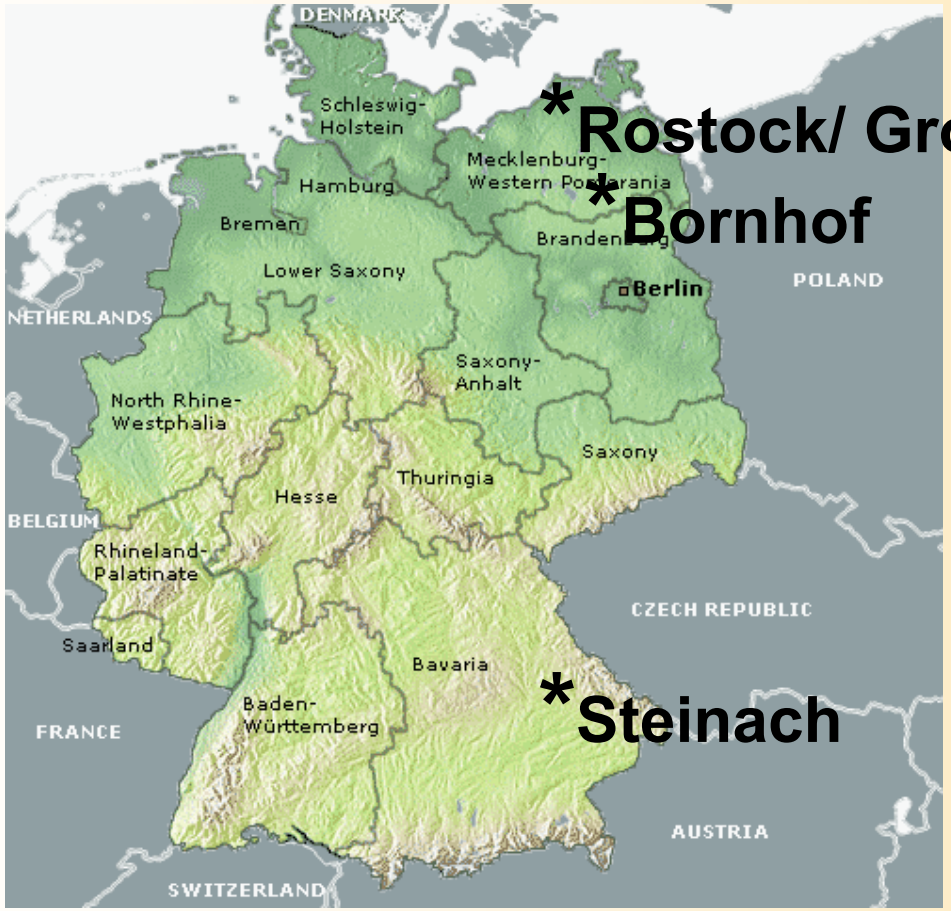
Polyploidisation of breeding lines

- No tetraploid/polyploid plants so far
- Tetraploid tissue

Root diseases and Resistance tests

- unambiguous classification of symptoms
- unclear differentiation between tolerant and resistant cultivars is unsatisfying.

Map



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Tab. 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	