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Breeding *Lupinus albus* for resistance to the root pathogen *Pleiochaeta setosa*

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Abstract Pleiochaeta root rot (PRR) caused by *Pleiochaeta setosa* is a serious, widespread fungal disease in lupin crops, especially in *Lupinus albus* (broad-leaf lupin, or white lupin). PRR resistance is common in the gene pool of *L. albus* with various landraces from the Mediterranean region being the most resistant, and suitable for use in breeding new cultivars. Heritability of resistance is sufficient to make good gains from selection but only when controlled-environment (CE) screening is used. Field disease nurseries on loamy soil gave much lower heritability of resistance. Field disease nurseries had spatially variable spore counts despite continuous lupin cropping, and this was partly responsible (along with climatic conditions) for their reduced precision compared to tests conducted in a CE. Giving infected *L. albus* roots a single, most-severe-lesion score on a 0–9 scale was adequate for CE screening but not as precise or discriminating as the more time-consuming method of six scores per root. Replication in CE experiments was reduced to two pots of 16 seedlings each without sacrificing genotype discrimination.

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Keywords White lupin · Heritability · Plant improvement

Abbreviations

BLS	Brown leaf spot
CE	Controlled environment
DN	Disease nursery
PRR	Pleiochaeta root rot
REML	Residual maximum likelihood

Introduction

Pleiochaeta setosa is a serious pathogen of lupins and causes significant damage world-wide to the cultivated species (Hill 1998; Noffsinger and Santen 2005; Tivoli et al. 2006). While the brown leaf spot (BLS) form of the disease is particularly important in narrow-leaf lupins (*Lupinus angustifolius*), it is the root rot disease (Pleiochaeta root rot—PRR) that has the major impact in commercial *Lupinus albus* (white lupin) crops grown on the loamy soils in eastern Australia and in other countries (Gondran et al. 1994). There have been very few reports on breeding PRR resistance in *L. albus* (Hill 1998; Sweetingham et al. 1996; Tivoli et al. 2006).

The *L. albus* breeding programme at Wagga Wagga aims to breed durable resistance to PRR. Two new commercial cultivars were released and commercialised in 2005, Luxor (resistant) and Rosetta

(moderately resistant). The parental source of resistance in these crossbreds was unknown at the time the fixed genotypes were produced. Sweetingham and Yang (1998) found PRR resistance in two landraces of *L. albus*: the Azores landrace, P27593, appeared to have a single dominant gene for PRR resistance, and the Cretan landrace, P25758, had at least two genes for PRR resistance. The relationships between the various genes for PRR resistance in the *L. albus* germplasm is not known. Luckett et al. (2008) described the refinement of a screening technique to test for PRR resistance.

The aims of this work were: to screen germplasm, parental material and crossbreds for resistance to PRR; to estimate the heritability of resistance; to correlate pot-trial results from controlled-environment (CE) experiments with field disease nursery (DN) results; and to estimate the natural spore concentration found in field DNs. Spore concentration and distribution in DNs is important (along with environmental conditions) in determining the disease pressure that plants experience compared to that applied in CE experiments.

Materials and methods

Fungal materials

A virulent, single-spore isolate of *P. setosa* (PS6), which sporulated well in culture, was previously described by Luckett et al. (2008) (DAR78139, Living culture collection, NSWDPI, Orange, NSW). Early screening experiments (see below) used a mixture of multiple fungal isolates but disease pressure was low in some of these experiments (data not shown). Subsequent screening used only PS6. New freeze-dried ampoules of *P. setosa* PS6 were opened for each experiment (Luckett et al. 2008) and grown on 20% V8 media plus 5.4 g of $CaCO_3$ and 20 g of agar l^{-1}. The plates were maintained with a 12-h photoperiod at 19°C. After 21 days the plates were sub-cultured onto V8 media to produce sufficient inoculum (conidia) after a further 21 days for each experiment. Spores were washed from Petri dishes with sterile water (including 0.1% Wettasoil®) by dislodging the spores with a flexible rubber spoon scraped over the surface. The concentration of the spore suspension was determined using a haemocytometer, so that a calculated measured volume could be added when potting mix was produced, giving a final concentration of 1,000 spores g^{-1}.

Plant materials

The most PRR-resistant landrace, P25758, was used as a resistant control in all screening experiments (Sweetingham and Yang 1998), along with the commercial cv. Kiev-mutant as a highly susceptible control. Various types of *L. albus* germplasm were screened: existing cultivars, breeding lines, parents used in crossing, and landraces sourced from around the world. A total of 212 germplasm lines with diverse origins were imported from the Australian Lupin Collection in Perth, Australia (Department of Agriculture & Food Western Australia, where seed can be sourced by interested researchers). Lines were seed increased at Wagga Wagga, and then assessed for PRR resistance. Many lines have high seed alkaloid content (bitter), in contrast to low-alkaloid (sweet) commercial crops. *Lupinus albus* is a partially-outcrossing species, so to avoid pollen-mediated contamination, the high-alkaloid types must be grown in containment or in an isolated location.

Scoring system

A 0–9 scoring scale was used (Luckett et al. 2008). The system is based on lesion severity on the main root of 21 day-old seedlings, where 0 = no lesion, and 9 = lesions that have completely severed the root (Fig. 1). Small lateral roots were ignored. A scoring system on a 0–5 scale was used in some early experiments conducted in 2003. The 0–5 scale was co-linear with the 0–9 scale; however, the latter was developed to provide additional resolution.

Root scoring was conducted in two ways. In Type A experiments, each seedling was assessed for lesion

Fig. 1 A 0–9 scoring scale for Pleiochaeta root rot disease lesions on the roots of 21 day-old *Lupinus albus* seedlings. The *horizontal scale bar* represents 1 cm

severity on the 0–9 scale within each cm from the soil surface for the first 6 cm (i.e., producing six scores per seedling root). Genotypes that were very susceptible often had the seedling root severed within the first 1 cm or 2 cm. For such plants, all root position scores below the point of severance were recorded as 9. The analysis of Type A lesion severity scores included the six-level factor of position-on-the-root (1 cm, 2 cm, … 6 cm).

When more throughput was required, each seedling root was scored only for the single most severe lesion present in the whole of the top 6 cm of the root (Type B experiment). With Type B scoring a seedling with a single major lesion (e.g. score 5) received a higher score than a seedling with multiple, less-severe lesions (e.g. score 2). This is appropriate since multiple low-score lesions are unlikely to result in seedling death whereas a lesion score above 6 is usually fatal. Consequently, the mean scores for Type B experiments were higher than for Type A.

Type A experiments consisted of four replicate pots, Type B experiments had two replicates, both Type A and Type B had 16 seedlings per pot (175 mm diam, 3.8 l volume).

Design and analysis

Experimental designs were generated using DiGGer software (Coombes 2002; Cullis et al. 2006) which allowed the pots to be spatially arranged in replicates, rows and columns in an optimal fashion.

Data analysis was undertaken using Genstat v8 statistical software (VSN International, Hemel Hempstead, UK). In Type A experiments residual maximum likelihood (REML) was used, fitting genotype, position-on-the-root and genotype-by-position as fixed factors, with replicate, pot, and seedling-within-pot as random factors. In Type B experiments, root position effects were not included. A meta-analysis (using Genstat REML) across multiple experiments was used to arrive at overall resistance ratings for genotypes. Ratings were used in selection decisions.

Lupinus albus is largely self pollinated and the genotypes tested in screening experiments were genetically fixed and uniform. Broad-sense heritability (h_B^2) was calculated using REML for each experiment with all components fitted as random. Heritability and its 95% confidence interval were calculated as $V_G/(V_G + V_E)$ (Simmonds and Smartt 1999) using the VFUNCTION command in Genstat.

CE growth room experiments

The desired spore concentration of 1,000 g^{-1} of soil was achieved by adding the calculated spore number to a measured mix of moist 80% double-washed river sand and 20% brick sand in a concrete mixer for 90 s. Sixteen seeds were sown per 175 mm-diam pot in a regular grid. Pots were not given any fertiliser or Rhizobia because seedlings were only grown for a short time and seed reserves combined with base-level soil fertility was sufficient for good, rapid growth. All experiments were conducted in a CE growth room (16-h photoperiod, 15°C day, 10°C night). Pots were stood in large stainless-steel tubs which enabled the pots to be given extended bottom watering after sowing to ensure uniformity, and encourage good infection. Tubs were drained of water three days after sowing. Pots were then watered from above as required. After 21 days seedlings were carefully removed from soil, rinsed and scored for PRR lesions using the 0–9 scale.

Field DNs

A paddock at the Wagga Wagga Agricultural Institute (35°03′07″ S; 147°21′06″ E) with a chromic luvisol (FAO-Unesco 1974) of pH5.0 was continually cropped to *P. setosa*-susceptible narrow-leaf lupins for 10 years. This was designed to build up the level of natural *P. setosa* inoculum in the soil so that field disease screening could be performed and compared with CE results. The actual disease level was not measured in the years prior to the DNs reported here. However, by observation of the foliage it was clear that BLS was present at high levels in wet seasons. Leaf fall from heavily-infected plants contributes to the soil spore population.

DNs were grown in 2003, 2004, 2005 and 2006 containing fixed advanced lines from the Wagga *L. albus* breeding programme, plus susceptible and resistant controls. About 60 seeds of each genotype were sown in a 10-m row, replicated twice. Each test row was separated by a single spreader row of an *L. angustifolius* cultivar mixture (cvv. Danja and Merrit) highly susceptible to BLS but moderately-resistant to PRR (as are all *L. angustifolius* cultivars). This spreader row was to encourage disease development

on leaves and to ensure that spore concentration in the soil was maintained from year to year. Group G Rhizobia were applied in-furrow in liquid form at the time of sowing.

In 2003 and 2004, seedlings were dug up by hand in the field about eight weeks after sowing (at the 6-leaf stage). In 2005 and 2006 a tractor-mounted implement was used to lift the seedlings, which could then be removed and scored much more quickly. Seedlings were rinsed in tap water to remove soil prior to scoring. Twenty random seedlings were scored per row using Type B scoring. Seedlings in spreader rows were not scored. Field data were analysed and then correlated with CE results for the same genotypes.

Spore density measurements

After scoring the DNs in 2004 and 2006, soil samples were taken from pre-determined positions across the experimental area. In the 2004 experiment, soil was collected from the top 0–2 cm and the next 2–5 cm. In 2006, only the top 0–2 cm was sampled. After forced-air drying (at 35°C in the dark) and homogenising the sample, a serial dilution was set up (Sweetingham 1991).

In 2004 a preliminary experiment was performed to determine an approximate soil spore concentration using a broad range of dilutions. This preliminary experiment indicated that the spore number g^{-1} of soil was in the range of 2000–3000. This information enabled a more targeted approach in the main experiment, and dilutions of 1/750, 1/1500, 1/3000, 1/6000 and 1/12000 were chosen, with 3, 5, 9, 5 and 3 replicate plates for each concentration, respectively. Increasing the number of plates in the centre of the expected concentration range makes the analysis more robust (Finney 1964). Semi-selective agar plates (100 ml of V8 juice, 400 ml of deionised water, 2.7 g of $CaCO_3$ and 10 g of agar, plus 5 ml of aureomycin [1% chlortetracycline hydrochloride], 1 ml of Metalaxyl [10% Apron XLS] and 1 ml of Bavistin [0.05% Bavistin]) were used for each sample. In 2004, six equally spaced positions across the DN were sampled at two depths giving 12 tests overall, or 300 agar plates in total.

In 2006, the experiment was conducted and examined in the same manner as in 2004 but the dilutions used were 1/500, 1/1000, 1/2000, 1/4000 and 1/8000. Plate frequencies were: 3, 4, 6, 4, and 3, respectively. Thirty equally spaced sampling positions were used in a regular grid across the DN paddock but were separated into two sections. The total number of plates incubated per section was 300 (to fit into the incubator). Each group was analysed separately.

After 10 days the agar plates were assessed for the presence or absence of sporulating *P. setosa* colonies by examination under a binocular microscope. The data were analysed using the Most Probable Number procedure in Genstat v8 for Windows.

Results

CE growth room screening experiments

The number of screening experiments completed successfully is given in Table 1. For each experiment the genotype main effects were always highly significant ($P<0.001$, Table 2). No genotype was discovered that was more resistant than the control P25758. Some genotypes were more susceptible than Kiev-mutant. Figure 2 shows the distribution of estimated genotype means for the Type A and Type B scoring systems. As expected, Type B scoring gave higher mean genotype scores. The meta-analysis of CE data showed generally continuous variation from very-resistant to very-susceptible genotypes.

Meta-analysis for Type A experiments

Meta-analysis allowed all the 213 genotypes (plus two controls) from eight experiments to be directly compared. Experiment, replicate-within-experiment,

Table 1 Summary of CE screening experiments to determine resistance to *Pleiochaeta setosa* in *Lupinus albus*

Year	PRR scoring scale used	Experiments	
		Type A[a]	Type B
2003[b]	0–5	3 (45)	Nil
2004	0–9	5 (171)	Nil
2005	0–9	1 (34)	7 (384)
2006	0–9	Nil	8 (568)
Totals		9 (250)	15 (952)

[a] number of discrete experiments conducted, and the number of test genotypes screened in total in parentheses (excluding controls),

[b] one glasshouse experiment is included from 2003

Table 2 Summaries of meta-analyses for Type A and Type B *Pleiochaeta setosa* CE screening experiments in *Lupinus albus*

Source	Statistic	Scoring	
		Type A	Type B
Genotype effect	df	212	395
	Wald/df	10.18	5.60
	χ^2 *P* value	<0.001	<0.001
Position effect	df	5	–
	Wald/df	283.27	–
	χ^2 *P* value	<0.001	–
Genotype.position effect	df	1060	–
	Wald/df	10.33	–
	χ^2 *P* value	<0.001	–
Overall	Grand mean	3.265	5.315
	Genotype range	6.929–0.428	8.778–0.589
	Kiev-mutant mean	4.732	7.028
	P25758 mean	0.520	1.069
	LSD (5%)	1.258	2.156

and pot-within-replicate were fitted as random effects, while genotype, (root) position, and genotype-by-(root)position were fitted as fixed effects (see Table 2 for summary). The main effect for position (1–6 cm) was significant ($P<0.001$). The means were: 3.010, 3.320, 3.028, 3.003, 3.366 and 3.865 for positions 1–6 cm, respectively. The LSD (5%) was 0.0465, indicating that while higher lesion scores were generally present further from the pot soil surface, there was an increased incidence at a depth of 2 cm. The genotype-by-position interaction was significant ($P<0.001$) indicating that not all genotypes were damaged to the same degree at the same soil depth. The table of genotype-by-position means (6×213 matrix) was inspected. There were genotypes throughout the spectrum of resistance that had increasing lesion scores with depth or vice-versa. This genotype-by-position interaction needs further experimentation to determine its importance.

Meta-analysis for Type B experiments

Six experiments containing 396 genotypes and employing Type B scoring were analysed together (see Table 2 for summary). Experiment, replicate-within-experiment and pot-within-replicate were fitted as random effects, and genotype as fixed. Genotype effect was highly significant ($P<0.001$). The means and associated errors tended to be higher than for the Type A scoring (as expected, Table 2); however, there was sufficient discrimination between genotypes for reliable selection purposes.

Spore density assessment, DN 2004

The overall *P. setosa* spore concentration was greater at 0–2 cm than at 2–5 cm (2217 v. 862 spores g^{-1} of soil) (Table 3). There was considerable variation

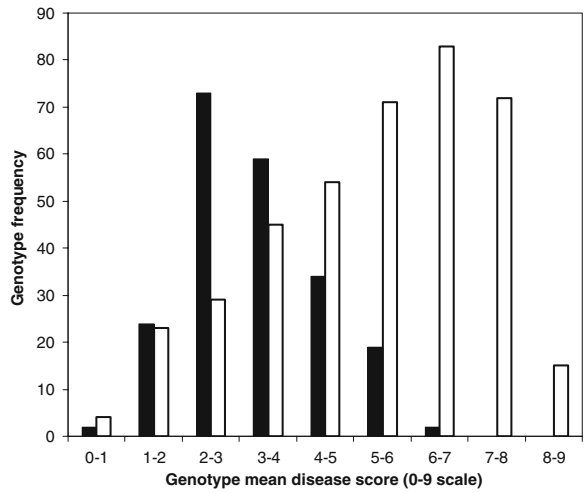

Fig. 2 Histograms of predicted genotype means for *Pleiochaeta setosa* resistance in *Lupinus albus*, scored on a 0–9 scale, using Type A (*solid bar*) or Type B (*open bar*) scoring system. See Table 6 for Kiev-mutant (susceptible) and P25758 (resistant) genotype means under the two scoring systems

between sampling sites across the paddock with a range from 955 to 4246 spores g^{-1} at 0–2 cm, and 424 to 1525 at 2–5 cm.

Spore density assessment, DN 2006

The average spore density in 2006 was much lower than in 2004. In the first section of sampling sites the estimated mean was 523 spores g^{-1} (range 147 to 1091). In the second section the mean was 166 spores g^{-1} (range 70 to 487) (Table 4). The concentrations were even more spatially discontinuous than in 2004.

DN results

In each of the four DN experiments (2003, 2004, 2005, & 2006) (Table 5) the main effect of *genotype* was significant ($P<0.001$, Table 6). The means of the controls (Kiev–mutant—susceptible, and P25758—very resistant) showed that the disease pressure was much lower in 2005, presumably due to a combination of low spore concentration (not measured) and environmental conditions (particularly rainfall leading to low soil moisture status).

Correlation of DN with CE data

In 2003 there were 46 entries in common between the DN and the CE experiments, including four controls. The correlation between the DN score and the average from the meta-analysis of CE scores was weak ($R^2=0.2434$, Fig. 3). Some genotypes were very inconsistent, e.g., WK223 was very susceptible in the CE compared to the DN.

Table 3 Results summary from *Pleiochaeta setosa* spore-density measurement in a DN in 2004 from six sites at two soil depths

Site	Soil depth 0–2 cm			Soil depth 2–5 cm		
	Most probable number estimate	95% confidence interval		Most probable number estimate	95% confidence interval	
		min	max		min	max
1	2332	1209	4125	1069	477	2059
2	3226	1766	5536	1525	739	2805
3	1523	1521	4847	737	274	1446
4	2809	771	2704	699	260	1624
5	955	430	1818	424	130	994
6	4246	2364	7212	945	426	1793
Overall	2217	1735	2797	862	620	1164

Table 4 Results summary from *Pleiochaeta setosa* spore-density measurement at 15 sites in two sections of a DN in 2006 at a single soil depth (0–2 cm)

Site	Section 1			Section 2		
	Most probable number estimate	95% confidence interval		Most probable number estimate	95% confidence interval	
		min	max		min	max
1	1091	533	1983	257	63	675
2	839	375	1616	324	100	758
3	612	261	1197	410	146	889
4	511	202	1048	147	24	455
5	683	310	1281	147	24	455
6	309	96	720	487	193	995
7	660	280	1304	156	26	485
8	845	378	1629	75	4	331
9	425	151	922	–	–	208
10	237	59	619	75	4	331
11	147	24	455	143	24	442
12	425	151	922	71	4	312
13	817	366	1568	–	–	208
14	307	95	717	257	63	675
15	419	150	910	70	4	309
Overall	523	421	640	166	116	230

– Estimate could not be calculated since all plates for that site had zero *P. setosa* colonies

Table 5 DNs conducted for *Pleiochaeta setosa* screening in *Lupinus albus* over 4 years

Year	PRR scoring scale used	Number of test entries[a]	Number of controls	Replicates
2003[b]	0-5	63	2	2
2004[c]	0–9	91	5	3
2005	0-9	45	7	3
2006[c]	0-9	35	5	3

[a] Not all entries from the DNs were in CE experiments, or vice-versa,

[b] 2003 DN contained both *L. albus* and *L. angustifolius* entries – only the *L. albus* results are reported here,

[c] years in which spore concentrations were measured

Table 6 Summary of statistical analyses of Pleiochaeta root rot in *Lupinus albus* measured in four DN experiments

Year	Replicate main effect	Genotype main effect	Grand mean (0-9 scale)	LSD (5%)	Kiev-mutant mean (susceptible)	P25758 mean (resistant)
2003	65.74[a] (1 df, $P<0.001$)	3.41 (62 df, $P<0.001$)	1.595	0.756	2.535	nt
2004	12.87 (2 df, $P<0.001$)	3.07 (95 df, $P<0.001$)	4.406	1.616	5.865	2.816
2005	0.67 (1 df, $P=0.413$)	4.58 (51 df, $P<0.001$)	2.113	0.328	2.292	1.058
2006	30.19 (2 df, $P<0.001$)	7.36 (39 df, $P<0.001$)	3.217	0.864	5.550	2.667

nt not tested

[a] Wald statistic (from Genstat REML analysis) divided by df is approximately distributed as χ^2

In 2004 the number of entries in common was much greater (89 plus five controls). However, disease pressure in the DN was low due to drought conditions and so the correlation was poorer than in 2003 (R^2=0.1654, Fig. 4). The number of common genotypes dropped to 21 in 2005 plus 5 controls; however, the correlation was greater than in the previous 2 years (R^2=0.4162, Fig. 5). In 2006, CE scoring and DN data were weakly correlated (R^2= 0.3711, Fig. 6). A number of genotype outliers were seen (e.g., WK290) which remain unexplained.

Sources of resistance

The meta-analysis of CE experiments reported here showed that the intravarietal selection Lucky-1 was the resistant donor parent for many of the resistant breeding lines, including cv. Luxor, which is currently the most resistant Australian cultivar. The resistance in lines P25758 and P27593 was confirmed and a further 21 genotypes were identified as being potential new resistance donors for breeding (Table 7).

Heritability

In eight Type A growth room CE experiments conducted between 2003 and 2005, heritability (h_B^2) estimates for PRR resistance ranged from 0.076 to 0.354. All estimates were significantly > 0 (as indicated by the 95% confidence interval) except the smallest one. The overall mean heritability was 0.256.

In six Type B CE experiments conducted in 2006, h_B^2 estimates were larger than in Type A experiments and ranged from 0.220 to 0.497 (mean = 0.370). Although low, these estimates were significantly > 0.

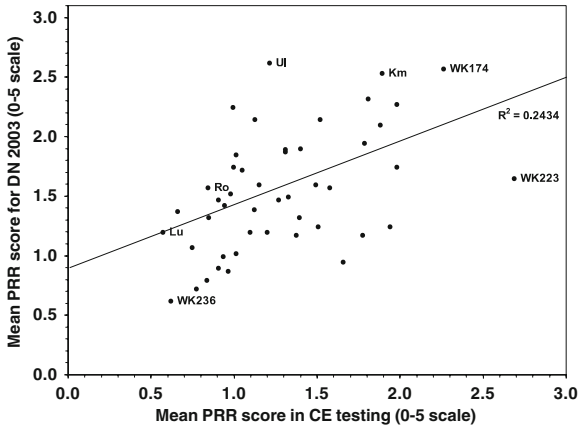

Fig. 3 Correlation between DN and CE screening experiments in 2003 for Pleiochaeta root rot resistance. In the 2003 experiments scoring was done on a 0-5 scale, prior to the development of the 0–9 scale (Luckett et al. 2008) which was used in all other experiments in this paper. Lu = Luxor, Ro = Rosetta, Ul = Ultra, Km = Kiev-mutant, WK… = Wagga breeding line

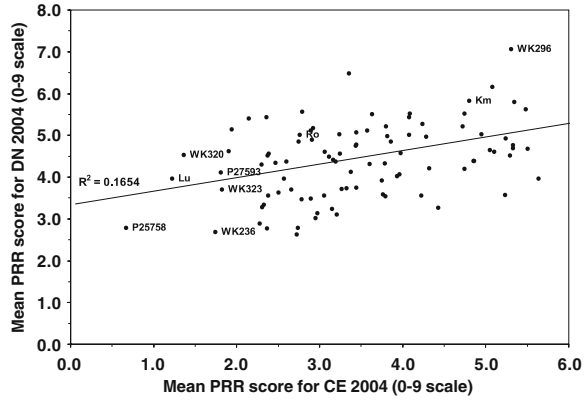

Fig. 4 Correlation between DN and CE screening experiments in 2004 for Pleiochaeta root rot resistance. Lu = Luxor, Ro = Rosetta, Km = Kiev-mutant, WK… = Wagga breeding line, P… = germplasm accession (see text)

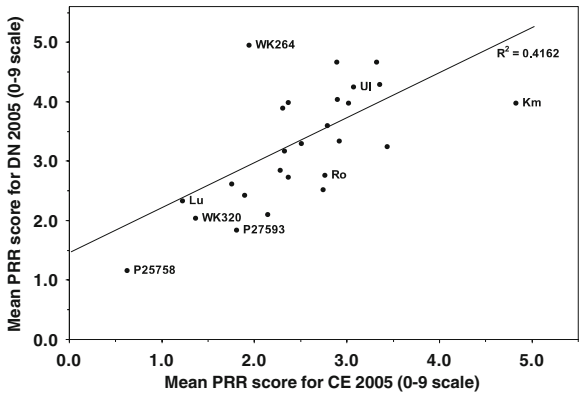

Fig. 5 Correlation between DN and CE screening experiments in 2005 for Pleiochaeta root rot resistance. Lu = Luxor, Ro = Rosetta, Ul = Ultra, Km = Kiev-mutant, WK… = Wagga breeding line, P… = germplasm accession (see text)

DN trials produced low estimates of heritability (0.058, 0.040, 0.081, and 0.096 for 2003 to 2006, respectively). All estimates were significantly > 0.

Discussion

The pathosystems of the main cool-season food legumes and their most common root pathogens have recently been reviewed (Infantino et al. 2006). These authors described how CE screening for breeding is more reliable than in the field for many diseases because the system is simplified and the most important environmental conditions can be regulated. However, CE screening is often of limited capacity and expensive (infrastructure, maintenance and energy costs). Field screening of some host-pathogen combinations works very efficiently, for example, Fusarium wilt resistance in chickpea, lentil and field pea—although in these cases root infection causes clear foliar symptoms so that plants can be scored repeatedly for wilting over time without the labour-intensive and destructive need for them to be dug up. In field nursery systems that are successful, artificial annual addition of disease propagules is widely practiced (Infantino et al. 2006). In *L. albus,* Fusarium resistance was measured in Egypt using a combination of CE and field experiments (Raza et al. 2000), and although the correlation between the two environments was poor for some genotypes, the most resistant ones were readily identified. This reflects our findings reported here for PRR in *L. albus*.

The screening experiments reported here confirmed preliminary evidence that *L. albus* genotypes displayed a wide range of susceptibility to the virulent *P. setosa* isolate PS6. The old commercial standard cvs Kiev-mutant and Ultra, were shown to be very susceptible and moderately susceptible to *P. setosa*, respectively. This supported anecdotal evidence from commercial *L. albus* crops in New South Wales. Two host landraces were confirmed as being very resistant (P25758) and resistant (P27593) (Sweetingham and Yang 1998). Wunderlich et al. (2008) showed that a component of resistance in P25758 was reduced recognition of the host by the pathogen. Two high-yielding cultivars from the Wagga Wagga breeding programme were also shown to be resistant (Luxor), and moderately resistant (Rosetta). The pedigree of these genotypes showed that they were not directly related to either of the best landraces, and so had obtained their resistances from different sources. Further screening experiments showed that Lucky-1 was the source of resistance in Luxor. It seems likely that the French cv. Lucky derived its resistance from Portuguese breeding material, which in turn may have had a contribution from Azores landraces (C. Huyghe, pers. comm.). In contrast, the source of PRR resistance in Rosetta is unclear. Bateman (1997) found little disease from *P. setosa* on *L. albus* hypocotyls unless wounding was employed at the time of infection. However, these experiments used French cultivars (Lucky and Lucyane) which are resistant to PRR.

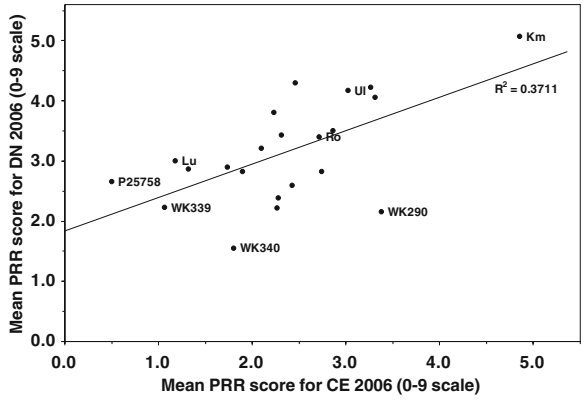

Fig. 6 Correlation between DN 2006 and Type A CE screening experiments for Pleiochaeta root rot resistance. Lu = Luxor, Ro = Rosetta, Ul = Ultra, Km = Kiev-mutant, WK… = Wagga breeding line, P… = germplasm accession (see text)

Table 7 Pleiochaeta root rot-resistant germplasm accessions of *Lupinus albus* identified from a meta-analysis of screening experiments conducted in a CE environment

Name[a]	Synonym	Country of origin	Type	Resistance rating[b]
LUXOR	WK142	Australia	Cultivar	R
LUCKY-1	-	France	Intravarietal selection	R
P25758	MJS304	Greece (Crete)	Landrace	VR[c]
P25863	97E58	UK	Breeding line	VR
P26734	CPI23300	Hungary	Landrace	MR
P26777	GRC5658B	Greece	Landrace	VR
P27154	ESP6056B	Canary Islands	Landrace	VR
P27267	MG110428	Italy	Breeding line	VR
P27272	MG111993	Italy	Breeding line	VR
P27273	MG111999	Italy	Breeding line	VR
P27277	MG112241	Italy	Breeding line	VR
P27279	MG115267	Italy	Breeding line	VR
P27438	SYR6261B	Syria	Unknown	MR
P27593	FRA6635B	Azores	Landrace	R[c]
P27840	SYR6728B	Syria	Landrace	R
P28096	SYR6728B	Syria	Landrace	VR
P28233	LA173	Ethiopia	Landrace	MR
P28981	MUTANT 47	Russia	Breeding line	R
P28983	POP-8066	Spain	Breeding line	R
P28985	ODORE-MARINA	Italy	Cultivar	R
P28991	US502648	Israel	Landrace	R
P28997	ESP4463	Spain	Landrace	MR
P28998	ESP4785	Spain	Landrace	R
P28999	PL095453	Poland	Breeding line	MR
P29002	US483075	unknown	Landrace	VR

[a] accession, Australian Lupin Collection, DAFWA, Perth, Western Australia, [b] VR = very resistant, R = resistant, MR = moderately resistant (rating obtained from meta-analysis of CE experiments in this study), [c] confirmation of resistance first described by Sweetingham and Yang (1998)

Type A scoring (six scores per root) was excellent as a detailed screening technique but Type B scoring (one score per root) was quite sufficient for screening large numbers of fixed lines, took less than half the time to measure and record, and allowed greater throughput. Broad-sense heritability of the PRR disease score was significant in both Type A and Type B CE experiments, indicating that a reasonable response could be expected when selecting for this character using these techniques. In narrow-leaf lupins, Cowling et al. (1997) found high broad-sense heritability for BLS in field experiments (0.89–0.94) but much lower heritability for PRR (0–0.53), while Bradley et al. (2002) reported high narrow-sense heritability (0.94) for field BLS in the same species, and concluded that the character was polygenically controlled.

Estimates of heritability from DNs in this work were low, and the correlation between DN and CE scores was generally poor. Field screening of breeding material under drought-prone conditions at Wagga Wagga was too unreliable for it to be useful as a stand-alone routine procedure. However, this scenario might be improved if spray irrigation and artificial application of conidia were employed (along with cold winter conditions) to encourage disease development. Severe drought conditions in Wagga Wagga, culminating in 2006, resulted in a large decrease in viable *P. setosa* spores in the soil of the DN. In addition, the high spatial variability in spore concentration, over even short distances, showed that well-designed and well-replicated experiments would be required to screen accurately for PRR in DNs. In addition, methods to artificially and uniformly supplement the soil spore population need to be investigated, for example, the importation and spreading of infected lupin plant residues.

The best traditional breeding approach is likely to be CE screening (possibly in segregating F_2 material)

followed by confirmation of resistance in subsequent CE experiments. Early-generation screening allows large numbers of individuals to be tested cheaply and quickly while acknowledging the disadvantage that some heterozygotes and escapees (false positives) will also be selected. These genotypes would be eliminated at later stages when testing is more expensive and throughput more limited.

Poor correlation of resistance ratings between DNs and CEs may be partly due to differences in the composition of the pathogen—only one strain (PS6) was used in CE experiments, whereas a more heterogeneous population with differences in virulence among strains would be expected in the field. This aspect of the technique requires further investigation. A robust and reliable field DN is preferable to confirm the resistance in lines identified with a single strain under CEs. Without such confirmation new genotypes run the risk of having limited significance in commercial crops.

It is unclear whether the PRR resistance reported here in *L. albus* genotypes also protects against the BLS form of the disease that can be a serious problem in some countries with autumn-sown *L. albus* crops (Gondran et al. 1994; Hill 1998). Further experiments to look at this aspect of resistance are required.

This work has identified geographically diverse sources of PRR resistance in the *L. albus* germplasm. The genetic relationship between these germplasm accessions is not yet known but new genetic combinations are being made at Wagga Wagga in an attempt to produce strong, durable resistance. Two mapping populations have been made: Kiev-mutant/P25758 and Kiev-mutant/P27593. Work is underway to phenotype and genotype these populations to map the resistance genes (QTL), and to identify closely linked DNA markers suitable for marker-assisted selection and to facilitate gene pyramiding. A combination of glasshouse and field screening was used to identify three QTL associated with resistance to *Aphanomyces* in pea (Pilet-Nayel et al. 2005) leading to good opportunities for marker-assisted selection. In *L. albus* the future availability of markers for resistance would allow selection of homozygous, potentially multigenic, resistant individuals from suitable segregating breeding populations.

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