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REGULAR ARTICLE

Enhancing white lupin (*Lupinus albus* L.) adaptation to calcareous soils through selection of lime-tolerant plant germplasm and *Bradyrhizobium* strains

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Abstract
Aims This study aimed to determine whether white lupin adaptation to moderately calcareous soils could be enhanced by lime-tolerant plants and Bradyrhizobium strains.
Methods Fourteen landraces from Italy, Morocco and Egypt and some cultivars were grown in moderate-lime (ML) and low-lime (LL) soil with each of two inoculants, one commercial and one including three Bradyrhizobium strains well-nodulating under ML soil (isolated from other lupin species). Grain yield and above-ground biomass were assessed in large artificial environments that mimicked field conditions. Shoot, root and nodulation traits at onset of flowering were studied in a pot experiment.
Results ML soil severely reduced plant yield, growth and nodulation but increased the harvest index relative to LL. Top-yielding genotypes for grain yield displayed significant rank inversion across soil types ($P < 0.05$). Lime-tolerant genotypes reduced their nodulation in ML soil less than limesusceptible ones. Some landraces outperformed the reference lime-tolerant cultivar Giza 1 in ML soil. One Italian landrace had a lime-tolerant response across agricultural locations. The Moroccan inoculant provided greater nodulation, more shoot residues but similar grain yield in ML soil, and less grain and shoot residues in LL soil, compared with the commercial inoculant.
Conclusions Lupin adaptation to ML soil can be improved mainly through selection of lime-tolerant plants.

Keywords Alkalinity · Genetic resources · Lime tolerance · Landrace · Plant–microbe interaction · Plant–soil interaction

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Introduction

Feed grain legumes are strategically important for European and North-African agriculture, to meet the demand for high-protein feedstuff and to increase the sustainability of crop–livestock systems (Carrouée et al. 2003). White lupin (*Lupinus albus* L.) exhibited higher crude protein yield than other rain-fed, autumn-sown species such as pea, faba bean and narrow-leafed lupin across climatically-contrasting sites of southern Europe, owing to its outstanding grain protein content (Annicchiarico 2008). In addition, there is an increasing interest in white lupin as an ingredient of functional or healthy food products (Arnoldi 2005).

Recent varieties featuring greater cold tolerance and novel plant architecture have improved the climatic adaptation and the yield of white lupin (Huyghe 1997), but its potential cropping area is severely constrained by narrow adaptation with respect to soil type. Lupin grain and biomass reduction is expected in soils whose active lime (i.e., the soluble fraction of calcium carbonate according to Drouineau's 1942 method) exceeds 1% (Papineau and Huyghe 2004), which are mildly calcareous, or in soils with an alkaline reaction (Tang and Thomson 1996). Active lime tends to have greater depressive effects than alkalinity (Duthion 1992; Liu and Tang 1999), but these factors are associated in calcareous soils. Although Ca^{2+} may directly affect *Lupinus* species (Jayasundara et al. 1998), the main effects of lime are indirect, through the precipitation of organic acids secreted by lupin cluster roots to mobilize and uptake phosphorus and iron (Dinkelaker et al. 1989) and through the inhibition of iron uptake by HCO_3^- (Tang and Thomson 1996). Indeed, lupin cluster roots increase their relative proportion in response to limed soils, although total root biomass decreases (Kerley 2000). Iron deficiency reduces leaf chlorophyll concentration (Tang et al. 2006) and photosynthesis (Kerley et al. 2002), but its main negative effect is to reduce nodulation (Tang et al. 2006). High soil Ca^{2+} concentration also has a negative effect on the growth and the nodulating ability of the nitrogen-fixing microsymbiont of lupins, *Bradyrhizobium* sp. (*Lupinus*) (Howieson et al. 1998). The optimal soil pH for root growth and nodulation of white lupin is between 5 and 6 (Jayasundara et al. 1998; Tang et al. 2006). The sharp decrease in nodulation that is observed near pH 7 (Tang and Thomson 1996) may be due to poor adaptation of *Bradyrhizobium* to alkaline soils (Howieson et al. 1998), the insufficient availability of iron, and direct or indirect effects of pH that might lead to reduced recognition of the host plant or reduced expression of nodulation genes (Tang et al. 2006).

The adaptation of white lupin to calcareous soils might be enhanced by the identification of plant genotypes that are tolerant to these conditions and can be exploited to breed adapted varieties. Various studies (Christiansen et al. 2000; Raza et al. 2000; Kerley et al. 2002) highlighted the adaptation of Egyptian germplasm to moderately calcareous or limed soils, which reflected their adaptation to conditions of their geographic origins. Variation in adaptation to limed or alkaline soils was also reported by Liu and Tang (1999) for commercial varieties and some accessions of indefinite origin. The high susceptibility to winter cold stress of Egyptian germplasm limits its exploitation in breeding autumn-sown varieties for subcontinental-climate or Mediterranean environments (Annicchiarico et al. 2010, 2011), raising the need for germplasm sources that combine agroclimatic adaptation to these environments and tolerance to moderately calcareous soils.

White lupin adaptation to calcareous soils might also be improved by identifying tolerant *Bradyrhizobium* strains and exploiting them to produce specific inoculants for the crop grown on such soils. Abd-Alla (1999) reported variation among *Bradyrhizobium* strains for the ability to promote white lupin nodulation in alkaline, iron-deficient soils. Raza et al. (2001a) isolated some strains which tolerated a pH over 9 and total $CaCO_3$ over 10% from Egyptian white lupin landraces grown in alkaline soils. The simultaneous inoculation with two such strains allowed good white lupin growth in a calcareous soil (Raza et al. 2001b). Dual inoculation also reduced the extent of plant genotype×strain interaction effects relative to inoculation with a single strain (Raza et al. 2001b), highlighting the usefulness of combined inoculation with a few elite lime-tolerant strains. Lupin species such as *L. atlanticus*, *L. cosentinii* and *L. pilosus*, which occur naturally in moderately calcareous soils (White 1990), may be particularly useful for identifying lime-tolerant *Bradyrhizobium* strains. Thami Alami et al. (2004a) selected some strains from *L. atlanticus* and *L. cosentinii* from moderately calcareous Moroccan soils which effectively nodulated white lupin in a calcareous soil. However, the advantage of the combined inoculation with these strains relative to a commercial inoculant requires verification.

The objective of this study was to verify whether white lupin adaptation to moderately calcareous soils could be enhanced by: (1) selection of plant germplasm, assessing the variation for tolerance to soil lime among landrace genotypes from Italy, Morocco and Egypt; and (2) the combined inoculation with elite *Bradyrhizobium* strains isolated from other lupin species by Thami Alami et al. (2004a), relative to inoculation with a commercial inoculant. Plant responses for yield, biomass, root and nodulation traits were investigated across moderate-lime (ML)

and low-lime (LL) soil types, assessing the interactions between soil type, lupin genotype and *Bradyrhizobium* inoculant factors.

Materials and methods

Isolation of rhizobia and preparation of inoculants

Three *Bradyrhizobium* strains isolated from Moroccan natural populations of *L. cosentinii*, *L. atlanticus* and *L. luteus* (coded as S 36, S 119 and S 16, respectively) were selected as components of the mixture of isolates from a collection of 159 strains on the grounds of their high infectivity on white lupin, adaptation to calcareous soil as measured from good nodulation and growth of white lupin under ML soil in a greenhouse experiment, tolerance to alkalinity and other stresses (salinity; some heavy metals), and similar multiplication rate (Thami Alami et al. 2004a; El Hilali 2006). The reference commercial inoculant was NPPL HiStick marketed by Becker Underwood (Toulouse, France), which includes a single *Bradyrhizobium* strain (as confirmed by molecular analysis: M. Zaccardelli, personal communication). In this and the following work, lupin seed was surface-sterilized by immersion for 20 min in a solution of 20% sodium hypochlorite followed by several rinsing stages in sterile distilled water. Each of the three Moroccan strains and the commercial strain were multiplied separately in liquid yeast mannitol. After 48 h incubation at 28°C, rhizobial suspensions with overall bacterial concentration of about 10^8 g^{-1} were prepared for the commercial strain and the mixture of the three Moroccan strains. These suspensions were used, at the rate of 1 ml g^{-1} of dry peat, to inoculate several sealed sterile bags, each including 50 g of dried peat and 30 ml of distilled water. The inoculation was performed by injection with a sterile syringe. After incubation for 48 h at 28°C, these bags, whose concentration of rhizobial cells was over 10^8 g^{-1} (as verified by the plate count procedure), were stored at 4°C until they were used as peat inoculants for *Bradyrhizobium* treatments.

Plant material

Seventeen white lupin genotypes (landrace accessions or cultivars) were tested under different soil types and *Bradyrhizobium* inoculants in two experiments (Exp. 1 and Exp. 2). They are listed in Table 1 along with the Italian cultivar Multitalia, which was included

Table 1 List of tested genotypes of white lupin (*Lupinus albus*) and their origin and seed source

Genotype	Material	Origin	Collecting site	Seed source[a]
Ita1	Landrace	Italy	Battipaglia	INRA Dijon
Ita49	Landrace	Italy	Pistoia	INRA Dijon
LA246	Landrace	Italy	—	INRA Dijon
LAP122	Landrace	Italy	Carpino Gargano	CRA Lodi
LAP123	Landrace	Italy	S. Croce Magliano	CRA Lodi
LAP124	Landrace	Italy	Soleto	CRA Lodi
Egy42	Landrace	Egypt	—	INRA Dijon
Egy64	Landrace	Egypt	—	INRA Dijon
Egy89	Landrace	Egypt	—	INRA Dijon
LA356	Landrace	Egypt	—	INRA Dijon
Giza 1	Cultivar	Egypt	—	Univ. Frederiksberg; IACR Rothamsted
Mar74	Landrace	Morocco	—	INRA Dijon
Mor28A	Landrace	Morocco	Bouznika	INRA Rabat
Mor29	Landrace	Morocco	Benslimane	INRA Rabat
Mor32	Landrace	Morocco	Ain Dakhka	INRA Rabat
Multolupa [b]	Cultivar	Morocco	—	INRA Rabat
Adam	Cultivar	France	—	—
Multitalia	Cultivar	Italy	—	—

[a] Accessions from INRA Dijon were collected and previously held by INRA Lusignan.

[b] Introduced in Morocco and multiplied locally for about 30 years.

only in Exp. 2. They included: (1) six Italian landraces originated in central or southern Italy and the Moroccan accession Mar74, chosen on the grounds of their good yield response in a previous evaluation across climatically-contrasting Italian sites having non-calcareous soils (Annicchiarico et al. 2010); (2) four Egyptian landraces which had shown fairly good climatic adaptation to Mediterranean environments of Italy (Annicchiarico et al. 2010) or Morocco (Thami-Alami, unpublished data); (3) the Moroccan landraces Mor28a, Mor29 and Mor32, collected by Thami Alami et al. (2004b) in locations featuring shallow soil (<30 cm) and calcareous parent rock; (4) the cultivar Multolupa multiplied in Moroccan environments with low-lime soil for about 30 years; (5) the French variety Adam; and (6) the Egyptian cultivar Giza 1, considered as a lime-tolerant control on the basis of earlier studies (Christiansen et al. 2000; Raza et al. 2000; Kerley et al. 2002). Moderately calcareous soils are widespread in areas of southern Italy from which most Italian landraces originated. Most landrace seed was obtained from the germplasm collection assembled by INRA Lusignan (Papineau and Huyghe 2004), which lacked collecting site information in some cases (Table 1). We used equal amounts of two seed sources for Giza 1 (Table 1). The Italian landrace LAP123 and the variety Multitalia were also tested across three Italian locations.

Experiment 1

This experiment aimed to assess grain yield and biomass responses under conditions similar to those of agricultural environments. It was performed in Lodi (northern Italy) in a set of four large (24.0 m × 1.6 m × 0.8 m deep) bottomless containers in concrete laid in a field, of which two were filled with local LL soil and two with ML soil imported from another site of northern Italy. The soils differed for total and active $CaCO_3$ as well as for texture, while having similar pH (Table 2). The phosphorus available was fairly high in all soils of this and other experiments, whereas nitrogen content was moderate in all cases except for LL and ML soils of Exp. 2, in which it was fairly low (Table 2). The containers of Exp. 1 having the same soil type were randomly assigned a different inoculation treatment, thereby obtaining four environments formed by the factorial combination of two soil types by two *Bradyrhizobium* inoculants. The soils were not sterilized but had hosted no crop having a symbiotic association with *Bradyrhizobium* for several decades. Within each artificial environment, the 17 genotypes were grown in a randomized complete block (RCB) design with three replicates. Each plot consisted of 27 plants arranged in a 9 × 3 lay-out and spaced 15 cm between rows and on the row, of which 21 plants formed the harvest area. Seed of each genotype was sterilized, subjected to peat dry inocu-

Table 2 Soil content of total and active lime, pH, phosphorus (Olsen method) and nitrogen (Kjeldhal method), soil texture class, and spring rainfall, for Experiment 1, Experiment 2, and three Italian test locations

Environment	Total $CaCO_3$ content (g kg^{-1})[a]	Active $CaCO_3$ content (g kg^{-1})[b]	pH (in H_2O)	P (mg kg^{-1})	N (g kg^{-1})	Texture class[c]	Spring rainfall (mm)[d]
Exp. 1, low lime	12	5	7.5	25	1.57	Sandy-loam	110
Exp. 1, moderate lime	123	30	7.6	32	2.02	Silty-clay	110
Exp. 2, low lime	2	~0	7.5	39	0.29	Sandy-loam	–
Exp. 2, moderate lime	146	39	8.1	37	0.38	Silty-clay-loam	–
Lodi (low lime)	2	~0	6.2	30	1.03	Sandy-loam	83
Sanluri (low lime)	10	3	7.4	28	1.13	Sandy-clay-loam	198
Foggia (moderate lime)	47	21	7.9	32	1.40	Clay	76

[a] As $CaCO_3$ in particles<2 mm, measured by Bernard calcimeter (Exp. 2) or De Astis calcimeter (other soils).

[b] As $CaCO_3$ reacting to neutral NH_4-oxalate (Drouineau 1942).

[c] According to FAO (1990).

[d] From 1 March to 15 June; value not reported for the greenhouse Exp. 2.

lation at the rate of 80 mg g^{-1} of seed, germinated in Petri dishes, and finally transplanted at the beginning of November 2005, taking all precautions to prevent the dispersal across containers of the tested rhizobium strains at any stage of the experiment. All environments were fertilized with 40 kg ha^{-1} of N, 52 kg ha^{-1} of P and 100 kg ha^{-1} of K prior to transplanting. Harvesting was performed in the first week of July 2006. The extent of low-temperature stress was limited by a moving shelter equipment which covered the four containers during most dry days in winter. The total precipitation over the crop cycle was 329 mm, whereas spring rainfall (1 March–15 June) was 110 mm. The following traits were recorded on a plot basis: (1) days from January 1 to the onset of flowering and to physiological maturity of about 50% of the plants; (2) plant mortality; (3) dry grain yield, shoot residues and above-ground biomass (i.e., grain+shoot residues), and harvest index, all estimated on oven-dried material; and (4) individual seed dry weight, estimated on a random sample of 100 seeds.

Experiment 2

The main objective of this experiment was to investigate root and nodulation traits, given the difficulty to reliably assess them in the field-type conditions of Exp. 1. In addition, Exp. 2 allowed for a second assessment of genotype and inoculant responses to the contrasting soil types, although on plant material at an earlier growth stage. This experiment was performed in Rabat using pots of about 1.8 kg soil capacity. It included two local soil types (one ML and one LL), the two *Bradyrhizobium* inoculants, and 18 white lupin genotypes (those of Exp. 1 and Multitalia). The ML soil was less favorable for lupin growth than that used in Exp. 1 on the basis of its higher total and active $CaCO_3$ and its alkaline reaction (Table 2). The experiment lay-out contemplated the combinations of soil types and *Bradyrhizobium* inoculants randomly arranged on main plots, and the individual genotypes randomly arranged on subplots, with three complete blocks per treatment. Each pot hosted four plants obtained from seed that was sterilized, germinated in Petri dishes and transplanted in late December 2005. The soils were sterilized by two cycles of autoclaving at 120°C for 60 min, fertilized at the rate of 26 kg ha^{-1} of P and 50 kg ha^{-1} of K, and added the relevant inoculant at the rate of 1 g Kg^{-1} of soil prior to transplanting. The pots were irrigated with distilled water across the trial. Harvesting of all plants occurred around mid-March, when all genotypes but the late variety Adam were at the onset of flowering, recording plant mortality, shoot and root dry weight, number of nodules, and nodule dry weight. Mean values per plant of each pot were subjected to statistical analysis.

Multi-location testing

Grain yield data of three earlier field trials were exploited to verify the tolerance to calcareous soils of LAP123 that was suggested by results of Exp. 1 and Exp. 2. The reference variety for comparison was Multitalia, rated as lime-susceptible by results of Exp. 2. The test sites were: (1) Lodi, LL soil with subcontinental climate; (2) Sanluri (Sardinia), LL soil with Mediterranean climate; and (3) Foggia (southern Italy), ML soil with Mediterranean climate (Table 2). The trials in Lodi and Foggia included 11 genotypes overall and were performed in the cropping year 2002–2003; that in Sanluri comprised 12 genotypes and was carried out in 2003–2004. The experiments included two sowing dates, i.e., an early- and a late-autumn sowing in Lodi and Foggia, and an autumn and a late-winter sowing in Sanluri. They were designed as a split-plot with three replications, assigning sowing dates to main plots and genotypes to subplots. Plots had 9 m^2 area and were sown at the rate of 45 germinating seeds m^{-2}. Seeds were inoculated with NPPL HiStick prior to sowing. Soil characteristics and spring rainfall of the sites are reported in Table 2, whereas additional information and results of the trials in Lodi and Sanluri were given by Annicchiarico et al. (2003; 2004).

Statistical analysis

An analysis of variance (ANOVA) that included the fixed factors soil type, *Bradyrhizobium* inoculant and genotype, and the random factor block within soil and inoculant, tested main effects and interactions for data of Exp. 1. In analogy with the ANOVA for genotypes grown in a RCB design in different environments (Gomez and Gomez 1984), block acted as the error term for soil type, inoculant and their interaction, whereas genotype and its interactions with the other

fixed factors were tested on an error term that pooled the interactions with block of these effects. A second ANOVA that included the fixed factor environment (as defined by the combinations of soil types and inoculants) beside genotype and block aimed to compare the four growing environments. Genotypes within each soil type were compared by separate ANOVAs that included the factors inoculant, genotype and block.

The ANOVAs for data of Exp. 2 were similar, the main difference being the block factor not nested within soil type and inoculant in this case. Soil type, inoculant and their interaction were tested on an error term that pooled their interactions with block, according to their lay-out as in a factorial experiment in RCB (Gomez and Gomez 1984). Genotype and its interactions with the other fixed factors were tested on an error term that pooled the interactions with block of these effects. Other ANOVAs were performed to compare the four growing environments and the genotypes within each soil type or inoculant. The ANOVAs excluded the data for Multitalia, to generate information perfectly comparable with that from Exp. 1. However, an additional ANOVA including Multitalia was performed for comparing all genotypes within each soil type.

Plant mortality values were subjected to the angular transformation prior to ANOVAs. Genotype×soil type interaction effects were assessed in terms of ratio of genotype values between ML and LL soil conditions, which is higher for genotypes better able to maintain regular growth in the calcareous soil. This ratio for genotype yield between stress and non-stress growing conditions has frequently been used for defining the genotype ability to respond relatively better to conditions of abiotic or biotic stresses, e.g., drought (Fischer and Maurer 1978) or plant competition (Annicchiarico 2003). Likewise, genotype×inoculant interaction effects were assessed as the ratio of genotype values between the Moroccan and the commercial inoculant. Simple correlations were computed to assess the relationships between: (1) genotype responses for the same trait in different soils; and (2) genotype×soil type interaction effects for different traits.

Genotype×soil type and genotype×inoculant interactions of crossover (or qualitative) type have special importance to breeders, because they imply the inversion of genotype ranks across soil type or inoculant conditions (unlike quantitative interactions). Their occurrence was verified for top-performing genotypes by Gail and Simon's (1985) test as described by Baker (1988).

LAP123 and Multitalia were compared in each location for grain yield averaged across sowing sites on the basis of an LSD value issued by an ANOVA that was performed on all tested genotypes according to the split-plot experimental lay-out.

All analyses were carried out using the Statistical Analysis System (SAS Institute 1999) software.

Results

Experiment 1

The final plant mortality was very low overall but higher under moderate-lime (ML) than low-lime (LL) soil (2.4 vs. 0.4%; $P<0.05$). Soil type affected all remaining traits except flowering time ($P<0.01$; Table 3). On average, the grain yield reduction in ML soil relative to LL was severe (−32%) but less pronounced than that for shoot residues (−51%) or above-ground biomass (−42%), because it was partly compensated by higher harvest index (0.532 vs. 0.447) associated with about four-day later maturity of the crop (Table 4). Later maturity in ML also implied heavier individual seed relative to LL (0.40 vs. 0.37 mg).

The crop response to inoculants tended to be soil-specific, with significant soil type×inoculant interaction observed for shoot residues, above-ground biomass, harvest index, and maturity time (Table 3). Compared with the commercial inoculant, the combined inoculation with the mixture of three Moroccan strains led to lower grain yield and shoot residues in LL soil, but comparable grain yield and more shoot residues in ML soil (Table 4). However, the increase in crop harvest index passing from LL to ML soil was higher in the commercial inoculant than the Moroccan one, and was associated with delayed maturity (Table 4).

Genotype variation across environments occurred for all traits ($P<0.01$). Genotype×soil type interaction was detected for all production and phenology traits. It was larger for grain yield, above-ground biomass and maturity time, according to P levels (Table 3) and the only moderate consistency of genotype response across soil types that was observed for these traits ($0.56\leq r\leq 0.78$; Table 5). The range of genotype

Table 3 Analysis of variance *F* test results for morphophysiological traits of 17 white lupin genotypes in Experiment 1 (environments formed by the factorial combination of moderate-lime or low-lime soil with two *Bradyrhizobium* inoculants, one commercial and one including three Moroccan strains adapted to calcareous soils)

Source of variation	*df*	Grain yield	Shoot residues	Above-ground biomass	Harvest index	Plant mortality	Flowering time	Maturity time	Seed weight
Soil	1	**	**	**	**	*	ns	**	**
Inoculant	1	*	ns	*	*	ns	ns	ns	ns
Genotype	16	**	**	**	**	*	**	**	**
Soil × Inoculant	1	ns	**	**	**	ns	ns	**	ns
Genotype × Soil	16	**	*	**	ns	ns	*	**	ns
Genotype × Inoculant	16	ns	ns	ns	ns	ns	ns	ns	ns
Genotype × Soil × Inoculant	16	ns	ns	ns	ns	ns	ns	*	ns

ns not significant

*$P<0.05$, **$P<0.01$

variation for mean onset of flowering was 7 days including the late cultivar Adam, and 3 days excluding it. Genotype variation for mean maturity date ranged 4 days overall. It ranged from 0.28 mg for Adam to 0.70 mg for LAP123 for individual seed weight, with a general trend for Italian germplasm towards heavier seed.

The top-performing genotypes for grain yield or above-ground biomass were different in the two soils. In particular, Ita1 was top-ranking for grain yield and high-yielding for above-ground biomass in LL soil, but drastically reduced its performance in ML soil (Table 6). This genotype exhibited significant ($P<0.05$) crossover interaction across soil types with LAP122 and LAP123 for grain yield, and with LAP122 for above-ground biomass. These latter genotypes featured the highest ML/LL ratio values (≥0.92 for grain yield and ≥0.74 for above-ground biomass: Table 6). Also LA246, which was top-ranking for grain yield and above-ground biomass in ML, displayed high ML/LL ratio values for these variables, whereas Multolupa was definitely lime-susceptible on the basis of its ratio values (≤0.25; Table 6). The intermediate ML/LL ratio values of Giza 1 suggested the presence of several genotypes with even better adaptation to moderately calcareous

Table 4 Mean value of white lupin morphophysiological traits in two experiments (environments formed by the factorial combination of moderate-lime or low-lime soil with two *Bradyrhizobium* inoculants, one commercial and one including three Moroccan strains adapted to calcareous soils)

Soil lime	Inoculant	Experiment 1					Experiment 2			
		Grain yield (t ha^{-1})	Shoot residues (t ha^{-1})	Above-ground biomass (t ha^{-1})	Harvest index	Maturity time (days from Jan. 1)	Shoot dry weight (g plant^{-1})	Root dry weight (g plant^{-1})	Nodule dry weight (mg plant^{-1})	Number of nodules per plant
Low	Commercial	4.23 a	5.30 a	9.53 a	0.439 c	179.9 d	1.47 a	0.39 a	37.0 a	49.6 b
Low	Moroccan	3.93 b	4.70 b	8.63 b	0.455 c	181.4 c	1.45 a	0.30 b	36.7 a	60.0 a
Moderate	Commercial	2.82 c	2.30 d	5.12 c	0.551 a	185.6 a	0.90 a	0.20 c	8.1 c	2.7 c
Moderate	Moroccan	2.74 c	2.59 c	5.33 c	0.514 b	184.3 b	0.90 a	0.20 c	20.8 b	41.0 b
SE[a]		0.074	0.083	0.150	0.0041	0.34	0.044	0.013	1.43	2.53

Column means followed by different letters differ at $P<0.05$ according to LSD

[a] *df*=8 for Experiment 1, and 6 for Experiment 2

Table 5 Correlations for morphophysiological traits of 17 white lupin genotypes in moderate-lime (ML) and low-lime (LL) soil and for the ratio of genotype values in the former condition relative to the latter (ML/LL), in two experiments (E1 and E2)[a]

Trait 1	Trait 2	*r*
Grain yield, ML, E1	Grain yield, LL, E1	0.71**
Shoot residues, ML, E1	Shoot residues, LL, E1	0.83**
Above-ground biomass, ML, E1	Above-ground biomass, LL, E1	0.78**
Flowering time, ML, E1	Flowering time, LL, E1	0.92**
Maturity time, ML, E1	Maturity time, LL, E1	0.56*
Grain yield, ML/LL, E1	Shoot residues, ML/LL, E1	0.96**
Grain yield, ML/LL, E1	Above-ground biomass, ML/LL, E1	0.99**
Grain yield, ML/LL, E1	Maturity time, ML/LL, E1	−0.38 ns
Shoot dry weight, ML, E2	Shoot dry weight, LL, E2	0.79**
Nodule dry weight, ML, E2	Nodule dry weight, LL, E2	0.36 ns
Nodule number, ML, E2	Nodule number, LL, E2	0.26 ns
Shoot dry weight, ML/LL, E2	Nodule dry weight, ML/LL, E2	0.63**
Shoot dry weight, ML/LL, E2	Nodule number, ML/LL, E2	0.54*
Above-ground biomass, ML/LL, E1	Shoot weight, ML/LL, E2	0.50*

+$P<0.10$, *$P<0.05$, **$P<0.01$ ($df=15$)

[a] For traits showing significant genotype × soil interaction (see Tables 3 and 7)

Table 6 Mean value of morphophysiological traits under moderate-lime (ML) and low-lime (LL) soils and ratio of response in the former condition relative to the latter, for 17 white lupin genotypes in two experiments (values averaged across two *Bradyrhizobium* inoculants)

Genotype	Experiment 1						Experiment 2		
	Grain yield (t ha^{-1})			Above-ground biomass (t ha^{-1})			Shoot dry weight (g plant^{-1})		
	ML	LL	Ratio	ML	LL	Ratio	ML	LL	Ratio
Ita1	2.76	5.80	0.48	5.01	12.16	0.41	1.05	1.65	0.63
Ita49	3.71	5.19	0.71	7.24	12.18	0.59	1.04	1.90	0.55
LA246	4.49	5.34	0.84	8.58	11.56	0.74	1.20	1.64	0.73
LAP122	4.02	4.37	0.92	8.03	10.33	0.78	1.14	1.60	0.71
LAP123	4.19	4.52	0.93	8.33	11.25	0.74	1.53	1.73	0.88
LAP124	3.88	4.70	0.83	6.95	9.99	0.69	0.62	1.17	0.53
Egy42	2.04	4.75	0.43	4.00	10.16	0.39	0.96	1.39	0.69
Egy64	3.17	4.39	0.72	5.69	9.47	0.60	1.43	1.79	0.80
Egy89	2.22	3.08	0.72	4.32	7.22	0.60	0.97	1.36	0.71
LA356	1.94	3.64	0.53	3.68	8.02	0.46	0.85	1.38	0.61
Giza 1	1.45	2.50	0.58	2.84	5.58	0.51	0.67	1.20	0.55
Mar74	3.03	4.24	0.71	5.56	9.67	0.57	0.80	1.49	0.54
Mor28A	3.37	4.60	0.73	6.04	9.99	0.60	0.74	1.57	0.47
Mor29	2.03	2.66	0.76	3.57	5.88	0.61	0.70	1.19	0.59
Mor32	2.77	3.99	0.69	4.92	8.39	0.59	0.72	1.21	0.60
Multolupa[a]	0.85	3.41	0.25	1.62	7.40	0.22	0.55	1.33	0.41
Adam	1.31	2.22	0.59	2.44	5.18	0.47	0.30	1.20	0.25
SE[b]	0.230	0.431		0.443	0.762		0.089	0.111	
LSD ($P<0.05$)	0.650	1.218		1.252	2.154		0.252	0.314	

[a] Introduced in Morocco and multiplied locally for about 30 years

[b] $df=64$

soils than this reference lime-tolerant cultivar, including some Moroccan landraces and the Egyptian landraces Egy64 and Egy89 (Table 6). The genotype ability to maintain regular growth in the calcareous soil concerned simultaneously grain yield and shoot residues, according to the high correlation between ML/LL ratios for these variables (Table 5).

The lack of genotype×inoculant interaction for any trait (Table 3) indicated that the effects of the inoculants were independent of the host plant. Genotype×soil type×inoculant interaction was detected only for maturity time (Table 3).

Experiment 2

The final plant mortality was very low (1.5%) and did not differ between soil types or inoculants ($P>0.05$). On average, ML soil implied much lower shoot (−32%), root (−42%) and nodule (−61%) dry weight and fewer nodules per plant (−60%), in comparison with LL soil ($P<0.01$; Table 4). The inoculants did not differ for shoot dry weight in either soil (Table 4), whereas their effect on root or nodule traits was affected by soil type×inoculant interaction (Table 7). In particular, the inoculants differed for root weight only under LL soil, where the commercial inoculant led to heavier roots (Table 4). Compared to inoculation with the Moroccan strains, the commercial inoculant implied similar nodule weight and fewer nodules in LL soil, as well as greater reduction of nodule weight and especially of nodule number in ML soil (Table 4). The few nodules per plant that it produced in this soil were outstandingly large (mean dry weight of 2.95 mg, compared with mean values between 0.51 and 0.75 mg for the other inoculant-soil type combinations).

Genotype variation occurred for all traits ($P<0.05$) except plant mortality. Genotype×soil type interaction was found for shoot dry weight, nodule dry weight and nodule number per plant (Table 7). It was moderate for the first trait and large for the other two traits, according to correlations for genotype values between soil types (Table 5). The correlations of ML/LL ratio values for shoot dry weight with those for nodule dry weight or nodule number per plant (Table 5) indicated that better genotype ability to tolerate the calcareous soil was associated with lower reduction of nodulation in ML soil.

No significant ($P<0.05$) crossover interaction between top-ranking genotypes was observed for shoot weight. However, the landrace LAP123 exhibited the highest ML/LL ratio for this variable (Table 6), confirming its lime-tolerant response which had emerged in Exp. 1. Multolupa had low ML/LL ratio for this variable in agreement with results from Exp. 1, but Adam currently featured even greater shoot weight reduction in ML (Table 6). On the whole, genotype values of ML/LL ratio for shoot weight in this experiment were moderately correlated with those for above-ground biomass in Exp. 1 ($r=0.50$; $P<0.05$). The Italian landraces LAP122, LA246 and LAP123, and the Egyptian landrace Egy64, consistently outperformed ($P<0.01$) the reference lime-tolerant cultivar Giza 1 for grain yield and above-ground biomass in Exp. 1 and for shoot weight in Exp. 2.

Table 7 Analysis of variance *F* test results for morphophysiological traits of 17 white lupin genotypes in Experiment 2 (environments formed by the factorial combination of moderate-lime or low-lime soil with two *Bradyrhizobium* inoculants, one commercial and one including three Moroccan strains adapted to calcareous soils)

Source of variation	*df*	Shoot dry weight	Root dry weight	Nodule dry weight	Number of nodules
Soil	1	**	**	**	**
Inoculant	1	ns	*	**	**
Genotype	16	**	**	**	**
Soil×Inoculant	1	ns	*	*	**
Genotype×Soil	16	*	ns	**	**
Genotype×Inoculant	16	*	ns	*	*
Genotype×Soil×Inoculant	16	ns	ns	*	ns

ns not significant

*$P<0.05$, **$P<0.01$

Multitalia, evaluated only in Exp. 2, could be rated as lime-susceptible on the basis of its ML/LL ratio for shoot biomass, equal to 0.43 and comparable with that of Multolupa. Accordingly, its decrease in shoot biomass passing from LL to ML soil was much more pronounced than that of the lime-tolerant genotype LAP123 (63% vs. 24%, based on shoot weight data reported in Table 8).

Genotype×inoculant interaction was detected for shoot weight and nodulation traits (Table 7). Genotype response for shoot weight, however, was fairly consistent across inoculation treatments according to correlation results (r=0.80, P<0.01) and the lack of significant rank inversion between top-ranking genotypes across inoculants (data not shown). Lower consistency of genotype response across inoculants was observed for nodule dry weight and nodule number per plant (r=0.53, P<0.05 for both traits). Correlations for ratios of genotype values between Moroccan and commercial inoculants indicated that genotypes with relatively better shoot weight response with the former inoculant also tended to relatively higher nodule weight in that condition (r=0.72, P< 0.01). Conversely, genotype×inoculant interaction effects for shoot weight were unrelated to those for nodule number according to correlation results. Genotype×soil type×inoculant interaction occurred only for nodule dry weight (Table 7).

Multilocation testing

Grain yield data across Italian locations with LL or ML soils confirmed the lime-tolerant response of LAP123. The yield gap between this genotype and the lime-susceptible genotype Multitalia in the Mediterranean site with ML soil (Foggia) was outstanding and comparable with that observed for shoot weight under ML soil in Exp. 2 (Table 8). Multitalia outyielded LAP123 in the Mediterranean site with LL soil (Sanluri), whereas the genotypes did not differ in the subcontinental-climate site with LL soil (Lodi).

Discussion

As expected, mean lupin grain yield or shoot weight were markedly reduced in ML soil relative to LL one in Exp. 1 or Exp. 2. The distinct delay of crop maturity observed under ML soil in Exp. 1 may have contributed, by means of longer assimilate retranslocation from vegetative organs to seeds, to the increases in seed weight and harvest index that mitigated the grain yield reduction in this soil relative to LL soil. No earlier comparison of lupin crop maturity, seed weight or harvest index between ML and LL soils has ever been reported, preventing any verification of the observed plant responses. The delayed plant maturity in ML soil may have been favored, however, by greater soil moisture availability due to greater water-holding capacity of this clayish soil and to lower water use in earlier stages from generally smaller plants. The reduction of root biomass under ML soil in Exp. 2 agrees with earlier reports (Liu and Tang 1999; Kerley and Huyghe 2001). The concurrent, marked decrease of number and weight of nodules per plant is a well-known

Table 8 Shoot dry weight in Experiment 2 and grain yield in field experiments at three Italian locations for two white lupin genotypes under moderate-lime (ML) or low-lime (LL) soil conditions

Genotype	Shoot weight (mg plant^{-1})[a]		Grain yield (t ha^{-1})		
	ML	LL	Foggia (ML)[b]	Lodi (LL)[c]	Sanluri (LL)[d]
LAP123	1.529 a	1.730 a	1.68 a	4.02 a	3.26 b
Multitalia	0.567 b	1.314 b	0.58 b	4.01 a	3.68 a
SE	0.089	0.111	0.089	0.111	0.134

Column means followed by different letters differ at P<0.05 according to LSD

[a] Averaged across two *Bradyrhizobium* inoculants in Experiment 2. Standard error df=64

[b] Averaged across two sowing times. Standard error df=44

[c] Averaged across two sowing times (from: Annicchiarico et al. 2003). Standard error df=44

[d] Averaged across two sowing times (from: Annicchiarico et al. 2004). Standard error df=40

component of lupin misadaptation to calcareous soils (Tang and Thomson 1996).

The occurrence of genotype×soil type interaction of crossover type between top-ranking material for grain yield in Exp. 1, and the relatively better response in ML soils exhibited by LAP123 consistently across experiments and agricultural sites, provide further evidence for the presence of white lupin genetic variation for tolerance to moderately calcareous soils. The yield disadvantage of LAP123 over Multitalia in the LL-soil site of Sanluri was consistent with its worse climatic adaptation to Mediterranean sites (Annicchiarico and Carroni 2009). Therefore, better tolerance to soil lime appears to be the prime reason for a large yield advantage exhibited by LAP123 over Multitalia in the Mediterranean-climate, ML-soil site of Foggia. The much lower yield of LAP123 in Foggia relative to Sanluri was also due to less spring rainfall (Table 2) besides the unfavorable soil. This result suggested, however, that even lime-tolerant material could overcome only partly the misadaptation of white lupin to moderately calcareous soils.

The artificial environments of Exp. 1 allowed to assess interactions with soil type of white lupin genotypes or inoculants for grain yield under conditions similar to agricultural environments and climatically equal across treatments. Other studies tended to suffer of one of the following limitations: (1) lack of grain yield assessment due to the short growing cycle implied by the growing environment (pot or hydroponic solution: e.g., Kerley et al. 2002, or Exp. 2); and (2) grain yield assessment in contrasting soil types placed in different locations, with confounding of soil type and climatic effects (e.g., Christiansen et al. 2000, or the current multilocational testing). Experiments in pots or liquid culture, although necessary in many contexts, may also suffer from abnormal plant root growth (Kerley and Huyghe 2001) and fairly modest correlations with agricultural environments for shoot growth response of genotypes (Liu and Tang 1999). However, our artificial environments also suffered some potential limitations. It was impossible to sterilize their soils, to eliminate any indigenous strain of *Bradyrhizobium* sp. (*Lupinus*). The presence of such strains was unlikely, though, owing to the long-standing absence of prior crops hosting *Bradyrhizobium* sp. and the fact that white lupin, which is not grown traditionally in northern Italy, exhibited no nodulation in local soils when not inoculated (Annicchiarico 2006). The strong reduction of lupin root growth in calcareous soils (Kerley 2000) probably prevented any sizeable root growth beyond the 0.8-m layer of such soil in ML-soil environments. The same artificial environments could successfully reproduce the adaptive responses to soil type as observed across agricultural environments for cultivars of a deep-rooting species such as lucerne (Annicchiarico 2007).

Genotype×soil interaction for shoot biomass was detected across limed and non-limed soils, or acid and moderately alkaline soils, in most (Liu and Tang 1999; Raza et al. 2000; Tang et al. 2006) but not all (Kerley et al. 2002) earlier studies. Two studies (Liu and Tang 1999; Tang et al. 2006) also found genotype×soil interaction for root biomass, unlike here. Assessing genotype responses for root biomass in pots or liquid culture (as done here and in most earlier studies) is less reliable, although less expensive, than in tall soil chambers (Kerley and Huyghe 2001). The observed genotype×soil interaction for nodule number, and the trend of lime-tolerant genotypes towards lower nodulation reduction in ML soil, are consistent with results by Raza et al. (2000) and Tang et al. (2006). One mechanism for better genotype ability to maintain nodulation in calcareous soils might be the greater capacity for acidifying the rhizosphere, which favors *Bradyrhizobium* colonization by improving its growing environment and the availability of iron from the plant (Tang et al. 2006). Such mechanism, however, also implies a high metabolic cost for the plant (Dinkelaker et al. 1989). Further work would be necessary to identify the physiological traits, among the several possible ones (Kerley et al. 2002), that feature the current lime-tolerant germplasm. The large seed exhibited by LAP123 and other Italian germplasm is associated with greater early root and shoot growth (Huyghe 1993), which may have greater importance for plants grown in the unfavorable ML soil.

The only moderate consistency of lime-tolerance response of genotypes across Exp. 1 and Exp. 2 (as measured by correlations for ML/LL ratios relative to plant weight) may be accounted for by the different plant growth stages of the assessments (crop maturity and onset of flowering), the different environmental conditions (for temperature, soil nitrogen availability, etc.), the mentioned limitations of pot experiments, and experimental error effects. Climatic misadaptation may have emphasized the effect of the soil lime-

stressing treatment on Adam in Exp. 2, leading to its outstanding shoot weight reduction. Various procedures of indirect selection for lime tolerance have been devised in lupin species to overcome the difficulties of direct field-based selection that arise from fairly high experimental error caused by natural soil heterogeneity (Kerley et al. 2001; Brand et al. 2002), but their application in white lupin is hindered by the complexity of the plant's tolerant response (Kerley et al. 2002).

Soil type×inoculant interaction effects for grain yield and shoot residues in Exp. 1 and for root weight and nodulation traits in Exp. 2 suggested, on the whole, the superiority of the commercial inoculant in LL soil and the lower adaptation of its *Bradyrhizobium* strain to ML soil relative to the combination of Moroccan strains. In the favorable soil, the greater plant root weight observed for the commercial inoculant relative to the Moroccan one in the presence of similar nodule weight (Table 4) may have resulted from greater N-fixing ability of the commercial *Bradyrhizobium* strain. In the long run, this characteristic would also contribute to greater above-ground biomass and grain yield of the commercial inoculant in this soil, as confirmed by results of Exp. 1. The drastic reduction in nodule number per plant exhibited by the commercial inoculant passing from LL to ML soil is typical for *Bradyrhizobium* strains poorly adapted to calcareous soils (Abd-Alla 1999). The concurrent outstanding increase in nodule size compensated only in part for the disadvantage of this inoculant relative to the Moroccan one in terms of nodule dry weight per plant under ML soil. Nodule weight per plant proved more related than nodule number to grain yield of white lupin plants subjected to different *Bradyrhizobium* inoculants (Raza et al. 2001b). The greater decrease in nodule weight per plant of the commercial inoculant passing from LL to ML soil could also be appreciated when expressing it as the ratio of nodule to root dry weight, to also take account of the different root development in the two soils. From LL to ML soil, this ratio, computed from data in Table 4, decreased sharply for the commercial inoculant (0.097 vs. 0.041) and mildly for the Moroccan one (0.122 vs. 0.107). Despite this difference, plant biomass in ML soil of Exp. 2 (or Exp. 1) did not differ between inoculants, supporting the greater N-fixing ability hypothesized for the commercial *Bradyrhizobium* strain.

Although the Moroccan inoculant outperformed the commercial one only for shoot residues in the ML soil conditions for which it was devised, the greater adaptation of its component strains to calcareous soils encourages further selection work on *Bradyrhizobium* strains isolated from lupin species adapted to calcareous soils. Actually, the tolerance to ML soil was only one of the attributes considered for selection of the current Moroccan strains (Thami Alami et al. 2004a; El Hilali 2006). A thorough evaluation of candidate strains also for N-fixing ability and suitability to combined inoculation might be indispensable to achieve also grain yield progress over a commercial inoculant in ML soil. The current lack of genotype× inoculant interaction confirms the wide compatibility with plants of inoculants including more strains that emerged in Raza et al. (2001b). We could not assess the relative extent of nodulation from each of the three strains on the different genotypes and plants within genotypes, but other work suggests that simultaneous infection by different strains may occur not only on the same plant but even on nearby root parts of the same plant (El Hilali et al. 2007). While justifying the further investigation of lime-tolerant *Bradyrhizobium* strains, the present findings indicate that white lupin adaptation to moderately calcareous soils could be enhanced to some extent through selection of lime-tolerant cultivars. Italian lime-tolerant landrace germplasm such as LAP123 could be particularly useful in breeding autumn-sown varieties for subcontinental-climate or Mediterranean environments (Annicchiarico et al. 2010; 2011).

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