

ISSN 1510-7809

***LOTUS* NEWSLETTER 2008**
Volume 38, Number 1



Editor: M. Rebuffo
INSTITUTO NACIONAL DE
INVESTIGACION AGROPECUARIA





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Front cover: The photograph on the front cover exhibit experimental plots of *Lotus* spp. in Chile. The response of *Lotus* to fertilization is reported by Dr. Hernán Acuña (pp.1-6).

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Response to Phosphorus, Potassium and Sulphur application on the productivity of *Lotus* spp. in two soil groups of central Chile

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Introduction

The *Lotus* forage species, *L. corniculatus* (Lc), *L. glaber* (Lg) and *L. uliginosus* (Lu), have several valuable characteristics for agriculture, among which is that they are adapted to poorly drained soils with low pH and fertility. In high fertility soils they are replaced by more productive species like white clover or lucerne. They respond to fertilizer application at low rates. The higher yield of Lc is reached with phosphorus and potassium application (Russelle, 1991). Another important element in *Lotus* nutrition is sulphur (Lowther, 1980).

In general legumes increase nodulation, N-fixation and growth when phosphorus availability in the soil increases, by phosphorus fertilizer applications or arbuscular mycorrhiza. The nodulating rhizobia genera for Lc and Lg is *Mesorhizobium* and for Lu *Bradyrhizobium*. Some authors propose using these species for “low input” production systems, where more productive species would not be successful. This performance is attributable to efficient soil phosphorus uptake by their large root systems (Blummenthal *et al.*, 1993). Hart *et al.* (1981) found that phosphorus concentration in Lu tissues is lower than the concentration in white clover tissues when both species grow under similar soil fertility conditions.

Experimental

Chilean research about the effects of mineral fertilizer applications on *Lotus* spp. is scarce. Two experiments were carried out in soils originating from volcanic ash, fixing phosphorus, medium texture and well drainage. One considered different levels of phosphorus application on Lc (Acuña, 1996) and the other studied the comparative response of phosphorus applications on Lc, Lg and two white clover cvs. (Acuña, 1998). Three experiments were carried out in poorly drained clay soil of a rice growing area, one per each species, to study phosphorus, potassium and sulphur application rates combined with three levels of soil water availability (Acuña *et al.*, 1998). Dry matter yield, availability of soil P, K, S and others elements, concentration of these elements on herbage and, in some cases, growth variables, such as the number of growing points and rhizomes or stolons, per unit area (Lu) and dimensions and appearance rates of leaves were measured. The dry matter yield (DM), soil nutrient availability and concentrations of phosphorus and other elements in herbage are reviewed in this paper.

Results and discussion

In the Lc experiment on volcanic soil (4 mg/kg of P; 123 mg/kg of K; 13.2 mg/kg of S- SO₄; pH 6.0) there was no phosphorus response despite very low soil phosphorus availability. Phosphorus applications of 0, 5, 10 and 15 kg/ha, as triple superphosphate, were compared. DM yield was recorded for three growing seasons. Because of the high soil potassium availability, there was also no response to this element. Figure 1 shows the sulphur effects on DM production of total and pure *Lotus* under generous application of phosphorus and potassium. DM yields increased when sulphur application rates were increased, despite high initial levels of soil sulphur availability. Soil sulphur increased from 13.2 to 17.1 mg/kg when 60 kg/ha was applied and to 22.4 mg/kg when 120 kg/ha was applied.

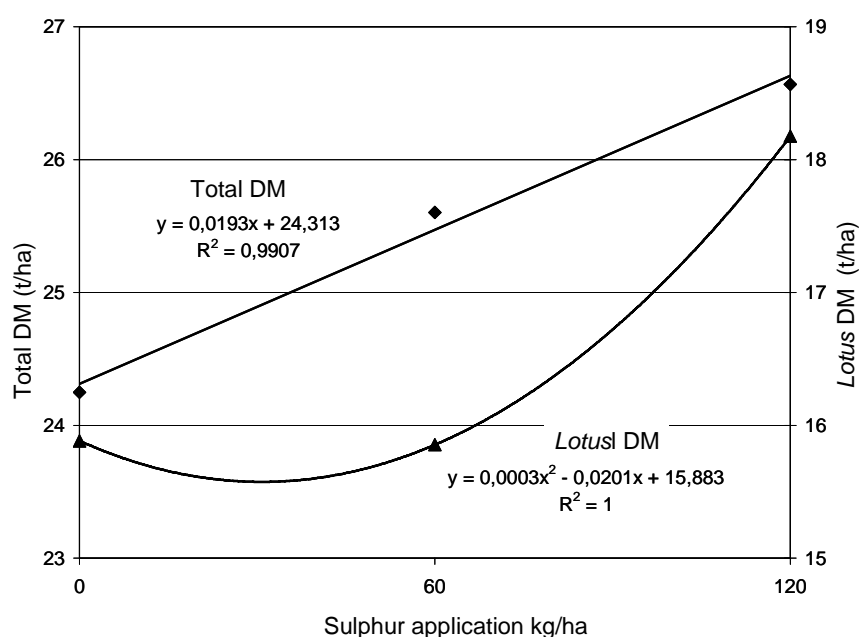


Figure 1. *Lotus corniculatus* cv. Quimey response to sulphur application in volcanic soils. Accumulated DM production in three growing seasons (t/ha).

When the response to P application of two *Lotus* species (*L. corniculatus* and *L. glaber*) and two *T. repens* cvs. (Ladino Italia and Huia) were compared in a volcanic soil, Lc reached high production levels (Table 1) with 7.5 kg/ha/year. The control without P yielded significantly less than 7.5, but it maintained the high level of production, 11.5 t/ha/year. Lg yielded 50% less than Lc, but its yields, including those with zero P treatment, were higher than those of white clover cvs. These results confirm that *Lotus* spp. are able to absorb phosphorus in a more efficient way than other forage legumes when soil phosphorus is available in small amounts. Table 2 shows a significant fall of soil P, after two years of production, in *Lotus* spp. treatments compared to clover cvs. treatments, due to greater *Lotus* P uptake as a result of its higher DM yield, given that the herbage P concentration differences between lotus and clovers do not explain this level of P extraction (Table 3). Phosphorus concentration in Lc was significantly less than white clover in all levels of P

application except 7.5 kg/ha; according to Hart *et al.* (1981) its performance would be similar to Lu. The Lg herbage P concentration values were higher than Lc concentrations.

Table 1. Comparison of two *Lotus* species and two white clover cvs. in volcanic soils. Dry matter yield (kg/ha) of pure *Lotus* in the second growing season.

cvs.	Annual rate of phosphorus application (kg of P/ha)			
	0	7.5	15	22.5
Huia	390	994	1602	1200
Italia	421	539	1864	2188
Quimey	11536	14749	12685	12552
Toba	3884	3589	4709	4756

120.3 s.e. for comparing P means within each cv.

399.3 s.e. for comparing cv. means within each P level or within different P levels (P x cv. means).

Table 2. Comparison of two *Lotus* species and two white clover cvs. in volcanic soils. Effects of *Lotus* spp. or white clover cv. on soil P changes (0- 10 cm).

cv.	Phosphorus in soil (mg/kg)	
	Second season	Third season
Huia	3.6	3.1
Italia	4.2	3.7
Quimey	3.5	2.6
Toba	3.4	2.6
s.e.	0.26	0.25

Table 3. Comparison of two *Lotus* species and two white clover cvs. in volcanic soils. Effects of P application on concentration of P in herbage (% in DM), in the second growing season.

cv.	Annual rate of phosphorus application (kg of P /ha)			
	0	7.5	15	22.5
Huia	0.170	0.200	0.222	0.235
Italia	0.172	0.180	0.225	0.225
Quimey	0.165	0.182	0.192	0.200
Toba	0.182	0.197	0.232	0.217

0.0033 s.e. for comparing cvs. within the same P level.

0.0022 s.e. for comparing P levels within the same cv..

The experiments in clay soils (5.4 mg/kg of P; 70.4 mg/kg of K; 1.73 mg/kg of S–SO₄; pH 5.5) tested 2 levels of P application at sowing, 10 and 20 kg/ha, plus annual applications, which reached 50 and 100 kg/ha by the third year, combined with 2 levels of K application at sowing, 20 and 40 kg/ha, plus annual rates, which by the third year totaled 60 and 120 kg/ha and combined with 3 levels of water availability, replenishing the soil with 50,100,150% of water evaporation (Class A evaporimeter) every ten days. Sulphur was applied (120 kg/ha) to all plots except to a control without S at higher levels of P, K and water. Phosphorus increased DM production at the 3 levels of water availability (Table 4), contrary to the literature which reports positive effects of phosphorus only at low application rates. Phosphorus herbage concentration increased significantly ($P < 0.001$) in the three species when P application was increased (Table 5). Lc shows the lower values and Lu the higher. There is no response of K application, except in the case of Lc, which increased yield in the first and third growing seasons. Potassium herbage concentrations of Lu doubled the concentration of Lg and Lc (Table 6). Calcium and magnesium herbage concentrations in Lc were significantly higher than Lg and Lu. There was no response to application in any of the three species.

Table 4. Phosphorus and potassium applications at three levels of soil water availability. Effects of P application x soil water availability interaction on DM production (t/ha) in the third growing season.

Species	Soil water availability (% of evaporation replenishing each ten days)						s.e.
	50		100		150		
	Low P	High P	Low P	High P	Low P	High P	
Lc	6.08	8.85	7.28	8.71	7.48	9.51	0.293
Lg	3.54	5.35	4.70	6.51	5.77	6.55	0.245
Lu	3.58	5.05	4.24	5.67	2.86	5.50	0.171

Table 5. Phosphorus and potassium application at three levels of water availability in clay soils. Effects of P application on the concentration of P in herbage (% in DM) in the three *Lotus* spp. (second growing season).

Species	Phosphorus levels		s.e.
	Low	High	
Lc	0.193	0.222	0.0018
Lg	0.236	0.273	0.0020
Lu	0.284	0.317	0.0016

Table 6. Phosphorus and potassium application at three levels of water availability in clay soils. Effects of P application on K, Ca and Mg concentration in herbage (% in DM)in the three *Lotus* spp. (second growing season).

	Levels of phosphorus application								
	Low			High			s.e.		
	Lc	Lg	Lu	Lc	Lg	Lu	Lc	Lg	Lu
K	0.60	0.67	1.71	0.62	0.70	1.70	0.008	0.019	0.053
Ca	0.60	0.37	0.32	0.59	0.40	0.30	0.010	0.007	0.007
Mg	1.50	1.13	0.73	1.59	1.20	0.70	0.037	0.033	0.016

Conclusions

The *Lotus* spp. response to P application was more evident in clay soils even though the soil P availability in both environments was equally low. This response was notably inferior to that of the clover, but the production levels reached by *Lotus* without P were very high compared to that of the clover under the same conditions. For that reason, the *Lotus* P uptake is very high compared to clover uptake, although the P concentration in *Lotus* herbage would be slightly lower or similar. There were no effects of K in DM production, contrary to the expectation in cutting experiments on soils with low K availability. Sulphur application improved the productivity in volcanic soils with high S availability; there were no S application effects on clay soils.

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The Sharnae greater lotus (*Lotus uliginosus* Schkuhr) germplasm – potential for low latitude environments

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Summary

This paper is a review of the scientific literature and industry experience with the Sharnae greater lotus (*Lotus uliginosus* Schkuhr) germplasm in Australia. The purpose is to assess the merits and limitations of Sharnae based on knowledge and experience that was not available when Sharnae was first registered for release in 1991. Sharnae was subsequently withdrawn from commerce in 1998. This review is intended to inform a consideration of the potential for commercialising Sharnae for use in low latitude environments, and to promote awareness of the availability of Sharnae (and other similar germplasms) for future cultivar development or evaluation work in low latitude homoclimates of eastern Australia.

Key words: pasture legume(s), plant improvement

Of the wide species diversity in the *Lotus* genus that comprises some 200 species (Kirkbride, 1999), two species (*L. australis*, *L. cruentis*) are endemic to Australia, and four exotic species (*L. angustissimus*, *L. suaveoleus*, *L. corniculatus*, *L. uliginosus*) have naturalised (Wilson, 1980). Of these, only *L. corniculatus* (birdsfoot trefoil) and *L. uliginosus* (greater lotus) are presently in commercial use as pasture plants (Blumenthal and McGraw, 1999).

While the potential zone of adaptation of both birdsfoot trefoil and greater lotus in Australia is substantial (Hill *et al.*, 1996), only greater lotus presently has significant commercial usage (Ayres *et al.*, 2006a; 2006b). Within greater lotus, only two cultivars are commercially available – Grasslands Maku (intraspecific hybrid tetraploid with 25% Mediterranean provenance bred in New Zealand) and Grasslands Sunrise (diploid with 25% Mediterranean provenance bred in New Zealand).

Greater lotus in Australia

Following commercial release in 1974 of Grasslands Maku, plantings of greater lotus-based pastures in Australia were of the order of 100 hectares per annum during 1978-1983, and increased to 30,000 hectares by 1990 (Harris *et al.*, 1992; 1993). The planted area

subsequently expanded to plateau at about 100,000 hectares by the late 1990's (Blumenthal and McGraw, 1999). The major application of greater lotus has been for beef and dairy cattle in high rainfall coastal districts. Minor applications include groundcover in orchard plantations and seed production (Harris *et al.*, 1993). Greater lotus is currently recommended in New South Wales for cattle and sheep grazing in high rainfall coastal districts and favoured moist sites on the Northern Tablelands (Ayres, 2004).

This relatively low level of usage of greater lotus has evolved through farmer experience with just one cultivar, Grasslands Maku, which has known limitations: Slow establishment and weak seedling vigour especially with cold conditions that accompany autumn planting (Keoghan and Burgess, 1987; Kelman and Bowman, 1996); susceptibility to summer moisture-stress (Blumenthal *et al.*, 1999); poor seed-set at low latitudes (Blumenthal and Harris, 1993); and susceptibility to frost damage (Schiller and Ayres, 1993).

More recently, a state-wide study in New South Wales (Ayres *et al.*, 2006a; 2006b) evaluating the adaptation of greater lotus and birdsfoot trefoil, and allowing for expected adoption levels, proposed that the greater lotus zone in New South Wales could expand to about 1 million hectares, provided that cultivar development achieved broad adaptation to extend the adaptive potential of greater lotus beyond favourable niche environments. Moreover, Ayres *et al.* (2007) proposed that breeding greater lotus for eastern Australia should target the environments most conducive to the warm season growth characteristics of greater lotus. That is, the low latitude summer rainfall region, including the high rainfall coastal zone and medium rainfall tablelands zone in northern New South Wales and south-eastern Queensland. In consequence, appropriate breeding objectives for this environment include better tolerance of summer moisture-stress, stronger seedling vigour and greater seed-set under low latitude conditions.

Species evaluation work in Australia with the *Lotus* genus has previously identified greater lotus as meriting further breeding work (Wilson, 1980; Kelman, 1993). To date, the selection work of Tony O'Brien at Grafton, New South Wales (O'Brien, 1974; 1978) led to the development of the Sharnae germplasm (Wilson, 1992), and the crossing work of Walter Kelman at Canberra, Australian Capital Territory (Kelman and Bowman, 1996) led to the development of the LUX97 germplasm. A recent characterisation study of a working collection of greater lotus cultivars and breeding lines found that these germplasms developed in Australia with high levels of Mediterranean provenance, express high year-round herbage growth, high winter growth activity, and set more seed than Grasslands Maku under low latitude conditions (Ayres *et al.*, 2007).

There is now a considerable knowledge base from research in eastern Australia with the Sharnae germplasm. The present paper reviews this knowledge base to assess Sharnae's potential for cultivar development, or alternatively for its use in future breeding for low latitude homoclimes in Australia and South America. The scientific literature on Sharnae comes mainly from Australian research. A significant number of published documents report on the development, characteristics and agronomic merit of Sharnae. For example: origins (O'Brien, 1978; Wilson, 1980); agronomic merit (Blumenthal *et al.*, 1991; Kelman *et al.*, 1992; Blumenthal *et al.*, 1993; Kelman, 1993; Bowman, 1993; Blumenthal *et al.*,

1994; Blumenthal *et al.*, 1995; Hill *et al.*, 1996; Kelman, 1996; Blumenthal *et al.*, 1999; Blumenthal and McGraw, 1999); seed yield capability (Arango *et al.*, 1998); seed quality (Kelman and Blumenthal, 1992); germination phenomena (Blumenthal *et al.*, 1996); nutritive value (Kelman and Tanner, 1990); and a registration paper provides a “cultivar description” of Sharnae (Wilson, 1992).

Moreover, recent research presents new data on regional adaptation (Ayres *et al.*, 2006a; 2006b), and the expression of morphological characteristics at low latitude (Ayres *et al.*, 2007). Also, a number of unpublished reports held by the senior author provide useful additional resource material. These include a report on the collecting expedition in 1974 that selected the parent germplasm (O’Brien, 1974); a briefing document that considered the case for commercialising Sharnae (Bowman and Wilson, 1992); and miscellaneous memoranda that describe experiences with commercial seed production that led to withdrawal of Sharnae from commerce in 1998 (K.A. Archer, *personal communication*).

The origins of the Sharnae germplasm

The first public report on the Sharnae germplasm appeared in the *Lotus Newsletter* (O’Brien, 1978). Tony O’Brien, an agronomist with New South Wales Department of Agriculture undertook a *Lotus* collecting expedition in 1974 to the western Mediterranean region (O’Brien, 1974). Amongst other *Lotus* material collected, O’Brien selected three vigorous early flowering greater lotus populations (CPI 67676, CPI 67677, CPI 67678):

‘I have selected a Lotus pedunculatus type from the Algarve area of Portugal distinct from the L. uliginosus of commerce’ (O’Brien, 1978).

Algarve is located at latitude 37°09’-37°11’ N, the soil at the collection site is of granite derivation, and average annual rainfall (AAR) is 850 mm. It was recorded that “...the site is kept wet by spring-bed soakages” (Wilson, 1992).

A composite of these 3 lines (PI 5303) was evaluated (Wilson, 1980) in north-eastern New South Wales and south-eastern Queensland. PI 5303 performed well but expressed a protracted flowering pattern, which was seen to be a potential problem for seed yield capability. Consequently, selection pressure was applied to CPI 67677 to achieve early and concentrated flowering. Sharnae was developed through long term selection (more than 10 generations) for early and concentrated flowering and high seed production.

Morphology of Sharnae

Sharnae is diploid, $2n = 12$ (Grant, 2004). A detailed morphological description is provided by Wilson (1992). Sharnae is a rhizomatous perennial legume that may have erect or decumbent growth habit, depending on the structure of companion vegetation. In comparison with the commercially prominent cultivar Grasslands Maku, Sharnae was noted to be morphologically similar with the exception of being i) less hairy (fewer hairs on the

leaves, calyx and peduncle tip), ii) fewer crown stems and rhizomes, and iii) smaller seed but a greater number of seeds/pod (Wilson, 1992).

'Sharnae is more robust and bulkier but forms a less dense sward than Maku' (Wilson, 1992).

Under relatively high latitude (34-35°S) conditions in southern New South Wales, Sharnae (and other Portuguese accessions) expressed greater plant height, equivalent autumn herbage growth but less summer growth, and less rhizomatous spread than Grasslands Maku (Kelman *et al.*, 1997).

In phytotron studies, Sharnae produced fewer, shorter but heavier rhizomes than Grasslands Maku – this was considered to provide an adaptive advantage in environments subject to low rainfall (Blumenthal and Harris, 1998). For environmental conditions where photoperiod ranged from 10-14 hours and temperature was varied diurnally through 15/10, 18/13, 21/16 and 27/22°C (maximum day temperature/minimum night temperature), neither photoperiod nor temperature changed the morphological distinctiveness of Sharnae.

Ayres *et al.* (2007) characterised Sharnae (and a set of other diploid and tetraploid greater lotus populations) under low latitude conditions in northern New South Wales. Vegetative characterisation and herbage yield data were obtained under glasshouse conditions over an extended period. Seed yield components were obtained from field sites at Glen Innes (29°42'S) and Armidale (30°31'S). In comparison with Grasslands Maku, Sharnae expressed larger leaf and thicker stem, greater seed-set, greater herbage yield in all seasons, but relatively weaker early growth vigour.

Seed production and seed quality of Sharnae

At three relatively high latitude sites in southern New South Wales (Nowra: 34°54'S/1166 mm AAR; Moss Vale: 34°28'S/1186 mm AAR; Canberra: 35°18'S/694 mm AAR) where Sharnae and some 38 other greater lotus lines were characterised, Sharnae (and other Portuguese lines) were earlier flowering, set more pods and expressed greater seed yield than Grasslands Maku (Kelman *et al.*, 1997).

Under low latitude conditions (Grafton 29°40'S) in north coastal New South Wales, Sharnae expressed earlier flowering and a more extended flowering pattern than Grasslands Maku (Wilson, 1992). Sharnae commenced flowering in early spring (mid-September) reaching peak flowering by mid-October, and then flowered intermittently until late summer. In this same Grafton environment, Bowman and Wilson (1992) reported seed yield of 314 kg/ha under plot culture. Sharnae expressed double the seed production of Grasslands Maku, smaller seed size, more seeds/pod and 38-45% hard seed content.

Seed yield of 400-800 kg/ha has been achieved in well managed crops grown in favourable seasons (McLaughlin and Clarke, 1989). By comparison, the seed yield of Grasslands Maku in New Zealand averages 250 kg/ha (Blumenthal *et al.*, 1993). At Cobbitty near Sydney

(33°55'S), Arango *et al.* (1998) reported seed yield under irrigated plot culture and intensive insect control of 1.1 tonnes/ha, and found that the combination of low plant density (<15 plants/m²) and late harvest (mid-January) maximised seed yield and seed quality. Blumenthal *et al.* (1996) reported 1000 seed weight of 0.743 g for Grasslands Maku compared with 0.532 g for Sharnae. Kelman and Blumenthal (1992) reported a higher level of hardseed in Sharnae (77%) compared with Grasslands Maku (47%) – a feature likely to be advantageous for long-term persistence.

In summary, with sound crop management, seed yield of Sharnae of some 300 kg/ha might be expected. Cultural practices for best seed production include planting in wide rows, keeping the maturing crop moist until 70-80% of pods are ripe, and maintaining vigilant insect control from flowering through pod-set (M.J. Blumenthal, *personal communication*).

Germination phenomena of Sharnae

Under controlled environment conditions, the base temperature for germination of Sharnae is 5°C compared with 6°C for Grasslands Maku (Blumenthal *et al.*, 1996). However, this difference was not considered to provide a major advantage for Sharnae in terms of establishment in cold environments. In this study, Sharnae showed a similar *germination rate*, *hours to first germination* and *hours to 50% germination* compared with Grasslands Maku. Both Sharnae and Grasslands Maku were found to have similarly high threshold temperatures for germination (49.8, 47.3°C, respectively), and the two lines germinated similarly under low moisture conditions.

It is especially noteworthy that the traits *germination rate* and *early flowering* are complementary. Kelman and Forrester (1999) reported that breeding for early flowering (as occurred with Sharnae and LUX97) has i) an indirect positive effect on germination rate and seedling vigour, ii) the expression of these effects is more pronounced in low latitude environments, and iii) germination rate is highly heritable.

Agronomic performance

The greater lotus zone in Australia has been most comprehensively studied in New South Wales. A description of the regions in New South Wales of relevance to greater lotus, and of the agronomic performance of Sharnae in these regions, is provided in the following.

Humid and sub-humid North Coast (28°11'S- 32°46'S)

The North Coast region comprises a high rainfall (1200-1600 mm AAR) humid subtropical coastal plain, and a medium rainfall (900-1200 mm AAR) sub-humid hinterland. The rainfall pattern is sharply seasonal with summer dominance, and soils typically are strongly acidic. The major constraints to grazing in this region are the late winter/early spring feed gap (due to erratic spring rainfall) and the low nutritive value of pasture in summer/autumn (associated with the low digestibility of maturing C4 grasses). In this context, the role for greater lotus is to increase the availability of winter/spring herbage and to enhance the quality of autumn pasture for “carry-over” into winter.

In plot trials on two soil types at Grafton (29°40'S/1089 mm AAR), Sharnae produced higher forage yields in late spring and early summer than Grasslands Maku (Bowman, 1993). In grazing trials in the Grafton hinterland, Sharnae persisted better, spread further and produced higher herbage yields than Grasslands Maku on a dry site. Conversely, on a relatively wetter site, Grasslands Maku outperformed Sharnae (Bowman and Wilson, 1992). On a sandy soil at Grafton in combination with Bahia grass (*Paspalum notatum*), Sharnae persisted for four years. In a kikuyu (*Pennisetum clandestinum*) sward at Wollongbar (28°49'S/1700 mm AAR) under dairy cattle grazing, Sharnae persisted for 3 years. However, at Grafton (29°40'S/1089 mm AAR), Sharnae as a companion legume with kikuyu under dairy cattle grazing, disappeared in one year (Bowman and Wilson, 1992). Sharnae outyielded Grasslands Maku at Wingham (31°53'S/1171 mm AAR) in the first year after planting (Bowman and Wilson, 1992).

There is evidence and industry experience that Grasslands Maku does not persist under the episodic drought conditions that prevail in north coastal New South Wales, whereas Sharnae's greater drought tolerance and higher seed production confers survival through adverse dry periods.

Cool temperate Northern Tablelands (28°55'S - 30°53'S)

The Northern Tablelands is an elevated (750-1400m) medium rainfall (775-1250mm AAR) landscape where the rainfall pattern is summer dominant, low fertility acidic soils predominate and there is a low photoperiod associated with low latitude and frequent cloud cover. Winters are intensely cold and include some 104 frosts over a 200 day frosting interval. The major stresses for pasture plants are summer moisture-stress, close grazing during winter and episodic drought.

In a grazing study (Ayres *et al.*, 2006a) in which Sharnae was compared with Grasslands Maku under a range of grazing management practices (grazing intensity, seasonal rest, spelling interval, grass competition):-

- a) The botanical presence of Grasslands Maku was markedly seasonal with high presence (ca. 40%) in spring but relatively lower presence (ca. 20%) in winter. Sharnae remained at a low (10-20%) but stable level.
- b) Grass competition affected Sharnae and Grasslands Maku differently. Whereas Grasslands Maku was restricted only by strong grass competition the botanical presence of Sharnae was restricted by both strong and weak grass competition
- c) Sharnae showed a positive response to autumn rest from grazing, especially under lax grazing intensity.

In this cool temperate environment, although the current area of usage of greater lotus is relatively small, greater lotus is considered to be an 'alternative legume' to white clover (*Trifolium repens*). In moist valley-floor landscapes and high altitude/high rainfall niche sites, Grasslands Maku becomes the dominant legume, persists into the long term (>5 years), and enhances the utilisation of low quality grasses (Ayres *et al.*, 2006b). Although there is no significant farmer experience with Sharnae in this region, Sharnae has characteristics that might be expected to confer adaptive advantage and lead to increased usage of greater lotus beyond favourable niche sites presently occupied by Grasslands Maku.

Temperate South Coast (32°13'S - 37°04'S)

Rainfall on the South Coast of New South Wales varies from 1,524 mm AAR on the coastal plain and hinterland highlands to 635 mm AAR in inland valleys; most districts receive at least 1,000 mm AAR. Rainfall distribution is slightly seasonal with more rainfall in warm season months than cool season months, and soils are mostly neutral to acidic. Pasture growth is markedly seasonal; low temperature constrains growth in winter and moisture stress limits growth in summer. The role sought for greater lotus in this environment is to provide late summer/autumn feed to offset the decline in growth and quality of white clover-ryegrass pasture (Blumenthal *et al.* 1991, 1993; Blumenthal and Harris, 1991).

In a field characterisation study (Kelman *et al.*, 1992), a collection of 40 accessions of greater lotus were evaluated at two sites (Nowra, 34°54'S/1200 mm AAR; Bowral, 34°28'S/1500 mm AAR). Of these 40 accessions, the New Zealand bred material (Grasslands Maku, G4703, G4704) and a group of Portuguese accessions (including Sharnae) were the most productive. The autumn production of Sharnae was highest at both sites.

Southern and Monaro Tablelands (34°44'S - 36°30'S)

In this 'Mediterranean type' climatic zone where AAR is 450-900 mm, winters are moderately cold and soils are typically acidic. Pasture growth is limited by low temperature in winter and hot/dry conditions in summer. The pasture feed-year comprises a flush of growth in spring, sporadic growth in summer-autumn and low growth in winter.

Sharnae outyielded Grasslands Maku at Tumut (35°18' S/907 mm AAR) in the first year after sowing (Bowman and Wilson, 1992). In an evaluation of *Lotus* accessions in the field at Canberra (35°18'S/632 mm AAR) to identify useful germplasm (Kelman, 1996), highlight results included: -

- a) Grasslands Maku was slightly more productive than Sharnae's Portuguese parent (CPI67677) in terms of early vigour and cool season growth, but produced significantly less herbage in summer
- b) CPI 67677 was taller and thicker in the stem, with longer internodes, but was less rhizomatous than Grassland Maku
- c) Unlike Grasslands Maku which produced flower buds but did not set seed, CPI 67677 set seed and recruited seedlings.

Referring to the Portuguese lines, Kelman (1996) said:

"...while not as productive as New Zealand accessions...these lines are a valuable source of germplasm for breeding cultivars of Lotus pedunculatus which would be better able to set seed over a wide range of latitude in Australia".

The Portuguese lines CPI 67676, CPI 67677 (Sharnae pre-cursor), CPI 67678 and the New Zealand lines G4703 and G4704 were subsequently crossed (Kelman *et al.*, 1992; Blumenthal *et al.*, 1993; Kelman and Bowman, 1996) to produce BL_{polycross} which performance is described in Ayres *et al.* (2007). Although greater lotus breeding work has

occurred at Canberra on the Southern Tablelands, rainfall conditions in this environment are considered to be too marginal for greater lotus to achieve significant commercial usage.

Regional adaptation studies with Sharnae

1. In a field evaluation (Blumenthal *et al.*, 1991; 1999) of Sharnae, Maku and G4704 at 6 high rainfall coastal sites between latitudes 27°30'S to 38°39'S in eastern Australia:

- a) At no site did Sharnae outperform Maku in terms of plant frequency or herbage mass
- b) Sharnae's performance was limited by poor seedling establishment from which it never recovered. It was considered that the small seed of Sharnae places it at a disadvantage in terms of seedling vigour.
- c) In high latitude temperate sites, where moisture conditions were more favourable and there was less C4 grass competition, Maku had an adaptive advantage provided that summer moisture-stress was not too severe.

2. In a study (Ayres *et al.* 2006 a) in 4 regions in New South Wales (subtropical North Coast, temperate South Coast, cool temperate Northern Tablelands, 'Mediterranean' Southern Tablelands) during a sequence of drought years:-

- a) The effectiveness of nodulation was lower for Sharnae compared with Maku at coastal sites (Casino, 28°53'S/1108 mm AAR, Nowra, 34°54'S/1136 mm AAR), and this was accompanied by a corresponding depression in seedling density
- b) At low latitude sites (Casino, 28°53'S/1108 mm AAR; Glen Innes 29°42'S/849 mm AAR), rhizome growth (rhizome number, rhizome extension) was greater for Maku than Sharnae
- c) There was no seedbank development or seedling recruitment for Sharnae (or Maku) in all 4 regions under study. However, Sharnae declined to low botanical presence at all sites in this sequence of dry years, so floristic expression was not reliably evaluated.

Forage quality

The aspect of forage quality that has received most attention in greater lotus has been the presence of condensed tannins (CT). Because of their protein-binding capacity, CT are associated with bloat protection in ruminants and with increased levels of by-pass protein during rumen digestion. However, high concentrations are also reported to affect palatability and reduce forage intake. In a large collection of greater lotus germplasm, relatively high levels of CT (8 %Dwt) were measured in 6 accessions of greater lotus, including Sharnae (Kelman *et al.*, 1997). In the total pool of accessions from the previous study, a significant negative correlation of CT with forage nitrogen and in vitro dry matter digestibility was found (Kelman 2006). Based on the presence of genetic variation for CT content and herbage productivity among accessions in this collection, a breeding and selection program utilising the Portuguese (Sharnae) accessions resulted in the development of new germplasm (LUX97) with lowered CT content (Kelman *et al.*, 2007).

Commercial status of Sharnae

The Sharnae germplasm was recommended for registration and release by the New South Wales Herbage Plant Liaison Committee in 1991 (Wilson, 1992). An application for Australian Plant Breeder Rights (PBR) as applying under the Plant Breeders Rights Act 1994 was accepted in 1993 and PBR was granted in 1995. However, PBR was surrendered in 1999 following the inability of the licensee to meet the terms of the marketing license. The main problem was the ongoing lack of success by the licensee in producing seed in commercial quantities, which in turn was attributed anecdotally to i) lack of harvesting expertise by contracted farmers with an indeterminate seed crop, ii) difficulties with weed control associated with uncertain herbicide tolerance of Sharnae, and iii) a perception of weak seedling vigour associated with low cold-tolerance.

Sharnae currently has no PBR protection. Accordingly, Sharnae is available i) for merit testing under a germplasm agreement with New South Wales Department of Primary Industries, and ii) for further improvement by crossing or reselection in conjunction with New South Wales Department of Primary Industries, as a pre-requisite to obtaining PBR or its equivalent in other countries and commercialisation. Breeder seed is held by New south Wales Department of Primary Industries at Glen Innes 'Centre for Perennial Grazing Systems'.

Acknowledgements

The authors are grateful to John Ryan (New South Wales Department of Primary Industries) for advice on intellectual property, and to Carmen Elvins for preparation of the manuscript.

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Understanding physiological mechanism of *Lotus creticus* plasticity under abiotic stress and in arid climate: a review

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Abstract

Lotus creticus (L.) (Leguminosae, Loteae) is a major pastoral and forage legume in the arid climate where salinity and drought are serious production problems. A review note was carried out to understand the physiological behaviour of this species face to salinity and drought. It has been shown through this note that *Lotus creticus* is fairly tolerant to salt at germination and growth phases. For instance, this taxon is able to support a level of salinity around 300 mM in germinative phase. In the growth phase, salinity affects growth of this plant. The fact that the roots were not affected by NaCl, was explained by a relatively greater proline accumulation during the salts stress. However, some research showed that under 70-140 mM NaCl, *L. creticus* grew even better than the control ones during the first month of growth. This aspect can be observed in halophytic and in some glycophytic succulent plants in which growth is stimulated by low to moderate salinity applied for a short time period. For 400 mM, *L. creticus* is able to produce and to allocate dry matter to the different organs. At higher salinity (140 – 400 mM NaCl), the high absorption and accumulation of ions caused important toxic effects and induced leaf tissue dehydration. The osmotic adjustment mechanism in *Lotus creticus* is a beneficial trait when the plants are treated with moderate levels of salinity (70 –100 mM NaCl). Moreover, the presence of hairy leaves allows keeping almost 81% of sprayed water and absorbing the 9% of the water retained, and decreased the epidermal conductance to water vapour diffusion. Research related to water deficiency showed that drought reduces the aerial part and root growth and leaf area. Moreover, water stress may influence the production of *Lotus* trichome increasing water foliar uptake in arid environmental conditions. The responses of *L. creticus* are hardening and osmotic and transpiration adjustments. An avoidance mechanism, which minimizes water losses when stomata are closed, was then considered. It can be deduced that *L. creticus* is a very useful species for revegetation in restored areas under arid and semi-arid Mediterranean conditions.

Keywords: *Lotus creticus*, Stress, Drought, Salinity, Arid Climate, Tunisia

Introduction

Arid climate is characterized by hot, dry summers and cool, cold winters, which limits the use of different species for soil revegetation (Savé *et al.*, 1999). Therefore, the use of native species for revegetation may be an interesting practice especially in those countries with dry climatic conditions, where salinity and drought are often serious problems because of the poor quality of irrigation water during the dry season (Sánchez-Blanco *et al.*, 1998). Salinity has long been known to influence the distribution of plant nutrients in legumes (Greenway and Munns, 1980). NaCl toxicity, the predominant form of salt in most saline soils, enhances the sodium content and consequently affects the absorption of other mineral elements (Greenway and Munns, 1980). Indeed, high levels of Na inhibit Ca and K absorption, which results in a Na/K antagonism (Rubio *et al.*, 1999). Ashraf and McNeilly (2004) suggested that maintenance of high tissue K/Na ratio as criteria for salt-tolerance. On the other hand, the relationship between salt tolerance and the macronutrient accumulation in vegetative organs of legumes was reported earlier (Cordovilla *et al.*, 1994). Plant species adapt to high salt concentrations in soils by lowering tissue osmotic potential with the accumulation of inorganic as well as organic solutes (Gerard *et al.*, 1991; Le Dily *et al.*, 1991; EL Haddad and O'Leary, 1994; Ullah *et al.*, 1994). Cations Na⁺ and K⁺ are known to be the major inorganic components of the osmotic potential (Asch *et al.*, 1999). Water deficiency is a major limiting factor of plant productivity in many arid regions of the Mediterranean basin (Boyer, 1982). Native species called Mediterranean plants are usually considered more tolerant and adapted to dry conditions and to soil salinity (Caballero and Cid, 1993). *Lotus* is a large (150 spp.), cosmopolitan genus that occupies two major centres of diversity, the Mediterranean region (including portions of Europe, Africa, and western Asia) and western North America (Allan *et al.*, 2004). It is one of about 10 genera within the tribe *Loteae* (Polhill, 1981; Sokoloff, 1998) and is the only genus in the tribe with an intercontinental distribution. Species of the genus *Lotus* are increasingly employed in pastures throughout the world because of their high productivity over a wide range of soils (Blumenthal and McGraw, 1999). There is potential for the use of *Lotus* in relation to both salinity and flooding tolerance. In addition, the interest in *Lotus* over the last decade has increased as greater emphasis is being placed on reducing N and P inputs into farming systems and lowering cattle stocking rates to reduce environmental pollution and land degradation (Blumenthal and McGraw, 1999). *Lotus creticus* is considered a good alternative to traditional covering plants because of its rapid growth and its need for little water (Sánchez-Blanco *et al.*, 1998; Cabot and Pages, 1997). It is an important naturalised legume in arid land of Tunisia. Until now, *Lotus creticus* ecophysiology originating from the Mediterranean countries such as Tunisia has been poorly documented (Rejili *et al.*, 2007). In this review, we have first emphasized arid regions and abiotic stress definitions followed by a description of *Lotus creticus* and its habitat relations. Physiological mechanisms of *Lotus creticus* plasticity under abiotic stress and in arid climate have been then reported and discussed.

Arid regions and arid climates

About one-third of the land area of the world comprises arid and semiarid climates (Johnson *et al.*, 1981). Arid desert soils were previously considered economically unimportant; however, during the past three decades, the economic and agricultural utilization of arid lands has emerged as a critical element in maintaining and improving the world's food supply (Skujins, 1984). In Tunisia, arid and semi-arid climate covers more than 3/4 of the total area of the country. Tunisia, located at the north of the 30th parallel, occupies the north part of the African continent, and its total area is a more than 164.000 km². The arid climate of Tunisia is characterized by high temperature, low relative humidity, high evaporation, and scanty rainfall. The desert lands also include saline areas; saline lands represent about 15% of the arid and semiarid lands of the world (Serrano and Gaxiola, 1994; Zahran, 1997). In saline areas, evaporation greatly exceeds precipitation, and soil salination may increase to a sufficient degree to eliminate most plants from these habitats (Batanouny, 1979; Zahran, 1997). Saline lands, like arid lands, have been largely ignored and are usually considered to be abandoned, non productive lands. Desert ecosystems are characterized by a lack of moisture and nitrogen, but drought and salt stresses are probably the most important environmental factors that inhibit the growth of organisms in arid and semiarid regions.

What is stress?

In physical terms, stress is defined as mechanical force per unit area applied to an object (Mahajan and Tuteja, 2005). In response to the applied stress, an object undergoes a change in the dimension, which is also known as strain. As plants are sessile, it is tough to measure the exact force exerted by stresses and therefore in biological terms it is difficult to define stress (Mahajan and Tuteja, 2005). A biological condition, which may be stress for one plant may be optimum for another plant. The most practical definition of a biological stress is an adverse force or a condition, which inhibits the normal functioning and well-being of a biological system such as plants (Jones and Jones, 1989). Drought and salt stresses are among the major stresses, which adversely affect plants growth and productivity.

Relation of *Lotus creticus* growth to different environmental factors

The genus *Lotus* L. contains approximately 100 species (Gunn, 1983; Polhill, 1994), distributed throughout of the World. It includes annual and perennial plants with strong branched taproots (MacDonald, 1946). The genus includes plant species adapted to an ample range of habitats from marine environments to high altitudes, from sandy soils to heavy saline soils (Heyn and Herrstadt, 1967; Heyn, 1970; Montes, 1988; Small, 1989). *Lotus creticus* has a definite capability to adapt to water stress but has specific requirements for water depth and flow rate of the current. These requirements, however, vary with different varieties. The requirement for water quality is not too strict; however, the discharge of phytotoxic chemicals from certain chemical industries is detrimental. Regions that once had an abundance of *L. creticus* plantings have been found to have no

Lotus due to discharges of toxic waste. *L. creticus* can adapt to most soil as long as there is no hardpan. It can grow at pH 5.6 to 7.5 but the optimum is at pH 6.5 (Charlton, 1973; William, 1988). *L. creticus* is a light loving plant and grows best when it is not shaded. During the *Lotus* growing period (April-August), average sunlight is between 4.6-9.0 hours per day. When the sunlight is abundant it also increases the temperature which favours the *Lotus* growth. Optimal temperature of *L. creticus* growth is between 20 and 30°C (William, 1988; Neffati, 1994) with water temperature at 21-25°C. During the early planting time the temperature should be at least above 15°C, otherwise both the seed germination and seedling growth are hindered, resulting in some decaying. The growth of the seedling then increases with the temperature. By June, when the temperature during the day reaches 30°C, the growth is the fastest (Charlton, 1973; William, 1988). Growth is slowed when air temperature is near 40°C. During the seed maturing of *Lotus creticus*, high day temperature and lower night temperatures are optimal (Charlton, 1973; William, 1988). Strong winds can break leaf and flower stalks. Broken leaf stems may allow rain water to enter the underground stem and cause decay. Rain water in the flowers spoils the flower appearance and hinders pollination. Young seedling may be affected by blue mould; buds or small leaves with mold on them may influence growth and photosynthesis. Certain underwater plant feeders may devour some buds. When the plants mature the flower and leaf stalks have some protective sharp protrusions to discourage them (Hovland *et al.*, 1982 *in*, William, 1988).

Response of *Lotus creticus* to drought condition

In general, the plant growth under water stress is reduced, nevertheless, it is known that plants have a suite of morphological and physiological adaptations that allow them to survive water stress and the degree of adaptation to drought may vary considerably between species. Bañón *et al.* (2004) showed that drought promoted significant differences in *L. creticus* stressed plants, reducing the aerial part and root. The reduction in leaf area occurred by effect of irrigation deficit and by high temperature in well-watered soil conditions. It has been considered an avoidance mechanism, which permits minimising water losses when stomata are closed (Blum, 1997). On the other hand, the degree of osmotic adjustment reached by the stressed plants (0.15MPa) was limited and it was insufficient to prevent a turgor pressure decrease and growth reduction. Grammatikopoulos and Manetas (1994) and Morales *et al.* (2000) have suggested that the leaf hairs of Mediterranean species may improve leaf water status by entrapping and retaining surface water, thus, assisting in its final absorption into the mesophyll, or reducing water loss by increasing the resistance of the boundary layer. The response observed in *L. creticus* plants can be considered as an advantageous feature that helps to improve leaf water status (Blum, 1997). Sánchez-Blanco *et al.* (1998), Savé *et al.* (1999), Morales *et al.* (2000), Franco *et al.* (2002) and Vignolio *et al.* (2005) showed that in water stress, the responses of *L. creticus* are hardening and osmotic and transpiration adjustments. Several authors reported the influence of the water deficit on photosynthesis (Flexas *et al.*, 1998; Sánchez-Blanco *et al.*, 2004; Chaves and Oliveira, 2004; Lizana *et al.*, 2006; Tambussi *et al.*, 2007; Yu *et al.*, 2007). Jaballah (2007) showed that the water deficit affects the rate of assimilation A in *L. creticus*. This result is in agreement with several reports on reduction

by water stress of the assimilation A rate (Bloch *et al.*, 2006) resulting from a reduction in the stomatic conductance (Chaves, 1991; Yordanov *et al.*, 2003; Campos *et al.*, 1999; Cornic, 2000) which limit the carbon diffusion by leaves. Moreover, water stress may influence in the production of *Lotus* trichome (Quarrie and Jones, 1977) increasing water foliar uptake in arid environmental conditions. Bañon *et al.* (2004) suggested that *L. creticus* plants exposed to dry soil conditions and low humidity would have more stomata than plants grown in the opposite conditions. In addition, the stomatal density of *L. creticus* plants decreased significantly with temperature on the abaxial surface in deficit irrigation conditions (Bañon *et al.*, 2004), but there was no effect on the adaxial surface. Sharpe (1973) reported that the adaxial and abaxial stomata differ in their responses to light, ambient temperature and water stress in cotton. According to Cihra and Brun (1975), the differences related to stomatal density observed between adaxial and abaxial surfaces can be a function of leaf expansion. This effect could also be related to leaf movements in relation to environmental conditions that show these plants (higher paraheliotropism; Palmer, 1985), which can be associated with greater drought avoidance (Savé *et al.*, 2000). It is clear that frequency and size of stomata vary as a function of leaf position and growth conditions (Jones, 1992). On the other hand, although the processes that regulate root water uptake are complex, it is clear that root anatomy and structure play an important role (Steudle and Peterson, 1998).

One indicator of the plant capacity to absorb and transport water is the density of the xylem vessels and tracheids in a cross-section of the stem or roots. The parameter vessels density provides an estimate of the mean diameter of these components of the xylem, a factor which is strongly related to water conductivity (Jones, 1992). In this sense, the vessels and tracheids developed in the seedlings in nursery conditions improve resistance of *L. creticus* plant to water deficit situations when plants grew in field conditions after transplanting. Franco *et al.* (2002) studied the influence of two irrigation treatments during nursery production on the post-transplant development of *L. creticus*. Their results showed that, during 96 days with irrigating 2 days/week with a total of 2.3 L of water per plant over the whole nursery period, plants had greater rootlength: shoot length ratio and higher percentage of brown roots, an indicator of more resistance to post-transplant stress. Similar results are obtained by Franco *et al.* (2002) in *Lotus* stressed plants after transplanting. In conclusion, *Lotus creticus* showed rather different adaptation responses to water deficit. An avoidance mechanism, which minimises water losses when stomata are closed, was deduced by reducing the transpiration rate resulting from a reduction in the stomatic conductance g_s . This knowledge can be used for improvement of cultivars and cultural practices for *Lotus* species especially in conditions where water deficit are features of the growing season.

Response of *Lotus creticus* to salt stress

Salinity is a serious threat to agriculture in arid and semiarid regions (Rao and Sharma, 1995). Nearly 40% of the world's land surface can be categorized as having potential salinity problems (Clemens *et al.*, 1983); most of these areas are confined to the tropics and Mediterranean regions. Increases in the salinity of soils or water supplies used for

irrigation result in decreased productivity of most crop plants and lead to marked changes in the growth pattern of plants (Clemens *et al.*, 1983). Increasing salt concentrations may have a detrimental effect on soil microbial populations as a result of direct toxicity as well as through osmotic stress (Tate, 1995). Soil infertility in arid zones is often due to the presence of large quantities of salt, and the introduction of plants capable of surviving under these conditions (salt-tolerant plants) is worth investigating (Delgado *et al.*, 1994). There is currently a need to develop highly salt-tolerant crops to recycle agricultural drainage waters, which are literally rivers of contaminated water that are generated in arid-zone irrigation districts (Glenn *et al.*, 1999). Salt tolerance in plants is a complex phenomenon that involves morphological and developmental changes as well as physiological and biochemical processes. Salinity decreases plant growth and yield, depending upon the plant species, salinity levels, and ionic composition of the salts (Delgado *et al.*, 1994). As with most cultivated crops, the salinity response of legumes varies greatly and depends on such factors as climatic conditions, soil properties, and the stage of growth (Cordovilla *et al.*, 1995a; Cordovilla *et al.*, 1995b; Cordovilla *et al.*, 1995c). Variability in salt tolerance among crop legumes has been reported (Zahran, 1991a; Zahran, 1991b).

Lotus creticus is cultivated in many countries; it is widely grown in arid and semi-arid region where soils contain high levels of salts. However, salt affected soils can be utilized by growing salt tolerant crops because such crops would allow expansion of crop production to areas where conventional reclamation procedures are economically or technically limited. In earlier report, we have shown that *L. creticus* is able to support a level of salinity around 300 mM in germinative phase (Rejili *et al.*, 2006). In 2007, Rejili *et al.* showed that salinity affected both biomass production and plant development. Concerning biomass production, Rejili *et al.* (2006) confirmed that dry matter of the aerial organs was significantly affected by NaCl levels exceeding the 100 mM. Le Hou  rou (1986) showed that *L. creticus* was able to support 100 mM of NaCl concentrations. S  nchez-Blanco *et al.* (1998) showed that the young plants treated with 70 and 140 mM NaCl grew even better than the control ones during the first month and the toxic effects of the Cl⁻ and Na⁺ appeared after a longer period of salt stress. This aspect can be observed in halophytic and in some glycophytic succulent plants in which growth is stimulated by low to moderate salinities applied for a short time period (Gorham, 1996).

The effect of salinity on *L. creticus* biomass depends on plant size and its relative average growth (RAG) (Rejili *et al.*, 2007a). The depressive action of salt on growth materializes in a significant reduction of the aerial organ growth activity (Rejili *et al.*, 2007a). For instance, shoots were more affected than stems for two different populations. Compared to the aerial organs, the roots dry matter was not affected by salt stress. The fact that the roots were not affected by NaCl is in accordance with the results obtained in many studies (Kumar and Bharadwaj, 1981; khavari-Nejad and Najafi, 1990; Munns and Termaat, 1986; Niemann *et al.*, 1988). This behaviour has been explained by a relatively greater proline accumulation in roots than in shoots during the salts stress. Proline plays an important role in the cellular osmoregulation and acts as a reserve of nitrogen to sustain root growth (Kalaji and Pietkiewicz, 1993; Misra *et al.*, 1996; Morales *et al.*, 2000).

Several authors suggested that, under saline stress, the osmotic effect is responsible for the aerial organ growth reduction (Munns and Termaat, 1986; Yeo *et al.*, 1991; Rengel, 1992). The response observed in *L. creticus* plants can be considered as an advantageous feature that helps to improve leaf water status under salt stress (Rejili *et al.*, 2007).

Plants exposed to saline stress were prone to an osmotic stress and to specific toxicity effects of Na⁺ and Cl⁻ ions (Bernstein and Hoyward, 1958; Shannon, 1984; Ayer and Westcot, 1985; Hajji *et al.*, 1999). Flowers *et al.* (1977) summarized the depressive effect of salinity on the growth by a nutritional and/or hydrous imbalance. The significant correlation between the aerial biomass production and its Na⁺ content suggest that, for *L. creticus*, the growth decrease was due to the ionic toxicity (Rejili *et al.*, 2007).

Generally, the most salt tolerant plants accumulate Na⁺ in their shoots whereas sensitive plants do not. In the first type, called "Includers", salt was trapped and accumulated in the aerial organs cells, mainly in its vacuoles (Yeo and Flowers, 1986; Levigneron *et al.*, 1995). In the second type, "Excluders", the salt conveyed to the shoots, fault to be trapped, was re-exported towards the roots by the phloemic tissue (Lessani and Marschner, 1978; Wieneke and Läuchli, 1980; Slama, 1982; Fortmeir and Schubert, 1995). Sánchez-blanco *et al.* (1998) and Rejili *et al.* (2007a) showed that *L. creticus* plants accumulated Na⁺ ions in its photosynthetic organs. The higher Na⁺ and Cl⁻ accumulation in leaves of treated plants and the absence of accumulation of amino acids and soluble sugars by saline effects indicated that the osmotic adjustment had been achieved by the elements provided in the saline water as Gibbs *et al.*, (1989) and Alarcón *et al.* (1994) reported. The osmotic adjustment by salt accumulation is less energy and carbon demanding than adjustment by organic solutes (Wyn Jones, 1981). For this reason, the capacity to include salts is considered a salt tolerance trait, when it is accompanied by the ability of plants to compartment NaCl in the vacuole, thus protecting salt-sensitive enzymes in the cytoplasm (Flowers *et al.*, 1977; Alarcón *et al.*, 1994; Wyn Jones and Pollard, 1983). Apparently, *L. creticus* treated with 140 mM NaCl is unable to sequester ions efficiently and the salts were accumulated leading to inhibition of growth (Sánchez-Blanco *et al.*, 1998).

The maintenance of suitable potassic nutrition to support growth of different organs requires a good selectivity, in the aerial organs, of K⁺ absorption, accumulation and transport compared to Na⁺. Many studies on halophytes and on some tolerant glycophytes plants showed that a high foliar K⁺/Na⁺ ratio is a salt tolerance criterion (Gorham *et al.*, 1990; Schachtman *et al.*, 1991; Wolf *et al.*, 1991; Yeo, 1998). Rejili *et al.*, (2007) showed that *L. creticus* is strongly selective for K⁺ ions. It is known that the capacity of plants to counteract salinity stress strongly depends on the status of their K⁺ nutrition. Increasing the K⁺ supply in the root environment may mitigate the reduction of plant biomass due to an increase in salinity (Chow and Tsang, 1990; Delgado and Sánchez-Raya, 1999). Potassium starvation regularly accompanies sodium toxicity (Flowers and Läuchli, 1983), and Peng *et al.*, (2004) have shown that the decline of salt tolerance under low-K⁺ conditions might have resulted from increased Na⁺ entrance through the high affinity K⁺ system.

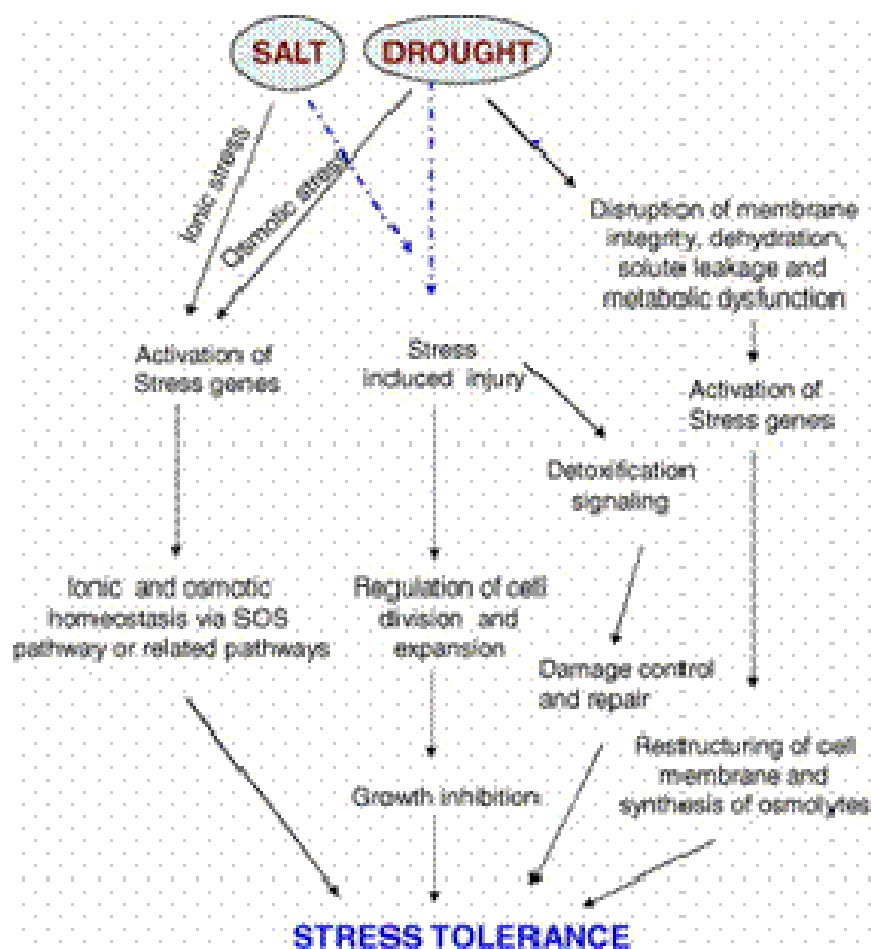


Figure 1. Generic pathway under salt and drought stress (Mahajan and Tuteja, 2005).

In conclusion, the present review has shown that *Lotus creticus* has a great resprouting capability and important growth rates under drought and salinity conditions. It can be deduced that this plant is a very useful species for revegetation in restored areas. Some effort must be conducted in improve its growth patterns under minimum irrigation in arid and semi-arid Mediterranean conditions. It is now well known that the stress signal is first perceived at the membrane level by the receptors and then transduced in the cell to switch on the stress responsive genes for mediating stress tolerance. Understanding the mechanism of stress tolerance along with a plethora of genes involved in stress signaling network is important for crop improvement. Recently, some genes of calcium-signaling and nucleic acid pathways have been reported to be up-regulated in response to both cold and salinity stresses indicating the presence of cross talk between these pathways (Figure 1). Salt and drought disrupt the ionic and osmotic equilibrium of the cell resulting in a stress condition. This triggers the process, which functions to reinstate ionic and osmotic homeostasis leading to stress tolerance. Stress imposes injury on cellular physiology and result in metabolic dysfunction. This injury imposes a negative influence on cell division and growth of a plant. This is an indirect advantage to the plant as reduction of leaf expansion reduces the surface area of leaves exposed for transpiration and thereby

reducing water loss. Stress injury and ROS generated in response to stress also triggers a detoxification signaling by activating genes responsible for damage control and repair mechanism therefore leading to stress tolerance. Moreover, other physiological factors such as rate of photosynthesis, protein assembly and general metabolic processes are severely hampered.

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