PHYSIOLOGICAL RESPONSE TO WATER AND SALT STRESS OF SOME WHITE LUPINE CULTIVARS (*LUPINUS ALBUS* L.)

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Abstract

Currently in Europe is a tendency to counteract some negative effects caused by intensive agriculture. One of these effects, genetic erosion, is determined by the existence in the culture of a small number of species and, within them, a relatively small number of cultivars. White lupine, by its traits (high quality protein content, ability to fix atmospheric nitrogen and to use low-phosphorus soils), could become very interesting species for farmers. The climate and edaphic changes, characterized by extreme temperatures, drought and saline soils represent a challenge for white lupine in extending surfaces.

In this study, we observed the reaction of 34 white lupine cultivars to drought and soil salinity. Plants were grown in vegetation pots (type Mitscherlich) until flowering. Regarding drought resistance, quantified by the amount of biomass accumulation, stomatitis conductance and assimilatory pigments concentration in green house conditionswere measured. Increased resistance to drought was found in the romanian cultivars Satu Mare and Bihor. From the foreigner, P12159, Lublanc and Družba showed also resistance to dryness.

NaCl excess in soil reduces vegetative growth because of lowering the amount of accumulated biomass and decreasing the chlorophyll concentration. Most of cultivars showed toxicity symptoms characterized by leaf chlorosis and necrosis.

Key words: *Lupinus albus* L., water and salt stress.

The genus *Lupinus* is large and complex, with over 200 species growing in a range of climatic and soil conditions. Several species are cultivated, and the grain is used as human food or animal feed.

Drought stress is an additional constraint to production of white lupin. Although Rodrigues et al. (1995) and Chaves (1994) evidenced some genetic variation for drought tolerance in this crop, the optimum strategy would be to escape drought, i.e. lupins should complete their growth cycle before severe water deficits occur. The more critical stages are at flowering where drought stress may lead to heavy flower abortion, and during pod growth. Escaping drought implies choosing between spring-sown and autumn-sown types and within each type to select for earliness in flowering. For the Western European countries, the selection of autumn-sown early flowering material allows the plants to set and feed the pods before severe water deficit and reduction of the photosynthetic activity occurs (Huyghe, 1997, Pinheriro, 2004). However, because of the duration of pod growth, white lupin, even with the best suited genotypes, often suffers from temporary water stress during pod growth and seed filling. Previous work in Lupinus albus L. by several groups, including our own, indicate that this species are mainly drought avoiders (Withers, 1979; Rodrigues et al., 1989; Chaves et al., 1991) and that its growth is severely inhibited at temperatures above 25 °C (Rodrigues et al., 1995). Drought avoidance includes stomata highly sensitive to water deficits and the shedding of old leaves as drought becomes more intense.

Dehydration avoidance characteristics were also reported in other lupin species, such as in *Lupinus angustifolius* L. by Henson and Turner (1989a) and in *L. cosentinii* by Henson et al. (1989b) as well as in annual or perennials legumes (Ludlow, 1989).

Another stress for the plants is the salt stress.

Soil salinity is a major limiting factor of agricultural productivity in many areas of the world. Legumes are highly sensitive to salinity (Maas and Hoffmann, 1977). Salinity causes two different types of stress. Osmotic stress resulting from the high solute concentrations and low soil water potential primarily limits plant growth during the first phase of salt stress (Munns, 1993; Sümer et al., 2004). Salt-stressed plants rarely suffer from wilting and turgor is not affected (De Costa et al., 2007). In a second phase of salt stress, ions accumulate in the plants and may reach toxic concentrations. Usually, sodium (Na^+) and chloride (Cl^-) are the predominant ions in saline soils. Both,

Na^+ and Cl^- may exhibit detrimental effects on plant metabolism and may cause growth inhibition of salt-sensitive species (Helal and Mengel, 1983; Marschner, 1986).

The aims of this study were: i) to track the physiological response of lupine plant to drought and salinity; ii) to characterize these cultivars on resistance to drought and salinity in order to further improvement in plant breeding.

MATERIAL AND METHOD

To examine the effects of drought and salt stress of *Lupinus albus* L. plants of 14 white lupine cultivars from different habitats were cultivated in two experiments, in 6 kg pots with chernozem cambic soil, in controlled condition. Each pot had three plants. Cultivars studied: Družba, Bihar, Kiew, Leblanc, Medi, Multolupa, Neutral, Neuland, Horizon, P12143, P12159, Satu Mare, Somes, Suislupine.

Water stress: water deficit was induced in a half of the pots. After the appearance of the first fully developed leaves, humidity was maintained by daily weighing and watering to 40% of field capacity, in comparison with the control plants (70% of field capacity). Plants were harvested after four weeks of water stress.

*Salt stress***:** was induced by adding 2.5 g NaCl per kg soil, to obtain an electrical conductivity of 10 dS m^{-1}**.** Plants were harvested after 12 days of salt treatment, and after the appearance of visible symptoms.

Further investigations: *Stomatal conductance* was measured twice a day (09.00 and 15.00 h). Measurements were carried out on the most recent fully expanded leaves and at least three individual plants were used for each data point.

The loss of water at leaf level was estimated based on the *dehydration rhythm* of the leaves by means of weighing to the precision balance respecting intervals of 1, 2, 3, 4 and 24 hours respectively (Toma et al., 1999).

The *chlorophyll concentration* was determined spectrophotometrically in acetone extract (Zörb et al.): Five grams of leaf fresh weight were homogenized in 80% acetone plus 0.1 g $MgCO_3$ using an Ultra-Turrax apparatus (20.000 U min^{-1}). The resulting suspension was immediately filtered using a Büchner funnel with fiberglass filter (Schleicher and Schuell). The chlorophyll concentration of the acetone filtrate was determined by measuring the absorption at 664 nm (chlorophyll a) and 647 nm (chlorophyll b).

Dry weight content was determined by weighing after dried plant material at 105°C to constant weight.

RESULTS AND DISCUSSIONS

Drought stress

Plants biomass

Until harvest, plants have accumulated between 12-20 g dry matter per pot (three plants).

The lowest values were recorded in cultivars Suislupine and Neuland (17 g) and the highest in Multolupa 21 g (*fig. 1*).

By water stress exposed plants, the dry matter ranged between 10-15g. The loss from dry weight, compared with the control plants was between 16.7 and 50%, smallest differences occurring in cultivars: Bihor, Družba, Lublanc, P12159 and Satmarean.

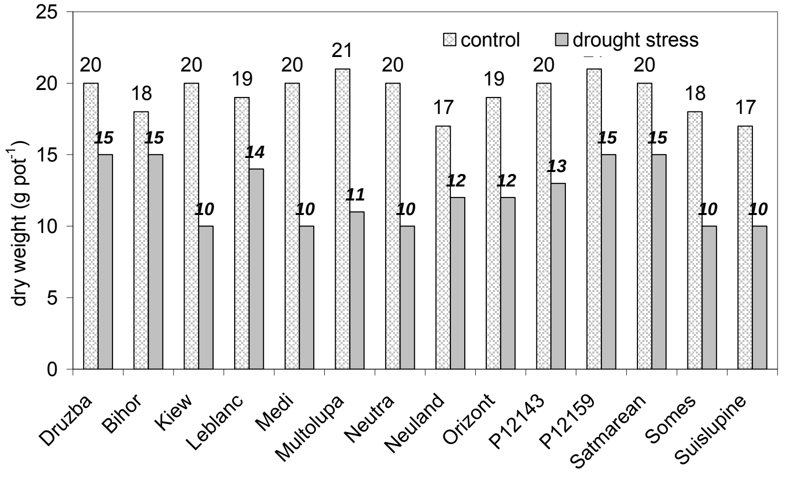

Figure 1 **Effect of drought stress on dry weight of *Lupinus albus* plants**

Stomatal conductance

It was found that the maximal stomatal conductance (morning values) started to decrease by the 6th day of the drought period, and the minimal (midday value) decreased after only two days of water stress. Similar results were obtained by Rodrigues et al. [1995], by other three lupine cultivars. The highest values of stomatal conductance were registered of Bihar, Družba, Lublanc, P12159 and Sătmărean cultivars.

Generally, except cultivar Neuland, reduced vegetative mass (*fig. 2),* is positively correlated with a decrease in stomatal conductance (*fig. 3*).

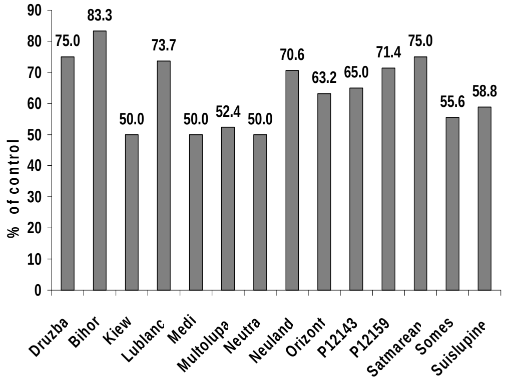

Figure 2 **Decreas in biomass in consequence of water stress**

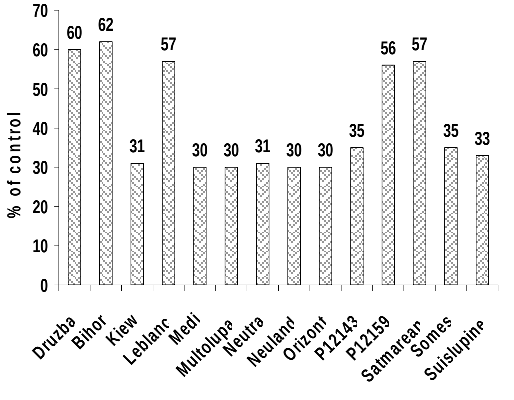

Figure 3 **Effect of water stress on stomatal conductance of white lupine cultivars**

Fig 4a **Salt stress effects of white lupine plants**

Fig. 4b **Salt stress effects of *Vicia faba* plants**

SALINITY

Toxicity symptoms

Ion toxicity was exclusively determined in the second phase of salt stress (Munns, 1993). Toxicity symptoms appeared as black necrotic spots on leaves with a sharp differentiation between green and necrotic tissue. First leaf injury appeared after 14 days of plant emergence. NaCl-treated plants showed also mild chlorotic symptoms predominantly starting on old leaves (Fig 4a). This phenomenon usually takes place in the case of amassment in excess of the sodium ions. At the level of the stomata cells, sodium blocks the potassium channels, thus preventing further the stomata movements (Bhandal et al. 1988, Maathius et al., 1999, Tester et al. 2003). The phenomenon could be observed to *Vicia faba* L. (Fig. 4b). (Slabu et al., 2009a) and *Tilia cordata* Mill. (Slabu et al., 2009b) plants exposed to the excess of NaCl.

The dehydration rhythm of the leaves

As a result of the repeated weighing of the leaves, it was found that, no matter the moment of determination, water losses to leaves with necrosis were significantly higher to the control leaves, which may prove that in this case the opening – closing mechanism of the stomata is damaged.

Chlorophyll concentration

The chlorophyll concentration decrease in the salt stressed plant compared to control plants. Reduced chlorophyll concentration as a result of a NaCl treatment was also previously described by Lutts et al. (1996) for rice, and by Slabu et al. (2009) for *Vicia faba* L. The reduction of chlorophyll in leaves after NaCl exposure may be explained as a result of high Cl^- concentrations in the chloroplasts, which is amplified by a simultaneously high Na^+ concentration.

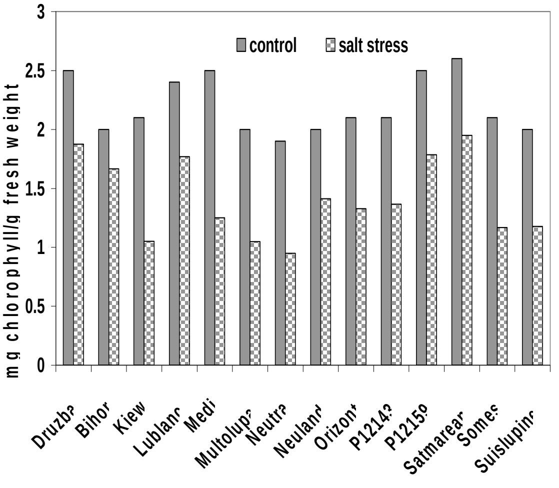

Figure 5 **Salt stress effect of the chlorphyll concentration**

Plant biomass and stomatal conductance

Under salt stress conditions plants accumulated less biomass compared to control. (*figure 6*) As in the case of water stress, the most resistant cultivars proved to be Bihor, Družba, Leblanc, P12159 and Satmarean. Also, stomatal conductance values decreased with 30-50% compared with control (*figure 7*). There is no correlation between them, and the reduction of plant vegetative mass.

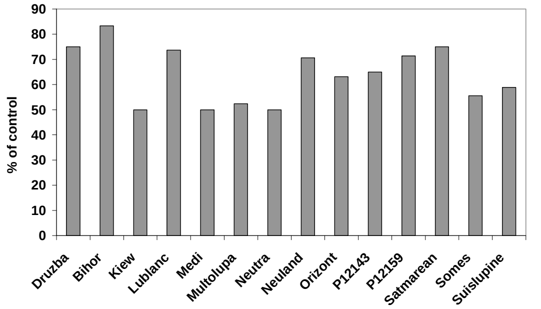

Figure 6 **Loss of dry weight content due to salt stress**

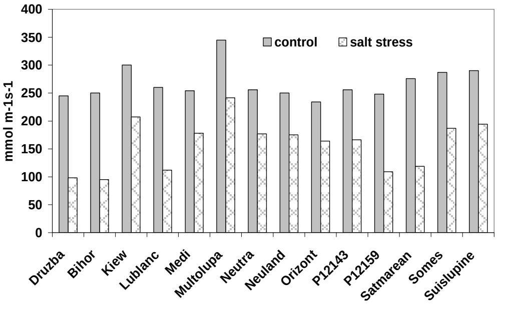

Figure 7 **Effect of salt stress on stomatal conductance**

CONCLUSIONS

Analyzed lupine cultivars respond to drought stress, by early stomatal closure follwed by a decrease of plants dry weight, the smallest reductions were recorded in cultivars: Bihor Družba, Leblanc, P12159 and Satmarean.

Under the saline stress, plants respond by chlorosis, caused by decreased of chlorophyll concentrations, followed by the appearance of point necrosis due to the toxic effect of sodium and chloride ions and decreased in biomass accumulation.

The more resistant to salinity, were found, as in the case of the drought resistance, cultivars: Bihor, Družba, Leblanc, P12159 and Satmarean.

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BIBLIOGRAPHY

Bhandal, I.S., Malik, C.P., 1988 - *Potassium estimation, uptake, and its role in the physiology and metabolism of flowering plants*, Int. Rev. Cytol. 110, p. 205–254.

Chaves, M.M., Rodrigues, M.L., Martins, J.M.N., 1991 - *Carbon and water economy of Lupinus albus* under drought c onditions, In: von Baer D, ed. *Proceedings of the 6th International Lupin Conference,* Temuco-Pucon, Chile, p. 262-266.

De Costa, J., Zörb, C., Hartung, C. W., Schubert, S., **2007** - *Salt resistance is determined by osmotic adjustment and abscisic acid in newly developed maize hybrids in the first phaseof salt stress*, Physiol. Plant. 131, p. 311-321.

Helal, H.M., Mengel, K., 1981 - *Interaction between light intensity and NaCl salinity and their effects on growth, CO_2 assimilation, and photosynthate conversion in young broad beans,* Plant Physiol. 67, p. 999-1002.

Henson, I.E., Jensen, C.R., Turner, N.C. 1989a. - *Leaf gas exchange and water relations of lupins and wheat. I. Shoot responses tosoil water deficits*, Australian Journal of Plant Physiology, 16 p. 401-413.

Henson, I.E., Jensen, C.R., Turner, N.C., 1989b. - *Leaf gas exchange and water relations of lupins and wheat.* III. Abscisic acidand drought-induced stomatal closure, *Australian Journal of Plant Physiology* 16, p. 429-242.

Huyghe, Ch., 1997 - *White lupin* (*Lupinus albus* L.), Field Crops Research 53, p.147-160.

Ludlow, M.M., 1989 - *Strategies of response to water stress.* In: Kreeb KH, Richter H, Hinckley TM, eds. *Structural and functional responses to environmental stresses,* SPB Academic Publishing, The Hague, The Netherlands, p. 269-281.

Lutts, S., Kinet, J.M., Bouharmont, J., 1996 - *NaCl-induced senescence in leaves of rice (Oryza sativa L.) cultivars differing in salinity resistance,* Ann. Bot. 78, p. 389–398.

Maathuis, F.J.M., Amtmann, A., 1999 - *K^+ nutrition and Na^+ toxicity: the basis of cellular K^+/Na^+ ratios*, Ann. Bot. 84, p. 123–133.

Marschner, H., 1995 - *Mineral Nutrition of Higer Plants*. 2. Aufl., Academic Press, London, p 254-260.

Munns, R., 1993 - *Physiological processes limiting plant growth in saline soils: Some dogmas and hypotheses,* Plant Cell Environ. 16, p.15-24.

Pinheriro, C., Passarinho, J.A., Ricardo, C.P., 2004 - *Effect of drought and rewatering on the metabolism of Lupinus albus organs,* Journal of Plant Physiology,161, p.1203-1210.

Rodrigues, M.L., Pacheco, C.M.A., Chaves, M.M., 1989 - *Carbon and water economy of two lupine linies unter induced stress,* The Hague, The Neterlands: SPB Academic Publising, p. 219-226.

Rodrigues, M.L., Pacheco, C.M.A., Chaves, M.M., 1995 - *Soil-plant water relations, root distribution and biomass partitioning in* Lupinus albus L. *under drought conditions*, Journal of Experimental Botany, Vol. 46, No. 289, p. 947-956.

Slabu, Cristina, Zörb, C., Steffens, D., Schubert, S., 2009a - *Is salt stress fo faba bean (Vicia faba L.) caused by Na^+ or Cl^- toxicty?,* J. Plant Nutr. Soil Sci. 172, p. 644-650.

Slabu, Cristina, Jitareanu, Carmen-Doina, Toma Liana-Doina, Robu, T., Marta, Alina-Elena, Radu, Mirela, 2009b - *Ecological impact of de-icing salt on Tilia cordata Mill. plants from roadside environment,* Lucr. şt., seria Agronomie, vol. 51, U.Ş.A.M.V. Iaşi, p. 40-44.

Sümer, A., Zörb, C., Yan, F., Schubert, S., 2004 - *Evidence of Sodium Toxicity for the Vegetative Growth of Maize (Zea mays L.) During the First Phase of Salt Stress.* - J. Appl. Bot.78, p. 135-139.

Tester, M., Davenport, R., 2003 - *Na^+ tolerance and Na^+ transport in higher plants*, Ann. Bot. 91, p. 503-527.

Toma, Liana-Doina, Milica C., Robu T., Jitareanu, Carmen-Doina, 1999 – *Fiziologie vegetală – Indrumator de laborator*, Edit. Ion Ionescu de la Brad, Iasi, p. 43 - 44.

Withers, N.J., 1979 - *Effects of water stress on* Lupinus albus. *L. Response of vegetative growth to water stress during a single growth stage at two humidity levels,* New Zealand Journal of Agricultural Research 22, p. 445-454.

Zörb, C., Wiese, J., Wiese, H., Krämer, C., Yan, F., Mühling K.H., Schubert, S., 2004 - *Biochemische Praktikumsversuche*. Verlag Grauer, Beuern-Stuttgart, p. 40-45.