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Effects of salinity and basalt powder interaction on seedlings of a white Lupin variety

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Abstract

The alleviation of abiotic stresses such as salinity, is one of the most important challenges to respond to the increased food demand of the growing world population and to ensure sustainable agriculture.

This work aimed to investigate the interaction effects of sodium chloride (NaCl) and basalt powder “Farina di Basalto®” (FdB) on germination and early seedling growth parameters of *Lupinus albus* L. Combined treatments were evaluated, by applying four NaCl concentrations (0, 75, 150, 300 mM) on lupine seeds that were previously coated by five FdB levels (0%, 1%, 3%,5% et 10%).

Analysis of variance showed that salinity had a significant negative effect on germination percentage and the studied parameters during the juvenile stage ($p<0.05$). This effect depended on the different NaCl levels compared to the control. The Basalt treatment showed a positive effect on growth parameters. Results revealed that in comparison with control, different concentrations of FdB stimulated hypocotyl elongation to reach the higher increase by 131% under 150 mM NaCl with 10% of FdB. In addition, Basalt in combination with salt stress have a significant effect on the salinity tolerance index and revealed the best results in salinity resistance in the presence of 10% of Basalt Powder.

Keywords: Salt stress, “Farina di Basalto→□, germination, early seedling, *Lupinus albus* L

Introduction

White lupin (*Lupinus albus* L.) belongs to the Fabaceae family. It is cultivated for human consumption, green manuring and as forage. This grain legume has Mediterranean origins, where drought, salinity and mineral deficiency are among the major constraints for lupines production [1]. Hence the interest of evaluating salt stress tolerance of white lupine to develop this culture in different regions threatened by soil salinization.

An increase in the salinity concentration in the soil solution leads to an increase in osmotic pressure, which necessitates an increased effort of the plants to absorb the nutrients, which negatively affected their growth and production [2, 3].

Plants are able to detoxify excessive oxidation caused by salt-stress conditions by complex enzymatic and nonenzymatic mechanisms that protect plant cells against oxidative damage [4].

The basalt is a tuff originates from volcanic projections [5]. It is sometimes observed as blocks or even ashes with black, red or even dark green color. This volcanic igneous rock is generally employed in different areas such as construction, industrial engineering and agronomy [6, 7]. In fact, the basalt is known as a natural fertilizer used to improve soil chemical proprieties [8]. This rock contains many chemical components required in agronomy and soil fertilization such as SiO_2 (37.76 to 59.64%), Al2O3 (11.77 to 14.32%), CaO (5.57 to 14.75%), MgO (5.37 to 9.15%), Fe_2O_3 (10.1 to 20.93%), K_2O (1.7 to 6.69%), Na_2O (1.4 to 3.34%) and TiO_2 (1.81 to 3.73%) [8].

This mineral used to restore soils fertility, has also shown a positive effect on plant growth, total yield and fruits quality [9, 10]. Besides, basalt was shown in many studies to confer resistance against pests and diseases to several crops, by stimulating their natural defence reactions in fields and even under storage conditions [8, 11, 12].

Furthermore, an alternative strategy to decrease the oxidative damage on plants is to use silicon (Si) fertilization, the principal component of Basalt powder, in plants subjected to environmental stresses [13]. Therefore, Si has been shown to be able to improve early growth and establishment of plants under stressful conditions through increasing antioxidant enzymes, photosynthetic capacity and low transpiration coefficient [14, 15]. Which incites studying the effect of Basalt powder on improving cultivated species tolerance to salt stress.

This study aims to evaluate the capacity of "Farina di Basalto®" to alleviate salinity effects on a white lupine variety, at the germination phase and early growth seedling by measuring various parameters under controlled conditions.

Material and methods

Plant material

Seeds of *L. albus* were obtained from the lupine collection of the Genetic Diversity and Molecular Characterization Laboratory of Higher School of Agriculture of Mograne.

Basalt powder "Farina di Basalto®"

Basalt powder or "Farina di Basalto®" type XF, produced by Basalti Orvieto s.r.l. from the basaltic effusions in Italy, was used in this work. It's composed of micronized particles that measure less than 20 µm obtained through an industrial process, by mechanical grinding of the basic volcanic effusive rock of Orvieto basalt, using ceramic elements, without adding other minerals or chemicals.

Known for its fertilizing effects applied in agriculture, "Farina di Basalto®" contains natural elements such as Silicon, Alumina, Potassium and Calcium [16]. Silica (silicon dioxide) is the main compound that characterizes FdB with percentages ranging from 45% to 49% [12]. The different components of Basalt powder are presented in the table below.

Table 1: Chemical composition of Basalt powder [12]

Components	Percentages
SiO_2	45 à 49%
Al_2O_3	20,5 à 25,6%
K_2O	8 à 10%
Fe_2O_3	5,2 à 8,5%
CaO	7,5 à 8,5%
MgO	1,9 à 2,6%
Na_2O	2,2 à 4,9 %
P_2O_5	0,6 à 0,7 %

Experimental design

This study was carried out in the Genetic Diversity and Molecular Characterization Laboratory of Higher School of Agriculture of Mograne located at 156 m of altitude, 10.092049° of longitude and 36.428272° of latitude. Randomly selected seeds were disinfected using 5% sodium hypochlorite for 3 min, then thoroughly rinsed with distilled water to remove sodium hypochlorite and placed in sterilized 9 cm ø petri dishes upholstered by two layers of Whatman® n°1 filter paper (5 seeds per petri dishes). Germination and juvenile growth were rated under four concentrations of sodium chloride and five basalt powder percentage, with four replicates for each treatment. Basalt powder, lightly moistened with distilled water, was mixed with disinfected seeds using five percentages 0, 1, 3, 5 and 10% corresponding to 0, 3.57, 10.73, 17.88 and 35.77 g/Kg according to the weight of the sampled seeds. Salt stress was applied by subjecting lupine seeds previously coated with different parentages of basalt powder, to 10 ml salt solutions of 75, 150 and 300 mM NaCl. Distilled water was used as control. Petri dishes were placed in the dark in an incubator at 20±1 °C and 40 % relative humidity for ten days.

Studied parameters

Seed germination was recorded every 24 h for 10 days. A seed was considered germinated when the radicle reaches 2 mm length [17]. At the end of the experiment, final germination percentage (GP) was calculated according to the formula cited by El Rasafi [18].

$$GP = \frac{\text{Germinated seeds}}{\text{Total seeds}} \text{ x } 100$$

Hypocotyl and radicle length were measured after 10 days, using a digital calliper. Seedlings fresh and dry weight were subsequently recorded. The dry weights were measured after drying fresh seedlings in a laboratory oven (KOTERMANN 2771, Uetze, Germany) at 70°C for 48h.

Salt tolerance index STI was calculated following the formula of Aghamir *et al* [19].

$$STI = \frac{\text{Seedling dry weight of stressed plant}}{\text{Seedling dry weight of control plants}}$$

Statistical analysis

A two-way analysis of variance (ANOVA) was carried out to test the combined effect of four salt stress treatments (0, 75, 150, 300 mM) and five basalt powder concentrations (0, 1, 3, 5, 10 %) on germination and early seedling growth of *L. albus*. Variation of data was statistically significant for Tukey's multiple range tests at the $p<0.05$ level. The statistical analysis of this data was done using 'PLAnt Breeding STATistical software' (PLABSTAT), version 3A of 2011-06-14 [20].

Results and discussion

Results presented in table 2 showed a significant effect of salinity and basalt treatments on the germination percentage of *Lupinus albus* seeds ($p<0.05$). In fact, a decrease of germination percentage was observed proportionally to the increase of NaCl concentrations as well as for the lowest basalt levels 1% and 3%. While, for basalt treatment, a significant re-increase of germination percentage was recorded at the rate of 5%, and this for all studied salinity concentration with an average of 83.75%. The interaction between the two treatments was non-significant for this parameter.

Analysis of variance revealed a significant interaction ($p<0.05$) between salinity and basalt treatments on cotyledon length of *L. albus* seedlings (Table 2). A significant enhancement of cotyledon length of stressed seedlings in presence of 10% of basalt application was demonstrated. At 300 mM NaCl, cotyledon length increased by about 5.5 % at 10% of basalt treatment compared to control. On the other side, FdB application stimulated hypocotyl and radicle length proportionally to the increasing basalt levels for all sodium chloride concentrations. Results recorded that hypocotyl and radicle lengths average increased by 49.8 % and 17% respectively, at 10 % of basalt treatment when compared to control. The influence of 10% of FdB was more effective on hypocotyl length at 75 mM and 150 mM NaCl, by an increase of 55 % and 131 % respectively compared to control.

L. albus seedlings fresh and dry weights vary according to the applied salinity levels. Fresh weight average recorded a significant decrease by 38.5 % at 300mM NaCl compared to control. However, dry weight average showed a significant increase by 16.6% for 300 mM of salt concentration when compared to control. While, both parameters did not have a statistically significant difference under basalt treatment.

Results showed a significant increase in salt tolerance index when 10 % of basalt powder is applied to seedlings exposed to 75 mM of sodium chloride by 15%, nevertheless STI drops by 15% under 300 mM NaCl, when compared to control. These findings confirm that coating seeds with 10 % of basalt powder alleviate especially low levels of salinity stress in white lupine at early growth phase.

Table 2: Combined effect of salinity and basalt treatments on germination and seedling charactersics of *Lupinus albus*.

Salinity (mM) **	**Basalt treatment (%) ****					**Means**
	0	**1**	**3**	**5**	**10**	
	Germination percentage (%)					
0	100	100	90	95	85	94.00 ^{a}
75	95	90	90	95	95	94.00 ^{a}
150	90	85	75	90	70	84.00 ^{a}
300	80	53.3	35	50	15	47.67 ^{b}
Means	93.75 ^{a}	82.08 ^{ab}	72.50 ^{b}	83.75 ^{ab}	67.50 ^{c}	79.92
	Cotyledon length (mm)					
0	24.51±0.23	25.04±0.66	18.26±1.26	21.5±2.61	23.41±0.28	22.54 ^{a}±1
75	23.58±0.44	19±1.47	21.34±2.2	20.03±0.97	23.69±0.28	21.53 ^{ab}±1.07
150	22.83±0.52	19.69±2.23	15.08± 2.10	19.04± 2.35	23.5±0.35	20.10 ^{b}±1.08
300	20.19±1.66	10.31±2.06	7.1±2.87	22.23± 0.55	21.31± 0.66	16.23^{c}±1.56
Means	22.78 ^{a}±0.46	18.5 ^{b}±1.60	15.45^{c}±2.10	20.79 ^{ab}±6.48	22.98 ^{a} ±0.39	20.10±1.69**
	Hypocotyl length (mm)					
0	34.53±2.79	40.92±2.54	27.03±2.21	36.22±6.11	41.16±2.52	35.97 ^{a}±3.17
75	23.52±3.58	29.51±4.11	25.07±3.02	32.51±3,39	36.64±2.27	29.45 ^{a}±3.27
150	11.29±1.64	19.11±5.20	18.62±2.96	21.14±2.21	26.1±1.92	19.25 ^{b}±2.79
300	0	0	0	0	0	0
Means	23.11^{c}±2	29.85^{b}±2.96	23.57^{c}±2.04	29.96^{b}±2.92	34.63^{a}±1.14	28.22±2.03
	Radicle length (mm)					
0	42.44±3.87	50.63±3.44	33.99±2.62	43.33±5.50	41.16±7.87	43.64 ^{a}±4.67
75	46.51±4.95	43.95±12.30	42.58±7.33	40.42±6.04	52.18±10.43	45.13 ^{a}±8.21
150	19.25±2.82	24.28±7.53	27.07±5.84	21.32±4.02	25.45±5.76	23.47 ^{b}±5.19
300	7.81±1.18	5.63±1.04	4.46±1.78	13±1.34	10.52±1.01	8.28 ^{c}±1.27
Means	29.00^{a}±2.91	31.12^{a}±6.07	27.03^{a}±4.39	29.52^{a}±4.52	33.99^{a}±6.26	30.13±4.83
	Fresh weight (mg)					
0	3.31±0.047	3.77±0.14	2.62±0.14	2.95±0.33	3.34±0.15	3.20 ^{ab}±0.16
75	2.82± 0.11	4.76±1.81	2.91±0.27	2.98±0.15	3.7±0.06	3.43 ^{a}±0.48
150	2.7±0.08	2.72±0.35	2.14±0.33	2.66±0.42	3.11±0.13	2.67 ^{b}±0.26
300	2.41±0.23	1.25±0.25	0.97±0.27	2.62±0.14	2.61±0.18	1.97 ^{c}±0.21
Means	2.81 ^{a}±0.11	3.12 ^{a}±0.63	2.16 ^{b}±0.25	2.80 ^{ab}±0.26	3.19 ^{a}±0.13	2.82±0.27
	Dry weight (mg)					
0	0.74±0.05	0.79±0.04	0.78±0.02	0.81±0.03	0.77±0.02	0.78 ^{c}±0.03
75	0.79±0.02	0.81±0.04	0.83±0.02	0.85±0.02	0.92±0.02	0.84 ^{b}±0.02
150	0.88±0.01	0.9±0.04	0.92±0.02	0.92±0.01	0.91±0.03	0.91^{a}±0.02
300	1.03±0.12	0.95±0.03	0.92±0.03	0.78±0.07	0.91±0.04	0.9 ^{a}±0.05
Means	0.86^{a}±0.05	0.86^{a}±0.02	0.86^{a}±0.2	0.84^{a}±0.03	0.86^{a}±0.02	0.86*±0.02
	Salt tolerance index					
0	1.00±0.07	1.07±0.034	1.06±0.03	1.10±0.05	1.04±0.16	1.06 ^{a}±0.06
75	1.07±0.05	1.09±0.02	1.12±0.03	1.16±0.03	1.24±0.04	1.14 ^{ab}±0.03
150	0.88±0.03	0.9±0.05	0.92±0.03	0.92±0.02	0.91±0.05	1.23 ^{b} ±0.03
300	1.39±0.04	1.29±0.03	1.25±0.02	1.05±0.04	1.18±0.09	1.23 ^{b} ±0.04
Means	1.16^{a}±0.04	1.17^{a}±0.03	1.17^{a}±0.02	1.14^{a}±0.03	1.17^{a}±0.08	1.16*±0.04

Each value represents the mean ± SE (n = 4).

Results of significant differences between accessions and treatments determined by two-way ANOVA (Tukey test, $p<0.05$) are indicated by: *: $p<0.05$; **: $p<0.01$.

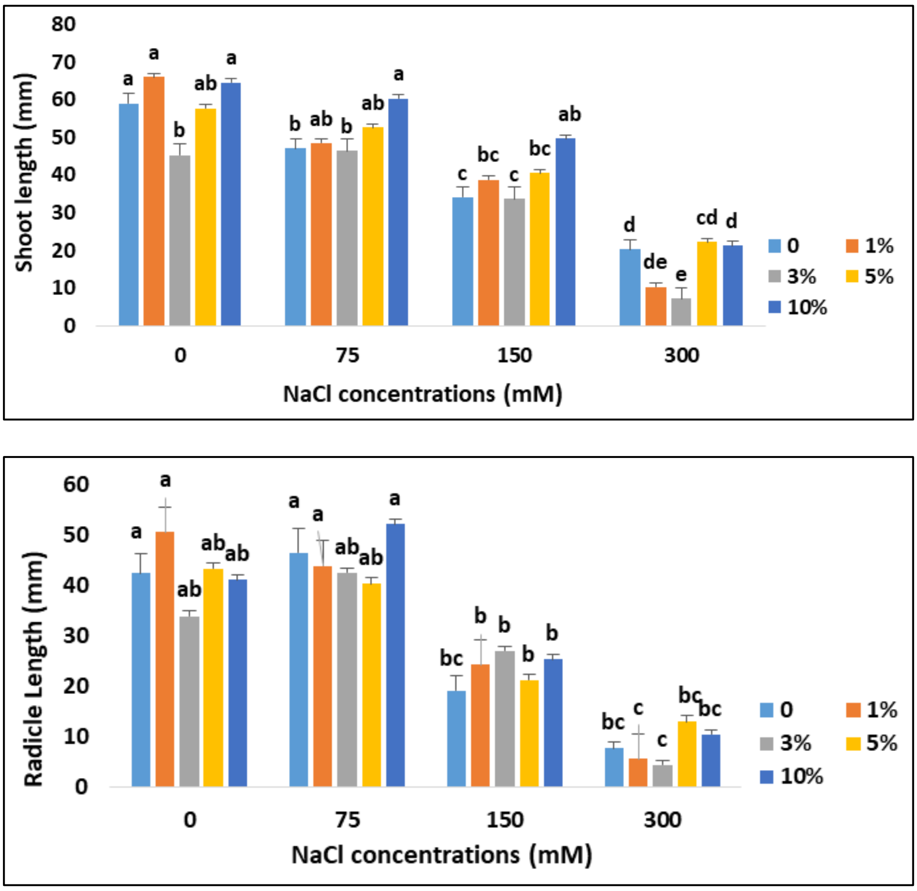

Fig 1: Effect of combined salinity and basalt treatments on shoot and radicle lengths of *L. albus*. Bars represent mean ± S.E. (n = 4). Different letters indicate a significant difference of treatments (p<0.05, Tukey's multiple range test)

Shoot and radicle lengths decreased along with increasing salinity (figure1). FdB showed a positive effect on these growth parameters for control seeds, with an increase of shoot growth by 12% at 1% basalt level. However, under salt stress, this increase of 12% is reached at 10% basalt treatment in the presence of a low concentration of sodium chloride 75 mM.

These findings agree with previous studies. In fact, the increase of salt concentration induces a high osmotic pressure, thus reducing the amount of available water to be absorbed by the seeds or by the presence of toxic ions in the solution, which negatively affects germination process and seedling growth [21, 22].

Regarding Basalt, its addition had a positive impact on growth parameter. Since the main component of basalt is silicon, this result is in line with previous reports, which have suggested that silicon has many positive effects on the growth and yield as well as physiology and metabolism in different plant species [23, 24, 25]. In addition, the study conducted by Zuccarini [26] to evaluate the effect of silicon on *Phaseolus vulgaris* L. under salt stress showed that Si application enhanced stressed plants growth significantly and that salinity alleviation was more effective at lower NaCl (30mM) concentration. Those positive results were observed in our study at 75 mM and 150 mM, especially by the stimulation of hypocotyl elongation according to the increase in "Farina di Basalto®" percentage, which demonstrates a high tolerance of this white lupine variety to salinity and the effectiveness of the studied basalt levels with the best results registered at 10%. Other studies have proven the beneficial effects of silicon on increasing leaf area and photosynthesis by decreasing the decomposition of photosynthetic pigments as well as antioxidant enzymatic activity under stress conditions [25, 27, 28, 29].

Conclusion

Seed treatment with Basalt Powder, rich in silicon, may increase significantly salt stress tolerance in white lupin at the juvenile growth stage. The present research highlighted a new method to alleviate salinity stress using a natural rock, which constitutes an ecological solution to increase white lupine production and allow to this crop to cope with abiotic constraints. The effectiveness of FdB for other lupine species as well as the optimal doses to use under controlled conditions and in saline soil may be the subject of further studies.

Conflicts of interest

The authors declare no conflict of interest.

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