Agronomical and Biochemical Responses of White *Lupinus albus* L. Genotypes to Contrasting Water Regimes and Inoculation Treatments

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Abstract: Two field experiments were conducted over the growing season November 15 – April 15- 2008-2009 and 2009/ 2010 at the experimental farm of Suez Canal University, Ismailia, Egypt. The purpose of this research was to study the effects of water stress and inoculation treatments on the yield, growth parameters and biochemical traits under field conditions and during two growing seasons. The experimental design for both seasons was randomized complete block in split-split plot arrangement with three replications. Where Irrigation treatments included normal (W0) and water stressed (Ws) were allocated to main-plots, two inoculation treatments: no-inoculation and inoculation with commercial inoculums were assigned to sub-plots. Five lupin genotypes including two cultivated varieties (Giza 1 and Giza 2) and three landraces (LR 1, LR 2 and LR 3) constituted the sub-sub-plots. Significant differences of irrigation, inoculation, genotype and their different interactions were detected for the most measured traits. Water stress reduced yield and growth parameters, whereas antioxidant enzyme activities were increased significantly as plants exposed to limited irrigation. Protein % was not affected by water limitation at both seasons, while 100-seeds weight was significantly affected in the first year only. There were potential beneficial effects of commercial inoculation, where it increased yield and growth parameters under water shortage condition and reduced enzyme activities. The landrace LR 1 is obviously, the best genotype in seeds yield, growth parameters over the two growing seasons and high activity of defense mechanism (activity of catalase and peroxidase enzymes) under water stress conditions and over all inoculation treatments. Thus it is considered a promising line under water limited environments.

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1. Introduction

White lupin (*Lupinus albus* L.) has been cultivated in Egypt at least for four thousand years (Gladstones, 1970). It is cultivated mainly for human nutrition because of their high protein and oil contents; as a green manure contributing to improved soil structure and as ruminant feed either as green forage or as grain introduced as protein supplements in the diets of ruminants. It is originated from the Mediterranean region where drought, salinity and mineral deficiency are among the major constraints for lupines production.

In such region, water is one of the important environmental factors regulating plant growth and development (Manivannan *et al*., 2007). Drought is therefore a major threat affecting the life of plants and is responsible for limiting crop yield globally. In addition, drought has a detrimental effect on nodulation and symbiotic N_2 fixation in legumes specifically (Denison & Kiers, 2004). Therefore, the nodulation ability of the host genotype is the key feature in sustaining nitrogen fixation under stresses.
Drought stress induces numerous morphological, metabolic, biochemical and physiological changes in plants. These include water status, growth, membrane integrity, pigment content, osmotic adjustment and photosynthetic activity (Zhang *et al*., 2007; Praba *et al*., 2009). It creates potential oxidative stress through accumulation of ROS, these may damage plants by oxidizing photosynthetic pigments, membrane lipids, proteins and nucleic acids (Reddy *et al*., 2004).

To eliminate ROS, all plants are endowed with detoxification mechanisms, including both enzymatic [superoxide dismuatase (SOD), ascorbate peroxidase (APX), peroxidase (POD), glutathione reductase (GR), etc.] and non-enzymatic (anthocyanins, carotenoids, ascorbic acid, etc.) antioxidants (Johnson *et al*., 2003). Kalefetog lu Macar and Ekmekçi (2009) recorded a significant increase in SOD, GR, APX and POD activities at all drought compared to control treatments in two chickpea cultivars. They concluded that the drought tolerant cultivar revealed higher antioxidant activities. In narrow-leafed lupins, Yu and Rengel (1999) found 21% increase in total SOD after 2 days of withholding water and a further increase was noted with an increase in severity of stress.

Mediterranean-type environment have shown that the number of pods, shoot length, nodule, root dry weight and seeds dry weight are dependent on developmental conditions like plant density, temperature or water stress (Lo´pez-Bellido *et al*.,

2000). Carvalho *et al*., (2004) found that, lupines cultivars tended to accumulate crude protein and carbon compounds in seeds at the end of the water stress period (15 days after anthesis), however, Jansen (2008) recorded non significant effect of water stress on protein content when imposed at the same stage. Seeds yield and harvest index are reduced due to pod and seeds abortions (Palta *et al*., 2007) under drought conditions, therefore the same authors (2003) suggested that seeds yield of lupines may be increased in low-rainfall by selecting cultivars with high pod retention.

Although lupines is an important legume crop worldwide, information on the effects of water stress on this species in limited. Moreover, little breeding efforts are devoted to study this species in Egypt as there are only two cultivated varieties. Therefore, the present work was designed to study the morphological and biochemical characteristics that may affect seeds yield under different water regimes including normal irrigation and artificial water stress. Therefore, the objectives of this study were to screen the lupines genotypes for yield, growth parameters, nodulation ability and enzymes activity under irrigation and inoculation treatments over two growing seasons in order to identify genotypes with superior yield and growth parameters under water stress.

2. Material and Methods

Plant material and treatments

Five lupine genotypes (Lupinus albus, ssp. termis) were used for this study, including two cultivated varieties and three landraces. The two cultivars Giza 1 and Giza 2 were obtained from Department of Legume Crops, Agricultural Research Center (ARC), Giza, Cairo. Both cultivars were evolved through individual selection from local landraces; Giza 1 is adapted for cultivation in northern region of Egypt, whereas Giza 2 is adapted for Upper Egypt region planting. The three landraces were collected from farmers' fields at Ismailia (LR1), Al-Salhia (LR2) and Almhsma (LR3) province.

Two field experiments were conducted over the growing seasons (November, 15-April, 15) 2008/2009 and 2009/2010 at the experimental farm of Suez Canal University, Ismailia. Rainfall, maximum and minimum temperatures for the two growing seasons are presented in Fig. 1.

The tested genotypes were subjected to two contrasting water regimes and two inoculation treatments. Water regimes included normal irrigation (W_0) where experiments were irrigated regularly depending on weather conditions and plant needs. Whereas water stress (Ws) treatment was irrigated when plants showed drought symptoms including loss of leaves. Irrigation treatments were started when plants reached 40 days after planting. Soil moisture content was determined for each irrigation regime gravimetrically by weighting method (Black, 1973). Mean soil moisture content for control and Ws treatments were 2.67% and 1.72% for the first season, whereas for the second season values were 2.00% and 1.35%, respectively. Inoculation treatments consisted of un-inoculation control, and commercial Bradyrhizobium inoculum obtained from department of microbiology, ARC, Giza. For inoculation purpose, seeds were mixed with 15 % glucose solution and inoculum mixture. The control plots were sown first to prevent cross-inoculation. At sowing, 357.14 kg ha^{-1} superphosphate was broadcasting (375 kg per hectare), while 178.57 kg of potassium sulphate (125 kg per hectare) was added at flowering and seeds filling stages. No N fertilizers were added.

The experimental design for both seasons was randomized complete block in split-split plot arrangement with three replications. Main plots were two irrigation regimes, split-plots were inoculation treatments and split-split plots were the five lupine genotypes. Each plot has two rows of 3 m length with 20 cm inter-row spacing and 50 cm between rows.

Soil analysis

Soil texture of the experimental area was sandy with a pH of 8.1, 89.9% coarse sand, 5.7% fine sand, 2.7% silt and 1.7clay. Bulk density (g cm^{-3}), EC_s (dS m^{-1} in saturated soil paste) and total nitrogen (N) were: 1.59, 1.2 and 0.028%, respectively.

Growth measurements

Sixty days after planting, five plants from each plot were dug out and pink nodules were detached from the roots carefully, Roots and nodules were washed in running water and dried at 70 ºC to estimate dry weights per plant (g). At maturity, five plants from each treatment were uprooted for recording the following data on single plant basis: number of branches, pods per plant, seed yield per plant and 100-seeds weight. Then plots were harvested by hand excluding one plant from both ends on 15 April, dried for 2–3 days for seeds yield per hectare determination.

A sub-sample of 50 g of grains was ground and the N concentration was determined using the A.O.A.C method (1990), then protein content of seeds was calculated by multiplying N% by 6.25.

Determination of activities of antioxidant enzymes

Fresh leaf samples (0.5 g) from each treatment were collected 60 days after planting and stored at - 20 ºC. Enzymes extraction was processed as described by Ni *et al*., (2001). Briefly, extraction was done using cold phosphate buffer (0.1 M, pH 7.0) containing 1% (w/v) polyvinylpyrrolidone and

1% (v/v) triton X-100. Then samples were macerated with 1 ml of the extracting buffer. Samples were further ground with another 1 ml of the extracting buffer. An aliquot (1.5 ml) of the extract was centrifuged at 10000 g for 10 min at 4 ºC and the resulted supernatant was immediately stored at – 80 ºC for future enzyme activity assays.

The protein concentrations in the leaf extracts were determined according to the Bradford (1976) method. Catalase (CAT) activity was determined via following the initial rate of disappearance of H_2O_2 at 240 nm (Luck, 1974). Peroxidase (POD) activity was determined according to Vetter *et al*., (1958). The reaction mixture contained 200 µl sample, 1 ml of 1% o-phenylenediamine (in 95% ethyl alcohol) and 1 ml of 0.3% hydrogen peroxide (in distilled water), the reaction is allowed to proceed for 5 min at which time it is stopped by adding 2 ml of saturated sodium bisulfate. The enzyme activity was expressed as the change in absorbancy at 430 nm and expressed as O.D. units X 10^3 min^{-1} mg $protein^{-1}$.

Statistical analysis

Analysis of variance was carried out for each growing season and combined over seasons using the statistical Package MSTATC to study the main and interaction effects of the studied factors on the measured traits. Mean separation was obtained using least significant difference test at the 0.05 probability level when significant F-tests ($P< 0.05$) were observed.

3. Results

Yield and growth parameters

During the 2009/2010 growing season, the experiment received more rainfall compared to the first season. The maximum temperature during the pod growth and development was higher in the second season (Fig 1).

Analysis of variance for yield and growth parameters in each year demonstrated significant effects of irrigation, inoculation, genotypes and their interactions on the measured traits in both seasons (Table 1). Irrigation treatments showed significant effects on yield, nodulation and growth parameters in the first year. In the second year, only yield and nodule dry mass were significant. Significant differences among genotypes were recorded for all variables in both years, except number of branches in the first year. Inoculation did not affect 100-seeds weight in both years, branches number (in the first year) and pods number (in the second year), but showed statistically significant differences in other measurements. Significant irrigation x genotype interactions was noted for all traits except branches number, pods per plant and 100-seeds weight. Irrigation x inoculation x genotypes interactions were significant for seeds yield, nodule mass, protein % and branches per plant in the second, whereas in the first year, only pods per plant,100-seeds weight and nodule mass per plant differed significantly.

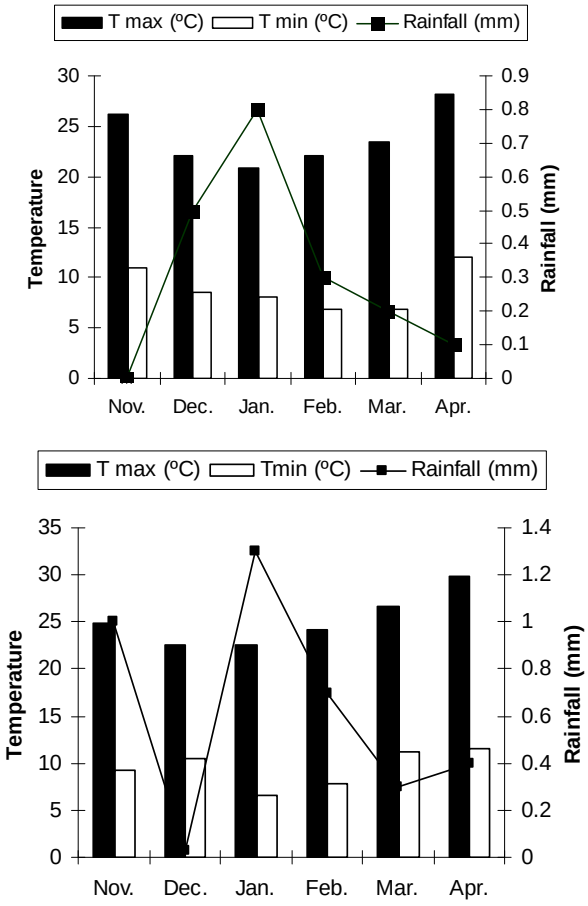

Fig.1 Monthly rainfall (mm), and maximum (T_{max}) and minimum (T_{min}) temperatures (ºC) at Ismailia, Egypt during the two growing seasons, 2008/2009 (above) and 2009/2010 (below)

Water stress resulted in a decrease in all studied traits in both years with varying values, except protein % that is showed 4.51% and 2.42 % increase at the first and second year, respectively (Table 2-9). On the other hand, commercial inoculation increased yield and growth parameters under water deficient condition in both the seasons compared to un-inoculated treatment. The highest reduction was noted for seeds yield per plant (23.93 and 24.06 %), seeds yield per hectare (23.98 and 24.00 %), pods per plant (33.97 and 13.52 %), branches per plant (11.21 and 3.92, respectively) and nodule dry weight (24.81 and 23.33 %) in the first and second year, respectively. The data for nodule dry weight demonstrates that even without Rhizobium inoculation, infection was occurred,

presumably due to a root infection by native Rhizobium strains derived from the soil or from the adjacent plot in which inoculation was practiced. There was a slight reduction in 100-seeds weight due to water stress (3.71 and 5.14 %), but inoculation caused a slow increase in this trait in both seasons (3.69 and 1.12 %). The two commercial varieties and LR 1 recorded a higher seeds yield per hectare in the second season, whereas, LR 2 and LR 3 showed lower yield. Under water stress condition Giza 2 and LR 1 out performed other genotypes in number of pods per plant and root and nodule dry weight in both seasons, protein % and seeds yield per hectare in the second season over all inoculation treatments. However, in the first season, higher seeds yield per hectare and heavier 100-seeds weight were produced by LR 1 and LR 2. The genotype LR 1 showed the lowest reduction for number of pods per plant, nodule dry weight, 100-seeds weight, number of branches per plant, increasing in protein %, and root dry weight. This favorable behavior was also confirmed by the values of the low estimated for yield per plant and hectare (10.8 and 10.76 %, respectively) during the second year.

Under normal irrigation, the same genotype produced high pods per plant, nodule dry weight at both seasons, high 100-seeds weight, seeds yield per plant at the first season only. The genotype LR 2 gave combined high number of branches (4.40), 100-seeds weight (43.86 %), protein % (35.26 %), seeds yield per plant (11.85 gm) and seeds yield per hectare (987 kg) at the first season. The commercial cultivar Giza1 with high values for all measured traits under control irrigation in the second season appears as instable genotypes when examined for the same traits under stress conditions. Poor yielding genotypes in stressed and non-stressed treatments at the second year were the landraces LR 2 and LR 3 (718.90 and 657.27; 583.40 and 492.34 kg per hectare, respectively).

Antioxidant enzyme activities

Significant differences were detected for catalase and peroxidase activities due to irrigation and genotype effects (Table 1). In contrast, peroxidase activity was not affected by nodulation, but catalase activity was statistically differed. Significant differences in the activity of both enzymes were recorded due to all types of interactions.

The activity of catalase and peroxidase enzymes was increased with water stress, where they showed 31.46 and 19.07 % increase for both, respectively (Table 10). Water stress caused increasing in catalase activity of uninoculated genotypes (49.78 %), whereas, there was slight increase incase of inoculated one (11.82 %). The same trend was observed for peroxidase activity where it recorded 20.71 and 17.48 % increase for uninoculated and inoculated treatments, respectively. Compared to controls, catalase and peroxidase activities of Giza 1, Giza 2 and LR 1 were more conspicuous as a result of water stress when compared across inoculation treatments. Over all irrigation and inoculation treatments, the same genotypes recorded the highest activities. In contrast, the LR 5 showed decrease in the activities of CAT and POD enzymes (43.62 and 1.98 %, respectively) when subjected to water deficient. There was a decrease in CAT activity in inoculated genotypes under water stress treatment except LR3, whereas POD activity decreased in Giza 2, LR 1 and LR 3 under the same environmental conditions.

4. Discussion

Yield and growth parameters

Two contrasting water treatments were used in this study, regular irrigation and water-stressed treatment which were applied when plants show wilt symptoms. In Egypt agriculture is dependent on irrigation as rainfall is very low (nearly 120 mm per year) and does not support crop productivity. The second growing season received rather more rains that the first one, whereas day temperature was higher one –two degrees in pod filling and development period. So, the first season is considered drier than the second one. Drought stress caused an observed detrimental effect on plant growth and productivity; on the other hand addition of commercial inoculums increased all the parameters under investigation compared with the uninoculated treatment. In addition, inoculation mitigated the harmful effect on yield and growth traits when plants were exposed to water stress. The effect of water stress on measured traits varied from year to year, also lupines genotypes responded to the treatment differently. It is clear that, in the first year the effect of water stress was more severe and recorded a higher decrease in pods and branches per plant; and nodule dry weight, although there was a similar decrease in seeds yield. This may be due to slight effect of water limitation on 100-seeds weight. Carvalho *et al*., (2004) attributed that the uninfluenced water stress on seeds biomass to the stems of lupines can temporary be storage sites for assimilates which are later used in seeds filling and therefore seeds weight remains unaffected.

1.

Table 1 Analysis of variance for yield, growth parameters and enzymes activities of lupine genotypes grown under irrigation and inoculation treatments in 2008/2009 and 2009/2010 seasons

SoV	df	No of branches	Pods number per plant	100-seed weight	Seeds yield (gm per plant)	Root dry weight (gram per plant)	Nodule dry weight (gram per plant)	Protein (%)	Seeds yield (kg per hectare)	Catalase activity (nmol H_2O_2 min^{-1} mg $protein^{-1}$)	Peroxidase activity (O.D. units $X10^3$ min^{-1} mg $protein^{-1}$)
2008/2009 Season											
Irrigation	1	3.50	367.48*	34.43*	108.29*	8.12	1.61*	34.32	752028.64*		
(IR)		0.33	2.71	6.44	2.79	1.75	0.006	2.07	7997.37		
Error 1	2	0.42	14.77*	10.31	10.81*	7.98*	4.57*	132.08*	75023.30*		
Inoculation	1										
(Ino)		0.38	15.65*	5.27	2.68	1.28	0.67*	3.21	18792.79		
IR x Ino	1	0.32	52.07*	65.07*	8.69*	12.08*	2.39*	40.82*	60382.05*		
Genotype	4	0.12	23.70*	6.51	7.03*	6.41*	1.13*	39.01*	48815.18*		
(G)		0.12	0.67	8.13	1.32	1.60	0.75*	12.73*	9137.43		
IR x G	4	0.23	7.77*	33.25*	1.85	1.00	0.24*	3.79	12871.99		
Inoc. X G	4	0.25	1.86	6.01	1.45	1.13	0.003	2.02	5862.84		
IR x Inoc. X G	4										
Error 2	36										
2009/2010 Season											
Irrigation	1	0.44	29.47	48.71	113.63*	5.18	1.79*	8.73	788833.93*	29.59*	164326.67*
(IR)		0.04	4.57	13.56	0.25	0.47	0.001	1.43	1692.75	0.11	2337.07
Error 1	2	1.07*	7.74	5.36	17.66*	4.77*	6.09*	111.87*	122609.25*	26.93*	763.27
Inoculation	1										
(Ino)		0.01	0.01	13.88	2.03*	1.08	1.08*	5.83*	14056.93*	12.29*	836.27
IR x Ino	1	0.48*	32.66*	83.44*	76.34*	6.56*	3.05*	5.59*	530389.33*	25.57*	18789.36*
Genotype	4	0.07	3.39	2.39	7.45*	2.83*	1.47*	38.42*	51827.11*	15.82*	40087.62*
(G)		0.11*	4.82	9.45	3.54*	0.58	1.04*	30.44*	24554.38*	13.85*	53712.14*
IR x G	4	0.14*	3.44	4.66	0.91*	0.33	0.32*	6.24*	6358.60*	3.27*	28081.47*
Inoc. X G	4	0.03	2.06	5.16	0.21	0.39	0.001	1.07	1449.88	0.04	742.06
IR x Inoc. X G	4										
Error 2	36										

Table 2: Effect of irrigation and inoculation treatments on branches per plant of lupine genotypes grown under field conditions in two seasons (2008-2009 and 2009-2010).

	Branches per plant													
	2008/2009						Mean	2009/2010						Mean
	Ws			W0				Ws			W0			
Genotype	0 Inoc	Inoc	Mean	0 Inoc	Inoc	Mean		0 Inoc	Inoc	Mean	0 Inoc	Inoc	Mean	
Giza1	4.25	4.12	4.18	3.90	3.58	3.74	3.96	4.10	4.50	4.30	4.10	4.10	4.10	4.20
Giza2	4.47	4.55	4.51	3.90	4.20	4.05	4.28	4.20	4.07	4.13	3.55	4.13	3.84	3.99
LR1	4.28	4.38	4.33	3.93	3.20	3.57	3.95	4.40	4.87	4.64	4.12	4.73	4.43	4.53
LR2	4.57	4.23	4.40	3.93	3.80	3.87	4.13	4.10	4.43	4.27	4.19	4.53	4.36	4.31
LR3	3.87	4.10	3.98	4.13	3.40	3.77	3.87	4.22	4.38	4.30	4.10	4.00	4.05	4.17
Mean														
Inoc	4.28	4.27		3.96	3.63			4.20	4.45		4.01	4.30		
IR	4.28			3.80				4.33			4.16			
LSD 0.05														
IR	ns							ns						
Inoc.	ns							0.095						
G	ns							0.15						
IR x Inoc	ns							ns						
IR x G	ns							ns						
Inoc x G	ns							0.21						
IR x Inoc x G	ns							0.30						

Table 3: Effect of irrigation and inoculation treatments on pods per plant of lupine genotypes grown under field conditions in two seasons (2008-2009 and 2009-2010).

pods per plant														
	2008/2009						Mean	2009/2010						Mean
	Wo			Ws				Wo			Ws			
Genotype	0 Inoc	Inoc	Mean	0 Inoc	Inoc	Mean		0 Inoc	Inoc	Mean	0 Inoc	Inoc	Mean	
Giza1	8.64	10.08	9.36	7.51	7.82	7.67	8.52	10.87	11.80	11.33	8.73	8.80	8.77	10.05
Giza2	13.77	14.64	14.20	11.38	11.79	11.59	12.90	11.00	8.87	9.93	8.87	9.80	9.33	9.63
LR1	16.52	17.73	17.12	10.21	11.40	10.81	13.97	11.80	13.40	12.60	12.40	11.53	11.97	12.28
LR2	14.29	16.42	15.36	9.20	10.49	9.84	12.60	9.13	9.00	9.07	7.67	8.98	8.42	8.75
LR3	14.57	18.98	16.77	9.84	6.50	8.17	12.47	7.60	10.80	9.20	5.40	7.87	6.63	7.92
Mean														
Inoc	13.56	15.57		9.63	9.60			10.08	10.77		8.65	9.40		
IR	14.57			9.62				10.43			9.02			
LSD 0.05														
IR	1.83							ns						
Inoc.	0.71							ns						
G	1.12							1.18						
IR x Inoc	1.01							ns						
IR x G	1.59							ns						
Inoc x G	ns							ns						
IR x Inoc x G	2.25							ns						

Table 4: Effect of irrigation and inoculation treatments on 100-seeds weight of lupine genotypes grown under field conditions in two seasons (2008-2009 and 2009-2010).

100-seeds weight														
	2008/2009						Mean	2009/2010						Mean
	W0			Ws				W0			Ws			
Genotype	0 Inoc	Inoc	Mean	0 Inoc	Inoc	Mean		0 Inoc	Inoc	Mean	0 Inoc	Inoc	Mean	
Giza1	39.95	36.03	37.99	36.88	39.35	38.11	38.05	38.82	35.18	37.00	35.17	35.57	35.37	36.19
Giza2	37.21	40.93	39.07	37.66	40.01	38.83	38.95	36.83	39.40	38.11	36.15	37.48	36.82	37.46
LR1	46.36	40.81	43.58	39.49	43.89	41.69	42.64	36.13	30.97	33.55	33.03	32.71	32.87	33.21
LR2	42.30	45.43	43.86	41.54	39.21	40.37	42.12	33.07	31.98	32.52	30.03	29.78	29.90	31.21
LR3	36.81	40.61	38.71	36.52	36.74	36.63	37.67	33.99	33.52	33.76	30.64	31.30	30.97	32.36
Mean														
Inoc	40.53	40.76		38.42	39.84			35.77	34.21		33.00	33.37		
IR	40.64			39.13				34.99			33.19			
LSD 0.05														
IR	2.82							ns						
Inoc.	ns							ns						
G	2.02							1.87						
IR x Inoc	ns							ns						
IR x G	ns							ns						
Inoc x G	ns							ns						
IR x Inoc x G	4.04							ns						

ns: not significant at 5% probability level.

Table 5: Effect of irrigation and inoculation treatments on root dry weight (g) weight of lupine genotypes grown under field conditions in two seasons (2008-2009 and 2009-2010).

root dry weight (g)

	2008/2009							2009/2010						
	W0			Ws			Mean	W0			Ws			Mean
Genotype	0 Inoc	Inoc	Mean	0 Inoc	Inoc	Mean		0 Inoc	Inoc	Mean	0 Inoc	Inoc	Mean	
Giza1	7.87	8.70	8.28	6.33	5.00	5.66	6.97	5.51	6.09	5.80	4.43	3.50	3.96	4.88
Giza2	7.26	9.21	8.24	6.66	7.27	6.96	7.60	5.08	6.45	5.77	4.66	5.09	4.87	5.32
LR1	5.74	5.81	5.78	6.54	7.10	6.82	6.30	4.02	4.70	4.36	4.54	4.97	4.76	4.56
LR2	6.59	7.09	6.84	5.13	6.09	5.61	6.22	4.61	5.25	4.93	3.59	4.26	3.92	4.43
LR3	3.83	5.59	4.71	4.42	5.80	5.11	4.91	2.68	3.58	3.13	3.10	3.97	3.53	3.33
Mean														
Inoc	6.26	7.28		5.82	6.25			4.38	5.21		4.06	4.36		
IR	6.77			6.03				4.80			4.21			
LSD 0.05														
IR	ns							ns						
Inoc.	0.55							0.33						
G	0.87							0.52						
IR x Inoc	ns							ns						
IR x G	1.24							0.73						
Inoc x G	ns							ns						
IR x Inoc x G	ns							ns						

ns: not significant at 5% probability level.

Table 6: Effect of irrigation and inoculation treatments on nodule dry weight (g) weight of lupine genotypes grown under field conditions in two seasons (2008-2009 and 2009-2010).

nodule dry weight (g)

	2008/2009							2009/2010						
	W0			Ws			Mean	W0			Ws			Mean
Genotype	0 Inoc	Inoc	Mean	0 Inoc	Inoc	Mean		0 Inoc	Inoc	Mean	0 Inoc	Inoc	Mean	
Giza1	1.57	2.52	2.04	0.44	1.09	0.77	1.40	1.73	2.87	2.30	0.50	1.24	0.87	1.58
Giza2	0.89	1.24	1.06	1.27	1.06	1.16	1.11	0.98	1.41	1.20	1.55	1.21	1.38	1.29
LR1	1.14	3.07	2.10	1.14	1.83	1.48	1.79	1.26	3.50	2.38	1.30	2.09	1.69	2.03
LR2	0.75	0.84	0.80	0.64	1.06	0.85	0.82	0.83	0.96	0.89	0.73	1.21	0.79	0.93
LR3	0.39	0.89	0.64	0.67	0.82	0.74	0.69	0.43	1.01	0.72	0.76	0.93	0.85	0.78
Mean														
Inoc	0.95	1.71		0.83	1.17			1.04	1.95		0.97	1.34		
IR	1.33			1.00				1.50			1.15			
LSD 0.05														
IR	0.086							0.035						
Inoc.	0.028							0.016						
G	0.045							0.026						
IR x Inoc	0.04							0.023						
IR x G	0.064							0.037						
Inoc x G	0.064							0.037						
IR x Inoc x G	0.090							0.052						

significant at 5% probability level.

Table 7: Effect of irrigation and inoculation treatments on protein % of lupine genotypes grown under field conditions in two seasons (2008-2009 and 2009-2010).

protein %														
	2008/2009						Mean	2009/2010						Mean
	W0			Ws				W0			Ws			
Genotype	0 Inoc	Inoc	Mean	0 Inoc	Inoc	Mean		0 Inoc	Inoc	Mean	0 Inoc	Inoc	Mean	
Giza1	32.81	38.94	35.87	29.58	33.60	31.59	33.73	32.81	33.54	33.17	30.63	31.35	30.99	32.08
Giza2	33.25	33.51	33.38	36.31	35.70	36.00	34.69	31.72	36.46	34.09	31.72	33.54	32.63	33.36
LR1	28.88	32.04	30.46	30.10	35.88	32.99	31.72	24.06	32.81	28.43	32.45	37.88	35.17	31.72
LR2	34.30	36.23	35.26	34.30	38.59	36.44	35.85	29.17	34.64	31.90	30.26	32.45	31.35	31.63
LR3	33.08	34.12	33.60	37.28	40.95	39.11	36.36	33.18	30.26	31.72	32.81	33.18	32.99	32.36
Mean														
Inoc	32.46	34.97		33.51				30.19	33.54		31.57	33.68		
IR	33.71			35.23				31.86			32.63			
LSD 0.05														
IR	ns							ns						
Inoc.	0.74							0.54						
G	1.17							0.85						
IR x Inoc	ns							0.76						
IR x G	1.66							1.21						
Inoc x G	1.66							1.21						
IR x Inoc x G	ns							1.70						

ns: not significant at 5% probability level.

Table 8: Effect of irrigation and inoculation treatments on seeds yield per plant (g) of lupine genotypes grown under field conditions in two seasons (2008-2009 and 2009-2010).

Seeds yield per plant (g)														
	2008/2009						Mean	2009/2010						Mean
	W0			Ws				W0			Ws			
Genotype	0 Inoc	Inoc	Mean	0 Inoc	Inoc	Mean		0 Inoc	Inoc	Mean	0 Inoc	Inoc	Mean	
Giza1	7.73	10.32	9.02	9.06	8.96	9.01	9.02	12.81	15.67	14.24	8.63	9.90	9.26	11.75
Giza2	10.26	11.91	11.08	8.38	8.19	8.28	9.68	13.55	14.61	14.08	9.78	10.71	10.25	12.16
LR1	12.91	13.06	12.99	9.61	9.07	9.34	11.16	12.50	12.51	12.50	10.74	11.57	11.15	11.83
LR2	11.76	11.93	11.85	7.70	9.33	8.51	10.18	8.31	8.94	8.63	6.88	7.12	7.00	7.81
LR3	10.19	11.98	11.09	6.79	8.10	7.45	9.27	6.53	9.24	7.89	4.82	7.00	5.91	9.60
Mean														
Inoc	10.57	11.84		8.31	8.73			10.74	12.19		8.17	9.26		
IR	11.20			8.52				11.47			8.71			
LSD 0.05														
IR	1.85							0.55						
Inoc.	0.63							0.24						
G	0.99							0.38						
IR x Inoc	ns							0.34						
IR x G	1.41							0.54						
Inoc x G	ns							0.54						
IR x Inoc x G	ns							0.76						

ns: not significant at 5% probability level.

Table 9: Effect of irrigation and inoculation treatments on seeds yield per hectare (kg) of lupine genotypes grown under field conditions in two seasons (2008-2009 and 2009-2010).

	Seeds yield per hectare (kg)													
	2008/2009					Mean		2009/2010						Mean
	W0			Ws				W0			Ws			
Genotype	0 Inoc	Inoc	Mean	0 Inoc	Inoc	Mean		0 Inoc	Inoc	Mean	0 Inoc	Inoc	Mean	
Giza1	644.09	860.09	752.09	754.82	746.25	750.54	751.31	1067.59	1305.55	1186.57	718.96	825.05	772.01	979.29
Giza2	855.08	992.42	923.75	698.17	682.77	690.47	807.11	1129.21	1217.40	1173.31	815.05	892.71	853.88	1013.59
LR1	1075.65	1088.76	1082.21	801.19	755.89	778.54	930.37	1041.67	1042.22	1041.94	895.14	964.38	929.76	985.85
LR2	979.96	994.57	987.26	641.11	777.81	709.46	848.36	692.64	745.17	718.90	573.41	593.39	583.40	651.15
LR3	849.15	998.68	923.92	566.09	685.29	620.69	772.30	544.33	770.21	657.27	401.67	583.01	492.34	574.81
Mean														
Inoc	880.79	986.91		692.27	727.60			895.09	1016.11		680.85	77.71		
IR	933.85			709.94				955.60			726.28			
LSD 0.05														
IR	99.29							45.68						
Inoc.	39.93							19.86						
G	63.14							31.40						
IR x Inoc	ns							28.09						
IR x G	89.30							44.41						
Inoc x G	ns							44.41						
IR x Inoc x G	**ns**							62.80						

ns: not significant at 5% probability level

Table 10: Effect of irrigation and inoculation treatments on catalase and peroxidase activities of lupine genotypes grown under field condition

	Catalase activity ($nmol\ H_2O_2\ min^{-1}\ mg\ protein^{-1}$)					Mean		Peroxidase activity ($O.D.\ units\ X10^3\ min^{-1}\ mg\ protein^{-1}$)						Mean
	W0			Ws				W0			Ws			
Genotype	0 Inoc	Inoc	Mean	0 Inoc	Inoc	Mean		0 Inoc	Inoc	Mean	0 Inoc	Inoc	Mean	
Giza1	6.35	4.51	5.43	10.24	4.10	7.17	6.30	548.67	402.33	475.50	661.67	701.33	681.50	578.50
Giza2	5.53	3.12	4.32	8.97	7.63	8.30	6.31	529.00	596.00	562.50	751.00	700.00	725.50	644.00
LR1	5.14	4.23	4.68	9.17	5.48	7.32	6.00	446.33	637.00	541.67	790.33	684.00	737.17	639.42
LR2	2.90	2.98	2.94	3.97	3.50	3.73	3.34	494.33	640.67	567.50	409.67	666.67	538.17	552.83
LR3	3.42	6.31	4.86	2.53	2.94	2.74	3.80	688.67	504.00	596.33	655.00	514.00	584.50	590.42
Mean														
Inoc	4.66	4.23		6.98	4.73			541.40	556.00		653.53	653.20		
IR	4.45			5.85				548.70			653.37			
LSD 0.05														
IR	0.36							53.67						
Inoc.	0.11							ns						
G	0.17							22.26						
IR x Inoc	0.16							ns						
IR x G	0.25							31.77						
Inoc x G	0.25							31.77						
IR x Inoc x G	0.35							44.93						

ns: not significant at 5% probability level.

In this study, variation in seeds yield was observed among genotypes under water-stressed treatment at both years. The genotypes LR 1 and Giza 1 and Giza 2 maintained both high seeds yield per plant and plant under water limitation conditions at both seasons. The advantage of those genotypes was ascribed to high number of pods per plant, 100-seeds weight, root dry weight, nodule dry weight and rather low number of branches per plant. Palta *et al*., (2007) found that final pod number of the high-yielding genotypes was 85-92 % of its respective irrigated crops, whereas low –yielding genotypes recorded 48-60 %. They also reported that high pod retention genotypes resulted in high yield and consequently pod retention in lupines is an important yield-positive characteristic. The same finding was achieved by Dracup *et al*., (1998) who showed that terminal drought caused reductions in lupine through pod and seeds abortions. Although 100-seeds weight was reduced due to drought stress, the reduction was slight. Palta *et al*., (2007) found the same results as average seeds weight was unaffected by the rainfed conditions.

Nodule dry weight was decreased under water stress conditions in both years for both uninoculated and inoculated treatments. Similar results were obtained by Velagaleti and Marsh (1989) who reported a significant reduction in nodule dry mass under stress due to inadequate photosynthate supply to the roots caused by decreased plant dry mass production. Although, two genotypes Giza 2 and LR 3 showed 9.43 and 15%; 15.62 and 18.05 % increasing dry weight of nodules per plant compared to uninoculated treatment in the first and second year, respectively. However, under uninoculated treatment, all genotypes recorded some dry weight of nodule suggesting the presence of indigenous Rhizobium strains of lupin in the soil. Raza and Jørnsgård (2005) recorded 14-36 nodules per plant on lupines roots under uninoculated treatment in an experiment carried out at Ismailia governorate. The same genotypes; Giza 1, Giza 2 and LR 1 showed higher nodule and root dry mass under contrasting irrigation regimes, in addition to increasing and /or low reduction in protein %. The same conclusion was achieved by Rao *et al*., (2002) who suggest that genotypes with greater capacity for nodulation perform best under both unstressed and stressed conditions. However, the low effects of stress on Protein % may be a result of that plants were treated with water withholding from the beginning of their life. Jansen (2008) found a raise in protein % of narrow-leaf lupin due to high temperature (25 ºC). Also Carvalho *et al*., (2005) reported no effect of water deficit imposed at the beginning of seeds development (15-35 days after anthesis) on protein content of *lupinus mutabilis* and *lupinus albus*. The genotype LR 2 combined high values for seeds yield per plant, pods number per plant, nodule and root dry mass, 100-seeds weight and protein % (rather reasonable values) under water stressed conditions and over all inoculation treatments. This suggested that LR2 could prove to be good breeding material for further breeding programs aimed at the evolution of drought tolerance in lupin.

Antioxidant enzyme activities

Plants have several physiological and biochemical strategies, such as antioxidative defense and osmotic adjustment, to prevent the damaging effect of oxidative stress, induced by drought (Tan *et al*., 2006). In the present study, the defense mechanism used by lupin genotypes was activated. This was evident from the elevated activity of catalase and peroxidase enzymes. Water limitation caused a significant increase in both enzymes in Giza 1 (32.04 and 43.32 %), Giza 2 (92.13 and 28.98 %) and LR 1 (56.41 and 36.09 %) genotypes compared to control irrigation over all inoculation treatments, which is an indication for increased production of ROS.

The genotypes LR 2 showed 26.87 % increase and 5.17 % decrease in catalase and peroxidase activity, respectively. In contrast, the genotype LR 3 recorded decreasing values in both enzymes (53.41 and 1.98 %). Our results agree partly with those of Mourato *et al*., (2009) who recorded an increase in peroxidase and non significant increase in catalase activities in *Lupinus luteus* exposing to varying Cu concentrations and suggested that, peroxidase is involved in H_2O_2 elimination in yellow lupin species while CAT is not. They also concluded that SOD and POD have the major role in the antioxidative response of the investigated lupin species. Furthermore, Macar and Ekmekçi (2009) recorded a marked elevated activity in POD, GR, SOD and APX enzymes in two chickpea genotypes in all drought treatments. This indicates that the estimated H_2O_2 scavenging enzymes probably cooperated with each other during water deficit periods. However, the activities were higher in the drought tolerant genotype. In contrast, Chatterjee and Chatterjee (2000) reported a decrease in CAT activity in cauliflower leaves subjected to micronutrients. Therefore, increasing, decreasing and unaffected activity of protective enzymes is species dependent. Interestingly, the reducing catalase (all except LR3) and peroxidase activities (all except Giza 1 and LR 2) in inoculated genotypes under water stress conditions may be attributed to the ameliorative effect of Rhizobium. Malekzadeh *et al*., (2007) concluded a potential role of Arbuscular Mycorrhiza fungus in protecting plants exposed to heavy metal stress. Zahran (1999) mentioned that, one of the adaptations of legumes to arid lands (poor in N and P) and those with low moisture availability is their infection by mycorrhizal fungi in addition to Rhizobium.

In conclusion, our results emphasize the capability of lupin genotypes to withstand drought conditions, significant differences among irrigation, genotypes, inoculations and their different interactions. Water stress resulted in yield and growth parameters reduction and increasing in antioxidative mechanisms activity. Inoculation significantly increased yield and plant growth parameters under water stress due to its ameliorative effect against water stress effect. Out of the five tested genotypes, LR1 was distinguished by its high seeds yield per plant and hectare, improved growth parameters in addition to its high catalase and peroxidase activity. This line is considered to be the most tolerant genotype compared to other tested genotypes.

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