# Seed quality, water use efficiency and eco physiological characteristics of Lallemantia (*Lallemantia* sp.) species as effected by soil moisture content

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Abstract: This study investigated the effect of drought stress on the yield, water use efficiency (WUE), physiology, and seed quality of two species Lallemantia sp. Field experiments with three irrigation regimes were carried out in a split plot factorial in a randomized complete block design with three replications. Treatments included irrigation after 40 %, 60 %, and 100 % depletion of available soil water (ASW) ( $I_{40}$ ,  $I_{60}$  and  $I_{100}$ . respectively) as main plots and Lallemantia species L. iberica (M. Bieb.) Fisch. & C. A. Mey. (S<sub>1</sub>) and L. royleana Benth. in Wall  $(S_2)$  as subplots. Increment in depletion of ASW  $(I_{40}$  to  $I_{100}$ ) resulted in progressively less chlorophyll *a* content (Chl a), open stomata percentage (OS), and leaf area index (LAI). The highest Chl a and LAI were found to be 0.0087 mg g<sup>-1</sup>, and 2.68 mg  $g^{-1}$  leaf mass in I<sub>40</sub> treatment, respectively, while closed stomata percentage (CS) increased significantly as drought stress increased. The results of this experiment indicated that the appropriate yield of production was obtained in plots which were fully irrigated  $(I_{40})$  for all species of *Lallemantia*; however, the WUE increased as drought stress increased. The interaction of drought stress, Lallemantia species with grain yield and WUE was significant.

Key words: Lallemantia; drought stress; leaf area; water productivity

Vpliv vsebnosti vode v tleh na kakovost semen, učinkovitost izrabe vode in ekofiziološke lastnosti različnih vrst lalemancije (*Lallemantia* sp.)

Izvleček: V raziskavi so bili preučevani učinki sušnega stresa na pridelek, učinkovitost izrabe vode (WUE), fiziologijo in kakovost semen dveh vrst lalemancije (Lallemantia sp.). Poljski poskus s tremi režimi namakanja je bil izveden kot popolni bločni faktorski poskus z deljenkami s tremi ponovitvami. Obravnavanja so obsegala namakanja po 40 %, 60 %, in 100 % zmanjšanju razpoložljive vode v tleh (ASW, $I_{40}$ ,  $I_{60}$ , and  $I_{100}$ ) na glavnih ploskvah in vrste lalemancije, L. iberica (M. Bieb.) Fisch. & C. A. Mey. (S<sub>1</sub>) in L. royleana Benth. in Wall  $(S_2)$  na podploskvah. Povečanje pomanjkanja vode (iz  $I_{40}$  na  $I_{100}$ ) je povzročilo progresivno zmanjšanje vsebnosti klorofila a, zmanjšanje odstotka odprtih listnih rež (OS), in zmanjšanje indeksa listne površine (LAI). Največji vrednosti vsebnosti klorofila a in indeksa listne površine sta bili izmerjeni, 0,0087 mg g<sup>-1</sup>, in 2,68 mg g<sup>-1</sup> listne mase, pri obravnavanju I<sub>40</sub> medtem, ko se je odstotek zaprtih rež (CS) z večanjem sušnega stresa značilno povečal. Rezultati raziskave kažejo, da je bil najboljši pridelek dosežen na dobro namakanih ploskvah (I<sub>40</sub>) pri obeh vrstah lalemancije a učinkovitost izrabe vode se je s sušnim stresom povečevala. Medsebojni vpliv sušnega stresa, vrst lalemancije, pridelka zrnja in učinkovitosti izrabe vode je bil dokaj velik.

Ključne besede: *Lallemantia*; sušni stress; listna površina; učinkovitost izrabe vode

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# **1** INTRODUCTION

Lallemantia, commonly known as Balangu, belongs to the family of Labiatae and is represented by five species: L. iberica (M. Bieb.) Fisch. & C. A. Mey, L.baldshuanica Gontsch., L. royleana Benth. in Wall, L canescens (L.) Fischer & C.A.Mey., and L. peltata (L.) Fisch. & C.A.Mey. (Rechinger, 1982). It is found in different regions of European and Middle East countries, especially Iran (Zargari 1980; Naghibi et al., 2005). Lallemantia is an annual or perennial herb, a dwarf shrub (Anonymans, 2003), and is characterized by simple, ovate to round or sometimes linear, aristae-toothed bracteole, oblong, trigonous, smooth, and mucilaginous nettled leaves (Harley et al. ,2004). It is a good source of fiber, oil, and protein and has medicinal properties (Naghibi et al., 2005). This natural herb is used for the treatment of reflux of stomach (Anonymans, 2012), arthritis, joint pain, rheumatism, osteoarthritis, and abscesses (Mahmood et al., 2013). Balangu seeds are used in a wide range of traditional and industrial products such as a beverage namely Tokhme Sharbati and a bread in Iran and Turkey (Mahmood et al., 2013). The seed contains up to 30 % of a drying oil used for lighting, as a varnish, in paints as a lubricant (Usher, 1994), as a wood preservative, as an ingredient in oil-based paints, in furniture polishes, in printing inks, and in soaps. The oil could also be used for oil-foods and as a tanning agent. It is also used in the manufacture of linoleum (Anonymans, 2003). Sustainability and the overall management of water resources have been the greatest challenges of the century. World population has passed the six billion mark. Based on the proportion of young people in developing countries, the requirement for water will continue to increase significantly during the next few decades. This places an enormous demand on the world's limited irrigation water supply; thus, a precise knowledge of crop water requirements has become an essential prerequisite for the efficient use of water supplies (Reddy, 1999). Plants exposed to drought or drought stress have evolved a series of morphological and physiological adaptations which gives them tolerance to these stresses (Kumar et al., 2005). The degree of adaptation to the decrease in water potential caused by drought may vary considerably between species (Savé et al., 1995) and also within a species (Parker & Pallardy, 1985). Drought stress decreases the rate of photosynthesis (Kawamitsu et al., 2000; Rivero et al., 2007). Plants grown under drought conditions have a lower stomatal conductance in order to conserve water. Consequently, CO, fixation is reduced and photosynthetic rate decreases, resulting in less assimilate production for plant growth and yield. Diffusive resistance of the stomata to CO<sub>2</sub> entry is probably the main factor limiting photosynthesis in drought (Boyer, 1970). Certainly, under mild or moderate drought stress, stomatal closure causing reduced internal leaf CO<sub>2</sub> concentration is the major reason for reduced rates of leaf photosynthesis (Chaves, 1991; Cornic, 2000; Flexas et al., 2004; Romero-Romero et al., 2018). Another plant response to drought stress is to change the content of photosynthetic pigments that play important roles in light harvesting (Farooq et al., 2009; Ommen et al., 1999). Severe drought stress also inhibits plant photosynthesis by causing changes in chlorophyll content, by affecting chlorophylls components, and by damaging the photosynthetic apparatus (Iturbe-Ormaetxe et al., 1998). Drought stress causes a large decline in the chlorophyll a content, the chlorophyll b content, and the total chlorophyll (Manivannan et al., 2007). The decrease in chlorophyll under drought stress is mainly the result of damage to chloroplasts caused by reactive oxygen species (Ramireza et al., 2014). The effects of drought stress on chlorophyll and carotenoids content have been investigated some crops including cotton (Mssacci et al., 2008) and Catharanthus roseus (L.) G.Don (Jaleel et al., 2008). Water deficit reduces leaf area and the number of leaves per plant and leads to a reduction in crop growth. Loss of leaf area, which could result from the reduced size of younger leaves and the inhibition of the expansion of developing foliage, is also considered an adaptation mechanism to drought (Gebeyehu, 2006). In bean, reduced leaf area causes a decrease in canopy temperatures and a cooling of the plant growth environment (Ghanbari et al., 2013). Wentworth et al. (2006) determined the photosynthetic characteristics of two contrasting varieties of the common bean (Phaseolus vulgaris L.) under drought stress. Both varieties showed reduced chlorophyll content on a leaf area basis, a decrease in leaf area, and an increase in leaf thickness. Numerous biochemical changes occur in response to drought stress. Changes in mucilage (Rahimi et al., 2011) and protein accumulation and synthesis have been observed in many plants species as a result of plant exposure to drought stress during growth (Chen & Tabaeizadeh, 1992; Salehi et al., 2016; Omidi et al., 2018). The study of water use efficiency becomes particularly important in situations where growth is affected by limited water availability (Anyia & Herzog, 2004). Maximum water use efficiency is achieved at the limit between diffusional and metabolic limitations to photosynthesis (Flexas et al., 2004). However, there is still a lack of information about the morpho-physiological behavior of different Lallemantia species under conditions of limited water availability. Therefore, the main objective of this study was to determine the response of different species of Lallemantia to drought stress by evaluating their productivity and selected morphological, physiological, and biochemical parameters.

# 2 MATERIALS AND METHODS

# 2.1 CROP PRODUCTION, TREATMENTS AND EXPERIMENTAL DESIGN

The field experiment was conducted at the Research Farm of the Agricultural College of Shahed University, Tehran (Fig 1), during the years 2012-2014. Prior to preparing the plots, five points were sampled at a depth of 30 cm, and soil analyses were conducted. The soil type was loam-silty, the percentage of organic carbon was 0.77, the N content was 0.08%, the exchangeable K and available P content were 382 mg kg<sup>-1</sup> and 22.3 mg kg<sup>-1</sup>, respectively, and the pH was about 8. The climate was arid to semiarid with an average rainfall of 216 mm per year. Some physical properties of the soil layer at a depth of 30 cm are given in Table 1.

In this study, the *Lallemantia* species were used as the crop material. After field preparations, the experimental area was divided into 36 plots measuring  $3 \times$ 1.5 m in size. Between contiguous plots a distance of 1 m in each replication and 1.5 m between replications was maintained. The seeds were sown on the 14<sup>th</sup> of March 2012 at a row spacing of 25 cm and plant spacing of 5 cm in the rows. Cultivation practices, such as weeding, thinning, fertilizing, etc., were applied according to crop needs. The field experiment was designed as a split plot factorial in a complete randomized block design with three replications. The main factor was irrigation treatments, scheduled based on depletion of ASW over 30 cm soil depth including 40 %, 60 %, and 100 % depletion of ASW (abbreviated to  $I_{40}$ ,  $I_{60}$ , and  $I_{100}$ ). The second factor was two species of *Lallemantia L. royleana* (S<sub>1</sub>), *L. iberica* (S<sub>2</sub>). All plots received the same amount of water from germination to flowering. The irrigation treatments were applied at the onset of flowering. The percentage depletion of water (ASW) in the 0-30 cm layer of soil was estimated by measuring the soil water content gravimetrically using Eq. (1) (Martin et al., 1990):

Eq. (1) 
$$D(_{0_{0}}) = 100 \times \frac{Fc - \theta}{Fc - Wp}$$

Where *D* is depletion,  $F_c$  is the soil moisture at field capacity,  $\theta$  *is* the soil moisture, and *Wp* is the soil moisture at wilting point. The amount of water applied based on each treatment was calculated using Eq. (2) (Rostamza et al., 2011):

Eq. (2) 
$$I_n = \frac{(Fc - \theta) \times D \times A}{100}$$



Figure 1: Geographical location of experiment

Table 1: Some physical properties of 0-30 cm layer of soil

Particle s	ize distributio	n (%)		Bulk density	Field capacity	Wilting point		
Clay	Silt	Sand	Soil type	(g cm <sup>-3</sup> )	(% gravimetrically) (% gravimetrically			
18	24	58	Loam-silty	1.8	21.2	11		

Where  $I_n$  is the volume of irrigation water, *D* is the effective rooting depth, and A is the plot surface area. The surface area of each plot measured 4.5 m<sup>2</sup>. Each plot was watered individually through the furrows. Before applying irrigation treatments, the amount of water required was determined by monitoring changes in soil water gravimetrically. Every 2 days a soil sample was taken from a depth of 0-30 cm, dried in an oven at 105 °C for 24 h, and then the soil moisture was measured.

# 2.2 SAMPLING AND ANALYZING

### 2.2.1 Water use efficiency (WUE)

WUE is a quantitative measurement of how much biomass or yield is produced over a growing season, normalized with the amount of water used up in the process (Anonymous, 1998). Besides absolute yield, water use efficiency is an important agronomic factor, especially in agricultural irrigation systems and in climate areas where a limited amount of water from the rainy season has to last for the whole growth period as no further rainfall can be expected (Anonymous, 1998). In this experiment the WUE was calculated as the economical yield per unit of evapotranspiration of the crop.

(WUE = Y ET<sup>-1</sup>). ET was calculated using Eq. (4) (Garrity et al., 1982):

Eq. (4)  $ET = P + I - R - D_{\mu} \pm \Delta S$ 

Where *ET* is crop water consumption (mm), *P* is rainfall (mm), *I* is irrigation water (mm), *R* is surface runoff (mm),  $D_p$  is deep percolation (mm), and  $\Delta S$  is soil water content variation in crop root depth (mm). Therefore, total ET values were calculated using a summation of all ET during the growing season. In this study,  $D_p$  and  $R_f$  in Eq. (4) were assumed to be negligible. Since the slope of each plot was near zero and the amount of irrigation water was only enough to reach field capacity, it was also assumed that there was no deep percolation.

#### 2.2.2 Chlorophyll content

Chlorophyll content was measured in each irrigation treatment,  $I_{40}$ ,  $I_{60}$ , and  $I_{100}$ , and for every *Lallemantia* species. Measurements were performed on the appropriate method of Arnon (1994) with a modified formula (Porra, 2002). Chl was extracted by grinding 0.1 g of leaf material in 80 % acetone. The extract was centrifuged at 3000 rpm for 5 min, the supernatant was collected, and the process was repeated until the pellet became white or yellow in color. The supernatants were pooled, and absorption was read by spectrophotometer (Model Spectronic 20; Milton Roy Co., USA) at wavelengths of 645 nm, 663.6 nm, and 664.4 nm. Eventually the optical density was noted for the estimated chlorophyll content (Saraswathi & Paliwal, 2011) using Eq. (4).

Eq. (4): Chlorophyll a (µg ml<sup>-1</sup>) = 12.25 (A<sub>663.6</sub>) – 2.55 (A<sub>646.6</sub>) Chlorophyll b (µg ml<sup>-1</sup>) = 20.31 (A<sub>646</sub>) – 4.91 (A<sub>663.6</sub>) Total chl (µg ml<sup>-1</sup>) = 17.76 (A<sub>646.6</sub>) + 7.34 (A<sub>663.6</sub>)

### 2.2.3 Leaf area index (LAI)

Leaf area index is defined as the one sided leaf area of a plant per the ground area covered by canopy (Burton et al., 1991; Cutini et al., 1998). The area of each leaf was measured per  $2.0 \text{ m}^2$  of every plot with a leaf area meter (AM200, Bioscientific Co.). To calculate the leaf area, the number of leaves was multiplied by the average leaf area.

# 2.2.4 Leaf stomata variation

Optical microscopy was used to assess the response of *Lallemantia* species leaf stomata to irrigation regimes. A very thin layer of the abaxial epidermis was taken. After the chlorophyll was removed with bleach and distilled water, lam and lamella samples were prepared (Akbarinia et al., 2012). Then the percentage of closed and open leaf stomata was calculated per 1.0 mm<sup>2</sup>.

# 2.2.5 Root length variation

In June 2012, at the end of the experiment, plant roots were extracted, the attached soil was removed by washing, and the root length was determined using the line intersection method (Tennant, 1975) from which root length could be calculated for soil layer samples from a depth of 30 cm.

#### 2.2.6 Yield production

In this experiment, plants were harvested 3 months after planting, right after the physiological maturity of *Lallemantia* to avoid losing plant material. The effects of the drought treatments on seed yield were assessed. Samples were collected from 2.0 m<sup>2</sup> of plot.

#### 2.2.7 Seed quality

Seed chemical composition was determined as protein percentage by determining the nitrogen content using the Micro kjeldahl method (Cohen, 1910). The nitrogen percentage was multiplied by 6.25 to calculate the percentage of protein in the seeds. Seed mucilage was measured for every treatment using Kalnyasundaram's method (1982). Measures of protein yield and mucilage yield were obtained by multiplying the percentage of protein and mucilage by seed yield.

#### 2.2.8 Data analysis

Statistical software was used for data analysis, correlations, and all mean comparisons (Duncan). A p-value of 0.01 was considered statistically significant. All graphs were drawn using Microsoft Office Excel

# **3 RESULTS**

# 3.1 WEATHER CONDITIONS AND IRRIGATION

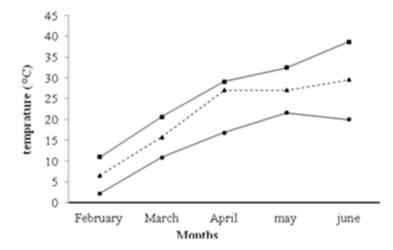
The average monthly maximum air temperature varied between 11 °C and 38.7 °C, and the average temperature exceeded 35 °C in June. The lowest average

monthly minimum temperature of 2.2 °C was observed in February (Fig. 2). The number of irrigation treatments and total water volume applied during the experiment are shown in Table 2. The interaction between irrigation treatments and species were observed on the LAI of the *Lallemantia* species (Fig 3).

# 3.2 THE EFFECT OF WATER STRESS ON GROWTH COMPONENTS OF LALLEMANTIA SPECIES

# 3.2.1. Leaf area index (LAI)

The variance analysis indicated that LAI of the Lal*lemantia* species was significantly affected (P < 0.01) by the interaction effect of irrigation regimes and species (Table 3). LAI was directly affected by the amount of water applied. The highest values were seen in  $I_{40}$  and they decreased as drought stress increased (Fig. 2). Under severe drought stress conditions  $(I_{100})$ , the LAI was the least in both species. There was no significant difference in the LAI of L. royleana in any irrigation system, however in L. iberica the LAI was significantly reduced as drought stress increased. As the water depletion increased in  $I_{60}$ and  $\rm I_{_{100}}$  , the LAI decreased 24.6 % and 49.62 %, respectively compared to  $I_{40}$  in L. iberica (Fig. 3). The mean comparison of LAI in Fig. 2 showed that in all species, the leaf area index decreased during plant growth as the intensity of drought stress increased. As the water depletion increased in  $I_{60}$  and  $I_{100}$ , leaf area index decreased. Among drought adaptation strategies, the minimization



**Figure 2:** Average monthly maximum (---), minimum (---) and mean ( $-\Delta$ -) temperatures (°C) for the experimental site in 2012-2014.

Treatments	Number of irrigation	Total amount of applied water (mm)
I40 S1	9	237
I40 S2	9	238
Mean		235.75
I60 S1	5	200
I60 S2	5	199
Mean		199.25
I100 S1	2	117
I100 S2	2	115
Mean		113.5

**Table 2**: Number of irrigations and amount of water applied per irrigation treatments to Lallemantia species plots after starting water regimes

**Table 3**: Analysis of variance of chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll (Ch T), percentage of closed stomata (CS), percentage open stomata (OS), leaf area index (LAI), root length (RL), water use efficiency (WUE), yield (Y) and thousand seed mass (TSM)

\$.O.V	df	Mean square									
		<u>Chl</u> a	<u>Chl</u> b	<u>Chl</u> T	%CS	%OS	LAI	RL	WUE	Y	TSD
Block	2	2.5×10-7 m.s.	7.77×10-7 m.s.	9.11×10 <sup>-7</sup>	41.49 <u>n.s.</u>	41.45 n.s.	0.105 <sup>n.s.</sup>	0.118 <sup>n.s.</sup>	0.234 <sup>n.s.</sup>	1481.6 <sup>n.s.</sup>	7× 10 <sup>-5</sup> n.s.
Irrigation	2	3.7×10 <sup>-6**</sup>	2.40×10 <sup>-6</sup> n.s.	1.82×10 <sup>-6</sup> n.s.	7936.59**	7936.92**	5.260**	0.806 <sup>n.s.</sup>	0.583*	29815.5*	312× 10-5 **
Lallemantia species	1	2.2×10-6**	1.84×10-6	7.93×10-6	210.73 n.s.	210.64 n.s.	6.890**	0.0001 <sup>n.s.</sup>	0.260 <sup>n.s.</sup>	909.03 <sup>n.s.</sup>	45× 10 <sup>-6</sup> n.s.
Irrigation ×species	2	6.25×10 <sup>-7</sup> n.s.	5.54×10 <sup>-7</sup>	4.34×10 <sup>-7 ns.</sup>	344.13 n.s.	344.08 n.s.	2.041**	0.282 <sup>n.s.</sup>	0.389*	6642.9 <sup>n.s.</sup>	17× 10 <sup>-4</sup>
Error	18	1.8×10-7	1.42×10-6	1.43×10-6	116.95	116.97	0.150	1.186 <sup>n.s.</sup>	0.176	2131.69	14×10 <sup>-6</sup> n.s
Total	35										

\*,\*\*: significant at 5 % and 1 % probability levels, respectively, and n.s: non-significant.

of water loss can be achieved through lowering either leaf area or stomatal conductance or by reducing the energy load of the plant (Rauf & Sadaqat, 2008). It is noteworthy that yield was not significantly different between  $S_1$  and  $S_2$ , but as drought intensity increased, LAI decreased in  $S_1$  significantly. Therefore, it is concluded that  $S_1$  is more sensitive to soil water depletion than  $S_2$ . Pandy et al. (2000) found that reducing LAI to decrease transpiration and deepening the root system to increase water extraction are adaptive strategies in maize plants under water stress. Hence, dry matter production decreases with a decrease in the leaf area index. Similar results were also

Table 4: Analysis variance of seed quality of Lallemantia species under irrigation treatments

S.O.V	df	Mean square	Mean square							
		Protein %	Protein yield	Mucilage %	Mucilage yield					
Block	2	1.30 <sup>n.s</sup>	2.40 <sup>n.s</sup>	0.068 <sup>n.s</sup>	5.65 <sup>n.s</sup>					
Irrigation	2	2.24 <sup>n.s</sup>	39.29 <sup>*</sup>	8.08 **	60.01 *					
Lallemantia species	1	61.09 **	30.28 **	54.02 **	16.78 <sup>n.s</sup>					
Irrigation species	2	8.87 *	8.90 n.s	0.772 <sup>n.s</sup>	20.32 n.s					
Error	18	1.55	2.70	1.217	9.82					
Total	35									

\*,\*\*: significant at 5 % and 1 % probability levels, respectively, and n.s: non-significant

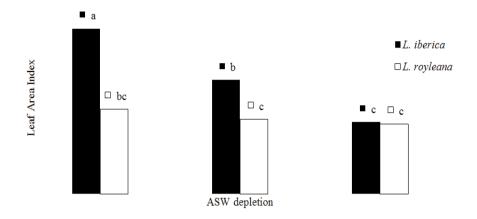


Figure 3: The interaction of water stress and *Lallemantia* sp on leaf area index (LAI). Different letters indicate significant difference (p 0.01) according to Duncan test.

reported by Tashiro and Wardlaw (1990). Stagnari et al. (2018) reported that the leaf area of radish reduced when the plants were exposed to drought stress. Rostamza et al. (2011) demonstrated a positive relationship between drought intensity and the reduction in LAI and dry matter from 7 % to 45.5 %. This can be associated with the fact that plants exposed to water stress served the photosynthetic sources to stay green and produce more dry matter. Furthermore, Hopkins et al. (2008) stated that leaf size could increase with increasing air temperatures. Large leaves have thicker boundary layers of air around their surfaces which insulate them and decrease water loss through transpiration.

# 3.2.2 Root Length

Results given in Table 3 showed no significant effect in root length among all irrigation regimes and *Lallemantia* species. Drought stress decreased root length, but not significantly. The same result was reported in strawberry cultivars by Klamkowski and Tender (2008) who demonstrated that cultivars 'Elsanta' and 'Salut' were not affected by irrigation regimes. Generally, growth inhibition in response to reduced water availability was higher in the above-ground portions of the plants than in the root systems. A deeper, more extensive root system enables plants to increase water uptake and survive during drought stress (Klamkowski & Tender, 2008).

Data of Chlorophyll a, Chlorophyll b, Total Chlorophyll, open and closed stomata, leaf area index, root length, water use efficiency, grain yield and grain qual-

**Table 5:** The mean chlorophyll a (Chl a), chlorophyll b (Chl b), total Chlorophyll (Chl T), closed stomata (CS) open stomata percentage (OS) per mm<sup>2</sup>, leaf area index (LAI), root length (RL) water use efficiency (WUE), yield (Y), thousand seed weight, percentage of grain protein, grain protein yield, percentage of grain mucilage and grain mucilage yield of *Lallemantia* species affected by different irrigation regimes

	Chl b												
	Chl a (mg	Chl T (mg	CS	OS	LAI	RL	WUE	Y	TSW	protein	Protein	Muci-	Mu- cilage
	$(mg g^{-1}) g^{-1})^{\circ}$	g <sup>-1</sup> )				(cm)	(kg m <sup>-3</sup>	)(kg ha-	; ha <sup>-1</sup> )		yield	yield lage	
Irrigatio	on level												
I40	0.0087 a 0.0098	a 0.0175	a 24.11 b	75.88 a	2.68 a	6.02 a	1.18 ab	208.37	a 0.888 a	21.57 a	7.27 a	5.46 c	11.22 a
I60	0.0082 a 0.0117	a 0.0199	a 64.61 a	35.38 b	2.02 ab	5.50 a	0.79 b	127.01	b 0.866 b	20.75 b	3.96 b	6.30 b	6.75 b
I100	0.0070 b 0.0107	a 0.0180	a 71.82 a	28.17 b	1.35 b	5.77 a	1.51 a	138.12	b 0.856 c	20.92 ab	• 4.34 b	7.10 a	8.95 ab
Lallema	ntia species												
S1	0.0079 b 0.011 a	0.0190	a 55.93 a	44.89 a	2.45 a	5.76 a	1.24 a	167.06	a 0.95 a	22.38 a	6.11 a	5.06 b	8.29 a
S2.	0.0084 a 0.009 a	0.0180	a 51.10 a	48.99 b	1.58 b	5.76 a	1.07 a	135.28	a 0.78 b	19.78 b	4.27 b	7.51 a	9.66 a

ity characteristics were averaged for both *Lallemantia* species (*L. iberica*)  $S_1$ , (*L. royleana*)  $S_2$ . Values within the column followed by the different letters are significantly different according to the Duncan test at 0.01 probability level.

# 3.2.3 Chlorophyll content

Results showed that the effects of irrigation and species were not significant on chlorophyll *a* content (Table 3). Drought stress also inhibited the photosynthesis of plants by affecting chlorophyll content and damage to the photosynthetic apparatus (Mafakheri et al., 2010). In this study, increasing the intensity of drought stress resulted in decreased chlorophyll a content.  $S_1$  (*L. iberica*) showed the significant, lower chlorophyll *a* content across all water stress treatments. The results of this study are in agreement with those of Nyachiro et al. (2001), who described a significant decrease in chlorophyll *a* caused by water deficit in six *Triticum aestivum* L. cultivars. The effect of species on chlorophyll *b* and total chlorophyll levels during drought stress and dependent upon the duration and severity of drought had been reported in other species (Kpyoarissis et al., 1995). The content of total chlorophyle is the stress of the stress of the stress of the stress and chlorophyle is a stress of the stress and chlorophyle in other species (Kpyoarissis et al., 1995).

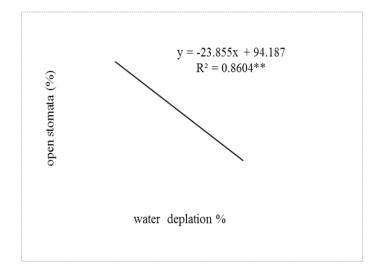
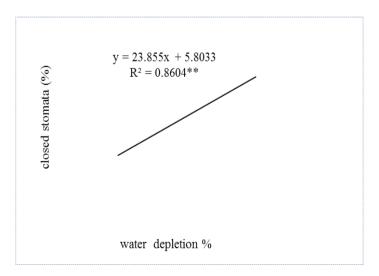


Figure 4: Linear regression between percentage of soil water depletion and percentage of open stomata in the fully expanded topmost leaf of the main shoot. The regression coefficient is significant at p < 0.01.



**Figure 5**: Linear regression between percentage of soil water depletion and percentage of closure stomata in the fully expanded topmost leaf of the main shoot. The regression coefficient is significant at p < 0.01.

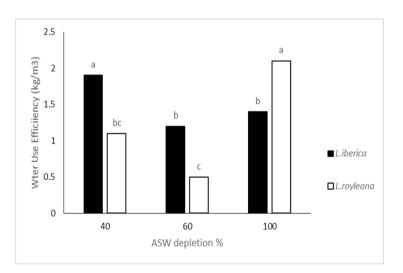
rophyll on leaf dry mass increased with an increase in the intensity of drought stress, but not significantly. An increase of total chlorophyll under drought stress implies an enhanced capacity for light harvesting. Since the production of a reactive oxygen species is mainly driven by excess energy absorption in the photosynthetic apparatus, this might be avoided by degrading the absorbing pigments (Herbinger et al., 2002).

#### 3.2.4 Stomata responses to drought stress

Variance analysis revealed that irrigation regimes had significant effects on the percentage of closed and open stomata (p < 0.01) (Table 3). There was a linear regression ( $R^2 = 0.86^{**}$ ) between the percentage of soil water depletion and the percentage of closed and open stomata. (As drought stress increased, closed stomata increased, reaching the highest value in  $I_{100}$  (Fig 5). Open stomata showed a 75.8 % reduction in  $\mathrm{I_{100}}$  compared to the well irrigation treatment (I $_{40}$ ) (Fig 2). Water availability is a limiting factor for a wide range of physiological processes in plants. One of the first responses of plants to drought is stomatal closure, which restricts gas exchange between the inside of the leaf and the atmosphere. Therefore, this is the main means of regulating water relations and carbon assimilation in plants (Hetherington & Woodward, 2003). Stomatal closure protects plants against excessive water loss, but also restricts the diffusion of CO<sub>2</sub> into the photosynthetic parenchyma (Chaves et al., 2003). S, not only had more open stomata but also showed less closed stomata than  $S_1$ , indicating a high ability of  $S_2$  to retain water in its tissues.

# 3.3. WATER USE EFFICIENCY OF LALLEMANTIA SPECIES

Water use efficiency is defined as the amount of dry matter production (grain) per unit of water consumed (evapotranspiration). The number of irrigation treatments and the total water volume applied during the experiment are shown in Table 2. The effect of irrigation regimes was significant (p < 0.05) on WUE (Table 3). The highest WUE was observed in  $I_{100}$  and the lowest was reported for  $I_{60}$  (Table 5). WUE decreased 33.05 % as drought stress intensity increased up to 60 % ASW. As water depletion increased to more than 60 %, WUE increased 21.85 % compared with I $_{40}$  which was not significant (Table 5). The interaction between drought stress, Lallemantia species was significant (Table 3). The highest WUE by 1.96 kg m<sup>-3</sup> was obtained in  $I_{100}$ , L. royleana ; the lowest WUE by 0.429 kg m<sup>-3</sup> was reported for  $I_{co}$ , L. royleana (Fig 6). In the present study, WUE varied significantly depending on water availability. The results shown in Table 5 revealed that the highest water use efficiency was obtained when irrigation was scheduled on minimum irrigation  $(I_{100})$ , while more soil water depletion of  $(I_{60} \text{ and } I_{100})$  improved WUE. The interaction between irrigation regimes showed that the behavior of Lallmantia species toward irrigation regimes was the same. The interaction of drought stress and Lallemantia species was significant. Our results are contrary to those reported in strawberry cultivars by Klamkowski and Tender (2008), who demonstrated that in drought stress, water use efficiency of the 'Salut' cultivar decreased. Our results were in agreement with the findings of Rostamza et al. (2011), Zegada-Lizarazu and Ijima (2005), and Nagaz et al.



**Figure 6**: The interaction of drought stress and *Lallemantia* species on Water Use Efficiency (WUE). Different letters indicate significant difference (p 0.05) according to Duncan test.

(2009) who reported that WUE increased under drought stress conditions. Chaves et al. (2003) stated that most plants tend to show an increase in water use efficiency under conditions of mild or moderate water deficiency. This increase results from the non-linear relationship between stomata conductance and carbon assimilation. Water loss is restricted earlier and more intensely than the inhibition of photosynthesis. High WUE reflects an ability to maintain photosynthetic capacity under conditions of water deficiency and a higher resistance to drought (Bota et al., 2001).

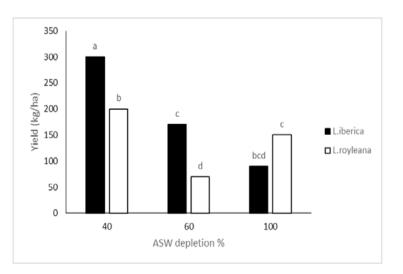
# 3.4. THE WATER DEPLETION EFFECTS ON YIELD OF *LALLEMANTIA* SPECIES

The effect of irrigation was significant (p < 0.05) on yield (Table 3). The yield response to drought stress is given in Fig 6. The yield of  $I_{40}$  was significantly higher than those of  $I_{60}$  and  $I_{100}$ , but there was no difference between the yields of  $I_{60}$  and  $I_{100}$  (Table 5). As water depletion increased to 60 % and 100 %, yield decreased 39.04 % and 33.71 %, respectively, compared with  $I_{40}$  (Table 5). Although  $S_1$  had more yield than  $S_2$  the amount was not significant. The interactions between drought stress and species were significant (Table 3). The highest yield, 297.22 kg ha<sup>-1</sup>, was obtained in  $I_{40}$ , *L.iberica*; the lowest grain yield, 63.40 kg ha<sup>-1</sup>, was related to  $I_{60}$ , *L. royleana* (Fig. 7).

Grain yield is the main objective of crop production. Grain yield of *Lallemantia* was significantly affected by drought stress. Mean comparisons showed that the maximum grain yield (208.37 kg ha<sup>-1</sup>) was obtained in  $I_{40}$  and the minimum was obtained when 100 % of AWS was evacuated. Treatments  $(I_{60})$  and  $(I_{100})$  gave similar results; however, there was no significant difference between these treatments. These results are similar with total dry matter and leaf area index. According to the results, even though grain yield was not significantly different in S<sub>1</sub> and S<sub>2</sub>, S<sub>1</sub> showed more grain yield compared with S<sub>2</sub>. This might be related to a correlation between grain yield with total dry matter and leaf area index. Weber et al. (1996) reported that both leaf area index and total dry matter were poor predictors of grain yield. Winter and Ohlrogge (1993) suggested that grain yield in each treatment increased when leaf area index and total dry matter increased. In this study, grain yield increased when leaf area index increased.

### 3.4.1 Thousand seed mass

Drought stress had a highly significant (p < 0.01) effect on 1000-seed mass (Table 3). The results of means comparisons indicated that the water stress treatments significantly decreased 1000-seed mass (Table 5). The results also demonstrated that there was significant difference between *Lallemantia* species. *L. iberica* showed the highest 1000-seed mass compared with *L. royleana*. Increasing severity of drought stress reduced load rea index, stomatal conductance, photosynthetic pigments, and photosynthesis that affected the transferring grain dry matter and 1000-seed mass.



**Figure 7**: The interaction of drought stress and *Lallemantia* species on grain yield. Different letters indicate significant difference (p 0.05) according to Duncan test.

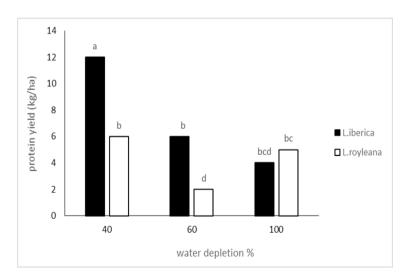
# 3.5 THE WATER DEPLETION EFFECTS ON SEED QUALITY OF SPECIES

#### 3.5.1 Mucilage

Data indicated the percentage of seed mucilage was affected by the irrigation treatments (Table 4). As drought intensity increased, the percentage of seed mucilage decreased (Table 5). The highest percentage of mucilage was related to  $I_{100}$ , and the lowest was reported for  $I_{40}$ . As the intensity of available soil water depletion increased to 60 % and 100 %, the mucilage percentage increased 13.33 % and 30.3 %, respectively. There was a significant difference in the percentage of seed mucilage between Lallemantia species. L. royleana with 7.51 % showed the highest percentage of seed mucilage compared with L. iberica. Mucilage yield was significantly affected by irrigation treatments. The highest and the lowest mucilage yields (11.22 kg ha<sup>-1</sup> and (6.75 kg ha<sup>-1</sup>) were related to  $I_{40}$ and I<sub>50</sub>, respectively. Although an increase in the severity of drought stress caused the seed mucilage yield to decline, there was no significant difference between the values of I40 and I100. It seems that increased seed coated mucilage in water deficit conditions occurs because of the genetic and morphological adaptation of plants to protect fertile new embryo in severe drought stress. Our results are in agreement with the findings of Rahimi et al. (2011) and contrary to those of Mousavi-Nik (2012). Data shown in Table (5) clearly indicates there were significant differences between drought stress treatments in seed mucilage yield. The decreasing trend of seed mucilage yield as the depletion of available soil water increases may be attributed to the fact that plants in  $I_{60}$  and  $I_{100}$  had fewer seeds/plant (grain yield) than those of  $I_{40}$ . In fact, the reduction of grain mucilage yield may be due to the accumulation of salt minerals around the plant roots in drought stress conditions, which impairs the absorption and transport of nutrients to the shoots and ultimately reduces the synthetics of active substance in plants.

# 3.5.2 Protein

Results showed the percentage of seed protein was affected by different irrigation regimes (Table 4). Increasing the intensity of available soil water depletion resulted in decreased seed protein percentage. The highest and the lowest protein contents were obtained in I40 and I60, respectively (Table 5). Increasing the intensity of available soil water depletion up to 60 % resulted in a significantly decreased protein content (3.80 %). Increasing the severity of available soil water depletion to 100 % resulted in a decreased seed protein content (3.01%), but the decrease was not significant (Table 5). The interaction effects of drought stress and Lallematia species were significant on protein content. Increasing the severity of drought stress resulted in a significant decrease in the protein content of L. iberica, however the decrease was not significant in L. royleana. Protein yield was affected by the irrigation regimes (Table 4). The effect of interaction between drought stress and Lallemantia species was significant (Fig 8). The highest protein yield was obtained in  $I_{100}$ , L. royleana, and the lowest was reported in  $I_{50}$ , L. royleana. Increasing the intensity of available soil water depletion up to 60 % resulted in a decrease in protein yield in both



**Figure 8**: The interaction of drought stress and *Lallemantia* species on protein yield. Different letters indicate significant difference (p 0.05) according to Duncan test.

species, but as water depletion increased to more than 60 %, protein yield increased. Drought stress is one of the most important factors that influence and decrease seed protein content (Daneshian et al., 2002). According to the results of our study, increasing the severity of drought stress resulted in decreased seed protein percentage, which is in agreement with the results of Johnson et al. (2002). One of the factors reducing seed protein content in plants exposed to drought stress may be severe drop of the photosynthesis productions and subsequent reductions in precursor protein production and may be attributed to a decrease in protein synthesis (Mohamadkhani & Haydari, 2008). In fact drought stress reduces protein content by increasing the activity of protein degrading enzymes and decreasing photosynthesis. The highest protein yield of I<sub>100</sub> was related to the increased adaption of plants to drought conditions from the beginning of growth compared to those reported for  $I_{60}$  and  $I_{40}$ . Our results were consistent with those of Musallam et al. (2004) and contrary to those obtained by Alghamdi (2003). The allocation of photosynthetic substrates was expected to be high among plants under  $I_{40}$  compared with  $I_{60}$ . The seeds of these plants were also expected to have less protein, but a higher yield.

# 4 CONCLUSION

The present study was designed to determine the effect of soil moisture content on ecophophysiological responses of Lallemantia species. This study has shown that L. royleana has tolerance to water shortage in soil. Despite drought reduced leaf area index, stomatal conductance, and photosynthetic pigments, which ultimately reduces grain yield, water use efficiency significantly increased with the increment in severity of drought stress especially in L.royleana. The evidence from this study suggests that Lallemantia is an efficient plant in low input systems of water supply. An implication of this is the possibility for producer to use Lallemantia as an important medicinal and industrial plant to grow in low input systems in which water scarcity is the most limiting growth factor. This research extended our knowledge about Lallemantia species responses specially water use efficiency under water shortage condition in soil. This is the first study reporting water use efficiency of two species of Lallemantia. However these findings are limited by the use of one seed lot of each species. It is recommended that further research be undertaken with 3 or more seed lots.

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