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Research article

Balangu (*Lallemantia* sp.) growth and physiology under field drought conditions affecting plant medicinal content

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ABSTRACT

Drought stress is one of the most important stresses adversely affecting plant growth and yield production. Due to the importance of global warming, the investigation of drought effects on the growth and quality of medical plants is of vital importance. Accordingly, a two-year field experiment was conducted in 2013–2014 to determine the effects of drought levels and plant species on Balangu (*Lallemantia* sp.) growth and physiological properties including medicinal content. The experiment was a split plot in which the drought levels (main plots) including control (D1, moisture field capacity, water potential at, 0.5 atm), moderate stress (D2, 6.5 atm) and severe stress (D3, 9.5 atm), and the Balangu species (sub plots) including *Lallemantia royleana* (Benth) (L1) and *L. iberica* (L2) were tested as the experimental treatments. Plant yield, oil content and the biochemical properties (i.e. medicinal content) including phenolic compounds, proline, carotenoids, and the activity of antioxidant enzymes including peroxidase (EC 1.11.1), super oxide dismutase (SOD, EC 1.15.1.1)) and ascorbate peroxidase (APX, EC 1.11.1.11) were determined. Drought stress significantly decreased crop yield and oil content. However, the production of phenolic compounds and proline as well as the activity of antioxidant enzymes, SOD and APX increased under stress. The species L2 was the more tolerant species under drought stress. The interesting point about this research work is the increased production of secondary metabolites (i.e. phenolic compounds) under stress, affecting both Balangu response and medicinal properties. Accordingly, it may be possible to regulate the production of secondary metabolites (medicinal contents) in Balangu species by adjusting the irrigating practices.

1. Introduction

Drought stress is one of the most important stresses adversely affecting plant growth and yield production. The unfavorable effects of drought stress on plant morphology and physiology, decrease crop yield. Under drought conditions plants use the following morphological and physiological mechanisms to alleviate the stress: 1) leaf rolling, reducing evapotranspiration, 2) regulation of leaf stomata activities, 3) production of different metabolites such as proline, affecting cellular water potential, 4) activation of different signaling pathways including the hormonal pathways, and 5) production of antioxidants (Ferdous et al., 2015; Forni et al., 2017).

Plants use two different strategies including avoidance and tolerance to grow under drought stress. In avoidant species, plants are able to have a normal growth, which is due to: 1) efficient and fast metabolism, 2) high uptake of water and nutrients, and 3) little production of secondary metabolites under deficient water conditions. However, in tolerant plant species, they maintain their regular growth, under drought stress, by: 1) adjustment of osmotic potential, 2) changes in cell wall properties, and 3) production of antioxidants and secondary metabolites (Varela et al., 2016). However, under prolong drought, and irrespective of plant type, plant produces higher rate of secondary metabolites, as a non-enzymatic mechanism. Such products are able to maintain plant activities, under oxidative stress, and in the presence of high rate of reactive oxygen species (Varela et al., 2016). Phenolic compounds, acting as antioxidants, can alleviate the stress by scavenging reactive oxygen species and maintaining cellular functionality (Agati and Tattini, 2010).

Medicinal plants, including Balangu*a* (*Llemantia* sp.), are cropped in different parts of the world including Iran (Samadi et al., 2007) for food and biodiesel purposes. The annual plants from this genus (Lamiacaeae family) are 15–50 cm tall, with the growth period of

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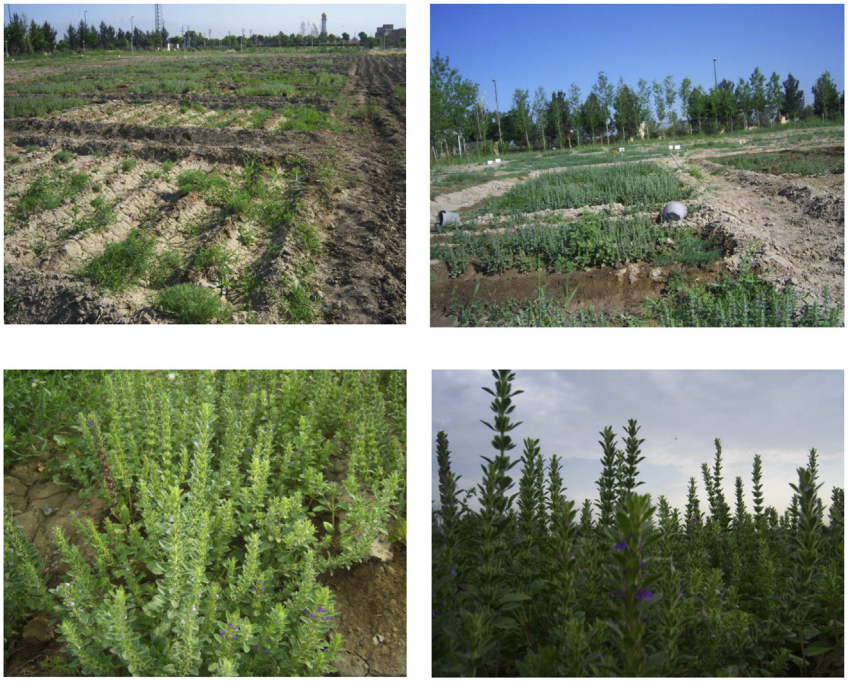

Fig. 1. Balangu seedlings and plants in the research field during the experiments.

90–120 d. These plants are sources of secondary metabolites and their seeds contain mucilage, polysaccharide, fiber, oil and protein (Moon et al., 2009; Zlatanov et al., 2012). Global climate change and the subsequent increasing drought significantly reduce medicinal plant growth and yield in different parts of the world. Accordingly, improving the stress tolerance of medicinal plants is an important issue, which must be considered during the cultivation of medicinal plants (Tilman and Clark, 2014; Elmendorf et al., 2015).

Investigating the effects of drought stress on the production of secondary compounds by medicinal plants is an important issue. According to the research work drought stress increases the production of secondary compounds affecting the quality of medicinal plants. This must be considered when developing tolerant medical plants under stress, especially if the quality of medical plants is of higher importance than their quantity. The tolerance of medicinal plant species is different under stress (Wilson and Roberts, 2014; Varela et al., 2016).

Different researchers investigated the effects of drought on the growth and quality of medicinal plants. For example, Kleinwächter et al. (2015) found that drought stress and stress signal tranducers (methyl jasmonate and salicylic acid) increased the production of secondary metabolites in different medicinal plants, and hence improved the quality of such medicinal plants. In another research work drought stress increased the production of secondary metabolites including polysaccharide, flavonoids, and terpenoids (in plant aerial part) and antioxidant enzymes in *Dendrobium moniliforme* enhancing the quality of the medicinal plant. The authors suggested that such physiological mechanisms make the plant survive the stress (Wu et al., 2016).

Kannan and Kul (2011) inspected the effects of drought on *Withania* (Solanaceae, a tropical medicinal plant) growth and physiology and found that drought significantly decreased plant biomass as well as photosynthetic pigments and activity, compared with control (non-stressed plants). The proteomic analysis of plants under stress indicated that the response of proteins (in the range of 34–40 kDa) was different. Using HPLC the differences in the root extracts of stressed and non-stressed plants were observed. Khorasaninejad et al. (2011) investigated the effects of drought stress on peppermint (*Mentha piperita* L.) growth and essential oil production. According to their results, drought stress significantly decreased plant growth as well as essential oil yield and fractionation, as indicated by GC-MS.

According to the above mentioned details the growth and quality of medicinal plants is affected by drought. For example, different research work has indicated that the production of phenolic compounds by medicinal plants usually increases under drought. The other important factor affecting the composition and the quality of secondary metabolites in medicinal plants is stress intensity and duration (Ncube et al., 2012; Varela et al., 2016; Yang et al., 2018).

Under drought stress the expression of flavonoid biosynthesis genes and the subsequent accumulation of flavonoids in different wheat genotypes increased (Ma et al., 2014). Flavonoids (polyphenolic secondary metabolites), with little molecular weight in plants, have different roles in plants, for example they act as signal molecules in the symbiotic process of N-fixation between legumes and rhizobium (Miransari et al., 2013). Interestingly, although according to research work the excretion of flavonoids by the legume host plant roots decreases under stress (Miransari and Smith, 2007, 2008, 2009), the overall production of flavonoids increases in the aerial part of crop and medicinal plants.

Due to the importance of drought and its effects on the growth and the quality of medicinal plants, in this research work the effects of stress intensity and plant species on the growth and physiology (including the medicinal contents) of Balangu plants were investigated in a two-year field experiment. It is because, to our knowledge, there are not much data on such effects. The objectives were to investigate the effects of drought stress on: 1) Balangu growth and yield, and 2) Balangu physiology including the activities of antioxidant enzymes, and the production of phenolic and proline compounds affecting plant medicinal contents.

2. Materials and methods

2.1. Field location

A two-year field experiment (2013–2014) was conducted to investigate the effects of plant species and drought levels on Balangu growth and medicinal contents. The experiment was performed in a research farm, in the northern latitude of 35° and 34′ and the eastern

Table 1
The physical and chemical properties of the experimental fields.

Texture	Clay (%)	Silt (%)	Sand (%)	K (mg/kg)	P (mg/kg)	N (%)	O.C. (%)	pH	EC (dS/m)
2013									
Loamy	20	36	44	270	7.6	0.05	0.57	8.1	3.75
2014									
Loamy	21	34	45	247	9.3	0.07	0.65	7.9	3.39

EC: electrical conductivity, O.C.: organic carbon, P: phosphorus, K: potassium.

longitude of 51° and 8′, 1190 m above the sea level in the southern part of Tehran province, Iran (Fig. 1). The physical and chemical properties of soil were analysed using the standard methods (Miransari et al., 2008) (Table 1).

2.2. Experimental design

The experiment was a split plot on the basis of a completely randomized block design in three replicates, using drought levels including control, (soil moisture = field capacity at 0.5 atm, D1), medium (D2 = 6.5 atm) and sever (D3 = 9.5 atm) as the main plots, and Balangu species including *Lallemantia royleana* (Benth.) (L1) and *Lallemantia iberica* (L2) as the subplots. The field was surface irrigated to acquire the desired levels of soil moisture.

2.3. Field practices

The field was prepared using moldboard and smoothed using disk, the plots (2 × 3 m) were established in the month of March 2013 and 2014. The seeds were planted in the depth of 1–1.5 cm in the plots, which were 1 m apart, and consisted of 10 rows (30 cm apart). The field was fertilized according to soil testing using urea (170 kg/ha) and ammonium phosphate (180 kg/ha). The plants were subjected to intermittent drought. The field drought was measured using an eco tensiometer, which was placed in the plant root area two months before establishing the stress. Following the installation of the instrument, the soil was saturated and the trend of soil suction and moisture content was investigated in a 60-day period each 1 h. The collected data was then transferred to a computer and analysed. The analysed data were used for establishing the desirable water potential, irrigation interval and the time of imposing the drought (at the end of flowering stage).

2.4. Collecting the samples

Seven samples were randomly collected from each plot at the time of physiological maturity, 12–24 h after imposing the final drought levels. For the measurement of morphological traits, the samples were air dried under the shade. For the measurement of antioxidant enzymes, the samples were immediately frozen in liquid nitrogen and kept in a freezer at −80 °C before conducting the biochemical analyses.

2.5. Yield and oil measurements

Grain yield (GY) was determined by collecting plants in a 5-m area at physiological maturity. Harvest index (HI) was also calculated by dividing biological yield by the grain yield. The amount of grain oil was also determined using the Soxhlet method according to the following details. The grains were first dried using autoclave and were then powdered. Using a cellulose cartridge the samples were placed in the upper part of the Soxhlet apparatus. The volatilization of the solvent diethyl ether, in the bottom of the apparatus, resulted in solubilizing the oil, which was then isolated, collected and weighed following the evaporation of the solvent (Soxhlet, 1879).

2.6. Carotenoids

The amount of leaf carotenoids was determined according to the following: 0.5 g of fresh leaf was treated with 20 ml of acetone 80% and the extract was collected. The extract was then filtrated using a filter paper and the amount of leaf carotenoids was determined at the wavelength of 480 nm using spectrophotometer (Gu et al., 2008).

2.7. Proline

Leaf proline was determined using the method of Bates et al. (1973); 0.5 g of fresh leaf was ground with quarts in porcelain pestle and mortar and treated with 10 ml sulfosalicylic acid; the homogenate was centrifuged at 13,000*g* for 10 min. Two mili liters of the filtrated extracts were transferred to tubes, with cover and treated with 2 ml of ninhydrin indicator and 2 ml of glacial acetic acid. The tubes were placed in boiling water at 100 °C for 1 h. The tubes were cool down and treated with 4 ml of toluene and shaken with a vortex for 15–20 S. The red surface layer and standard samples were simultaneously placed in a spectrophotometer and the absorption of the samples was determined at the wave length of 520 nm. The amount of proline (micromol/g fresh weight) was calculated using regression equations and the standard curves.

2.8. Peroxidase (EC 1.11.1)

The activity of peroxidase enzyme was measured according to the method of Wendel (1981); 0.5 g of plant tissue was homogenized with a buffered potassium phosphate (0.2 M at pH = 6.8) using a cold porcelain pestle and was then centrifuged at 12,000*g* for 15 min and the solution was used for the measurement of peroxidase activity. The reaction solution was incubated with 0.25 ml of raw enzyme, 0.25 ml of guayacol (20 g/l) and 5.2 ml of buffered phosphate (100 M at pH = 6.8) at 30 °C for 10 min. The increased absorption was measured 3 min after adding 0.25 ml of H_2O_2 with the final concentration of 5 mM using a spectrophotometer at the wavelength of 470 nm. The following standard solution (100 ml at pH = 6.8) was used: 0.25 ml guayacol 0.5%, 25 ml H_2O_2, 25 ml distilled water and 2.25 ml buffered phosphate.

2.9. Super oxide dismutase (EC 1.15.1.1)

The activity of super oxide dismutase (SOD) was determined according to the following details (Sun and Zigman, 1978). The frozen sample at 0.2 g was extracted using 3 ml of buffered HEPES-KOH (pH = 7.8) containing 0.1 mM EDTA. The extracts were centrifuged at 15,000*g* for 15 min at 4 °C. The supernatant was used to determine the activity of super oxide dismutase. The reacting compound contained 50 ml buffered HEPES-KOH (pH = 7.8), 0.1 mM EDTA, 50 mM sodium carbonate (pH = 10.2), 12 mM L-methionine, 75 mM nitroblue tetrazolium (NBT), 1 μM riboflavin and enzymatic extract. The amount of enzyme, which results in the inhibition of 50% NBT at the wavelength of 560 nm, is equal to the activity of one unit.

2.10. Ascorbate peroxidase (EC 1.11.1.11)

The activity of ascorbate peroxidase (APX) was determined according to the following details; 0.2 g fresh plant tissue was homogenized with 5 ml of a bufferic solution with 1 mmol ascorbate, 1mmole EDTA and 50 mmol potassium phosphate (pH = 7.0) using a cold porcelain pestle. The extract was then filtrated and centrifuged for 10 min at 10,000*g*, and the supernatant was used to determine the enzymatic activities. The solution contained 50 mmol potassium phosphate (pH = 0.7), 0.5 mmol ascorbate, 1 mmol H_2O_2, and 1 mmol EDTA and the total volume of the extracted enzyme was equal to 1 ml. The reaction initiated with H_2O_2, and the rate of absorption was measured for 10–30 S at the wavelength of 290 nm. The activity of enzyme was determined on the basis of μmole oxidized ascorbate per minute per mg protein (Asada, 1992).

2.11. Phenol

Measurement of phenol was conducted according to the following: 0.2 g of sample was mixed with 20 ml boiled water using a tube, which was then placed for 1 h in a bon mary. The samples were then filtrated using filter paper No. 1 and brought to the volume of 25 ml. Then one mili liter of extract was mixed with 5 ml indicator of Folin-Denis and 10 ml sodium carbonate ($NaCO_3$ 35%), and was brought up to volume using a 100-ml volumetric flask. The solution was left undisturbed under the room temperature for 45 min. Finally, the absorption of samples was calculated using the standard curve of galic acid (Faurobert et al., 2007).

2.12. Statistical analysis

Data were subjected to analysis of variance using SAS 9.4. Means were compared using Duncan's Multiple Range Test (P = 0.05).

3. Results

Different experimental treatments including year, drought and Balangu species as well as their interactions significantly affected GY, HI, oil, proline, carotenoids, peroxidase, SOD, APX and phenol (Table 2).

3.1. 2013

The amounts of GY, in 2013, was in the range of 101.56 kg/ha (*L. royleana* and D3) and 940.78 kg/ha (*L. iberica* and D1), which was significantly higher than the other treatments. Stress significantly decreased plant yield, however, *L. iberica* was the more tolerant genotype. Similarly, HI was the highest (24.65%) by *L. iberica* and D1, and it was the least by *L. iberica* and D3 (4.30%). The amount of oil was in the range of 26.65 kg/ha by *L. iberica* and D3 and 227.46 kg/ha by *L. iberica* and D1 (Table 3).

The largest proline amount (26.26 μmol g^{-1} FW) was resulted by *L. iberica* and D3 and the least (5.60 μmol g^{-1} FW) by *L. iberica* and D1. The amounts of carotenoids ranged from 20.7 (*L. royleana* and D3) to 98.2 (*L. royleana* and D1) mg g^{-1} FW. Peroxidase was in the range of 0.017 (*L. iberica* and D3) and 0.065 (*L. iberica* and D2) mM H_2O_2 mg^{-1} min^{-1} (Table 3).

3.2. 2014

In the second year of experiment the highest GY (519.81 kg/ha) was related to *L. iberica* and D1 and the least (194.43 kg/ha) to *L. roylena* and D3. However, HI was in the range of 7.25 (*L. iberica* and D3) and 20.16% (*L. iberica* and D2). The amount of oil was the highest by *L. royleana* and D1 (143.58 kg/ha) and the least (42.01 kg/ha) by *L. iberica* and D3 (Table 3).

Drought stress (D3) significantly increased the amount of proline in *L. iberica* (13.28 μmol g^{-1} FW), however, the least amount (2.70 μmol g^{-1} FW) was resulted under non-stressed conditions by *L. royleana*. Carotenoids ranged from 40.8 mg g^{-1} FW by *L. iberica* under stressed conditions to 91.2 mg g^{-1} FW by *L. iberica* under non-stressed conditions. The activity of peroxidase was the least under stressed conditions by *L. iberica* (0.024 mM H_2O_2 mg^{-1} min^{-1}) and the highest under non-stressed conditions by *L. royleana* (0.217 mM H_2O_2 mg^{-1} min^{-1}) (Table 3).

3.3. SOD, APX and phenol

With increasing the level of stress the activity of SOD increased as under D2 the species *L. royleana* and under D3 the species *L. iberica* resulted in significantly higher activity of SOD, than the control conditions (Fig. 2). The increased activity of APX under drought stress was more evident than SOD as in both genotypes with increasing the level of stress the activity of APX significantly increased, compared with the control treatment (Fig. 3). The amount of phenol was the highest under D2 in both genotypes compared with D1 and D3; however it was only significantly different from *L. royleana* under D1 (Fig. 4).

4. Discussion

A set of yield and biochemical properties of two different species of Balangu medicinal plant as affected by drought levels were determined. According to the analysis of variance the yield and biochemical properties of Balangu were significantly affected by drought, and there were significant differences between the two species. The superior genotype, in both years, was *L. iberica*, which resulted in significantly higher yield compared with *L. royleana* under D1 and D2. In the first year *L. Iberica* resulted in the highest HI under D1 and in the second year under D2.

Table 2
Analysis of variance indicating the effects of different experimental treatments on the yield and physiology of Balangu.

Phenol	APX	SOD	Peroxidase	Carotenoids	Proline	Oil	HI	GY	d.f.	S.V.
37.31**	1.57**	0.73**	0.155ns	2184**	5.71**	0.00004ns	1.05**	0.0001ns	1	**Y**
0.318	0.0223	0.0013	0.048	67.9	0.052	0.0002	0.045	0.0004	4	**Y (error)**
17.42**	27.24**	4.14**	6.11**	5913**	3.69**	0.000006ns	0.87**	0.01*	2	**D**
0.603*	0.117*	0.091*	0.018ns	147.7ns	0.21**	0.00009**	0.2ns	0.012*	2	**Y x D**
0.0945	0.023	0.0126	0.0049	65.25	0.0076	0.000008	0.059	0.0016	8	**Error (a)**
0.272ns	4.06**	0.0036ns	0.97**	1334**	1.21**	0.00003ns	0.673**	0.024**	1	**S**
12.94**	2.08*	0.789**	2.72**	177.1*	0.134*	0.000004ns	1.34**	0.021**	2	**D x S**
4.75*	0.55*	0.039*	0.49**	119.3ns	0.49**	0.00002ns	0.128ns	0.0006ns	1	**S x Y**
0.163ns	0.098ns	0.0006ns	0.208**	1549**	0.64**	0.00004ns	0.9**	0.008*	2	**D x S x Y**
0.574	0.0667	0.0053	0.006	27.71	0.007	0.00002	0.046	0.024	12	**Error (b)**
11.87	8.79	3.25	2.76	9.52	4.12	9.33	9.58	2.73		**C.V.**

S.V.: Source of variation, Y: year, D: drought stress, S: Balangu species, C.V: coefficient of variation, d.f.: degree of freedom, GY: grain yield, HI: harvest index, SOD: super oxide dismutase, APX: ascorbate peroxidase.

Table 3
Balangu growth, yield and physiology affected by different species and drought levels.

Peroxidase (mM H_2O_2 mg^{-1} min^{-1})	Carotenoids (mg g^{-1} FW)	Proline (μmol g^{-1} FW)	Oil (kg/ha)	HI (%)	GY (kg/ha)	S	D	Y
0.06b	52.9c	6.9de	227.46a	24.65a	940.78a	*L. iberica*	D1	**2013**
0.185a	98.2a	5.60f	60.77b	5.64bc	229.06b	*L. royleana*		
0.065b	32.1c	22.51b	67.75b	9.62a	272.46b	*L. iberica*	D2	
0.045c	47.2b	8.59d	44.23c	7.37bc	200bc	*L. royleana*		
0.017d	33.6c	26.26a	26.65d	4.30c	175bc	*L. iberica*	D3	
0.043c	20.7d	13.88c	43.83c	6.82bc	101.56c	*L. royleana*		
0.079b	91.2a	4.01d	124.9a	12.54b	519.81ab	*L. iberica*	D1	**2014**
0.217a	79.7a	2.70e	143.58a	12.09b	665.5a	*L. royleana*		
0.103b	44.3c	3.51d	135.96a	20.16a	501.78b	*L. iberica*	D2	
0.034d	61.9b	6.17c	79.05b	8.05c	315.75c	*L. royleana*		
0.024e	40.8c	13.28a	42.01c	7.25c	212.41d	*L. iberica*	D3	
0.037d	60.3b	7.47b	45.46c	11.94b	194.43d	*L. royleana*		

Y: year, D: drought stress, S: Balangu species, GY: grain yield, HI: harvest index.

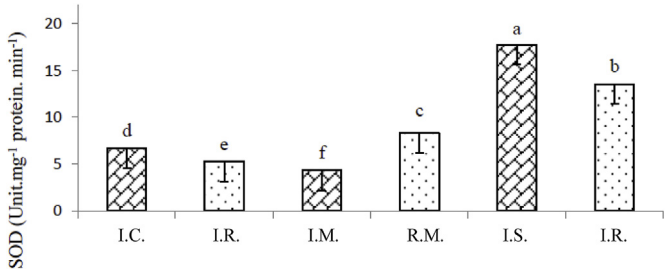

Fig. 2. Super oxide dismutase activity affected by different Balangu species and drought levels, columns with different letters are significantly different at P = 0.05, using Duncan's multi range test, I: *L. iberica*, R: *L. royleana*, C: Control drought, M: moderate drought, S: Sever drought.

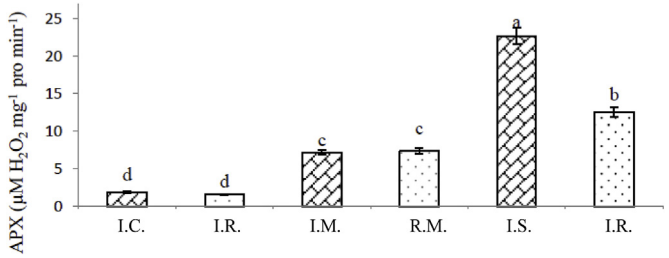

Fig. 3. Ascorbate peroxidase activity affected by different Balangu species and drought levels, columns with different letters are significantly different at P = 0.05, using Duncan's multi range test, I: *L. iberica*, R: *L. royleana*, C: Control drought, M: moderate drought, S: Sever drought.

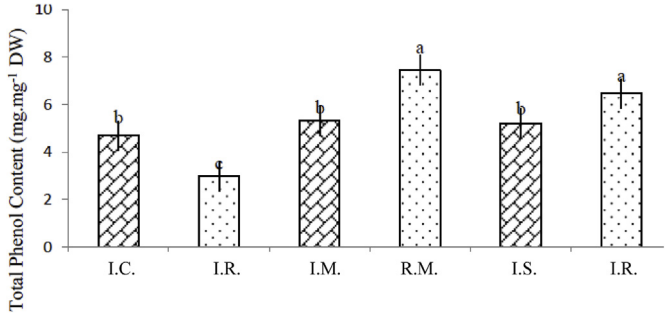

Fig. 4. Total phenol content affected by different Balangu species and drought levels, columns with different letters are significantly different at P = 0.05, using Duncan's multi range test, I: *L. iberica*, R: *L. royleana*, C: Control drought, M: moderate drought, S: Sever drought.

The amount of oil was the highest under non-stressed conditions, although in the second year, under D2, the species *L. iberica* also resulted in oil amounts, comparable to the amount of oil under D1.

Increased levels of drought significantly enhanced the rate of proline in both years by *L. iberica*. The increased production of proline, under drought stress, is among the most sensitive osmoticum responses of plants. Zali and Ehsanzadeh (2018) found that drought stress increased the production of leaf phenolic compounds, total soluble carbohydrates and proline, and the concentration of essential oils, and decreased plant growth, water related properties, and carotenoids and chlorophyll concentrations. However, the exogenous use of proline significantly increased phenolic compounds, chlorophyll, carotenoids, total soluble carbohydrate, proline, concentration of essential oil and total water content. Proline improved plant water properties by adjusting osmoregulation, because the genotypes with higher proline concentration and water content had a higher growth, when exogenous proline used. The authors indicated that the alleviating effects of exogenous proline is more pronounced in drought stressed plants as they contained higher rate of chlorophyll and relative water content.

Similarly, the results of this research work indicated that the rate of phenol was significantly higher in *L. royleana* than *L. iberica* under D1 and D2. Although, water stress may decrease the level of photosynthesis and the production of different metabolites, other research work has also indicated that the production of phenolic compounds increases under drought stress (Ncube et al., 2011, 2012; Gnanasekaran and Kalavathy, 2017), which is a mechanism used by plants to avoid the unfavorable effects of drought stress (Varela et al., 2016).

Soni and Abdin (2017) found that in drought stressed plants the concentration of proline, protein and lipid peroxidation as well as the activity of antioxidants including SOD and APX increased. Water stress induced the expression of proline biosynthetic genes such as pyrroline-5-carboxylase synthase1, 1-pyrroline-5-carboxylase synthase2 and 1-pyrroline-5-carboxylase reductase. However, under water stress, the activity of proline catabolic genes including proline 5-carboxyalte dehydrogenase and proline dehydrogenase1 decreased, which is a defensive strategy used by plant under stress. Similarly, our results indicated that the highest level of stress significantly increased the rate of SOD and APX in *L. iberica*.

The important aspect about this research work is that drought improves the medicinal content and hence the pharmaceutical properties of Balangu. This is of significance for planting medicinal plants under arid and semi arid conditions compared with moderate climatic conditions. The metabolic basis for the increased production of secondary materials under drought stress is according to the following. Under water deficient conditions, due to the closure of stomata, CO_2 uptake significantly decreases. Accordingly, the consumption of reducing $NADPH + H^+$ for the fixation of CO_2 by the Calvin cycle significantly decreases and results in the accumulation of $NADPH + H^+$.

Consequently, the production of highly reduced products including isoprenoids, phenols and terpenoids increases (Selmar and Kleinwächter, 2013). The authors indicated that although the moderate level of drought can markedly increase the production of secondary metabolites in medicinal plants, treating the plants with high concentration of CO2 (700 ppm) can also have similar effects. Accordingly, it is possible to adjust the rate of secondary metabolites and enhance the quality of medicinal plants by imposing the plants to drought stress.

In an interesting research work, Kumar et al. (2017) investigated the effects of climate change on the medicinal properties of *Aloe vera*. They found that the main phytochemicals in *A. vera* were phenols, flavonoids, terpenes and saponins. The highest rate of secondary metabolites was found under arid and semi arid conditions and the least under tropical conditions. The authors accordingly indicated that agro-climatic conditions as an important parameter can significantly affect the secondary metabolites in *A. vera*.

5. Conclusion

According to the results of this research work, growth and the biochemical properties including medicinal content of different species of Balangu, may be differently affected by drought stress. The plant uses some avoiding and tolerating mechanisms to survive under drought stress. The results indicated that the plant can tolerate the stress up to some level, and the genotype *L. iberica* was the more tolerant species under stress. According to the results such a tolerance is a result of some physiological and biochemical activities in the plant among which the increased production of phenolic compounds, proline, and antioxidant enzymes such as SOD and APX are the most important ones. The other interesting point is that the increased level of drought stress results in the increased production of secondary metabolites such as phenolic compounds, which are of both medicinal and defensive values. Accordingly, it may be possible to regulate the production of secondary metabolites in the medicinal plant Balangu by regulating the rate of irrigation. Future research may investigate how it is possible to alleviate drought stress on the growth of Balngu and in the meanwhile obtain Balngu plants, which are more efficient in the production of secondary metabolites.

Author contribution

The interesting point about this research work is the increased production of secondary metabolites such as phenolic compounds, under stress, which can affect both plant response as well as its medicinal properties. Accordingly, it may be possible to regulate the rate of secondary metabolites and hence the medicinal properties in Balangu by adjusting the amount and the time of irrigation.

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