 doi:10.20944/preprints202010.0110.v1

Article

Differential response of *Linum album* Ky. ex Boiss. accessions to long-term water deficit stress

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Abstract: *Linum album* is an important medicinal plant contains important lignan compounds such as podophyllotoxin as well as fatty acids. Despite the high medicinal value, it has not been studied in agricultural conditions so far. This study was conducted to evaluate the morphological, phenological, and physiological responses of six *L. album* accessions under water deficit treatments (100% available water, 75%, 50%, and 25%) in pot conditions. Based on the results the morphological properties of accessions reduced due to water deficit. Accessions of UTLA7 and UTLA9 showed higher seed yield and dry weight of the vegetative part. The occurrence of phenological stages in the accessions showed a significant difference. Maturity was accelerated in plants under stress conditions, and accession of UTLA9 completed its growth earlier than others. Physiological responses of the accessions did not have the same trend based on the measured traits, and significant differences were observed depending on the trait and accession. The most important result of this study was the diversity of responses in different accessions. The results showed that the effect of water stress on the measured traits depends on the level of stress and accession, which suggests that it is possible to select the tolerable accessions for the production of the desired product. Based on the results, plant breeders may be able to use the chlorophyll content as a marker to identify tolerate *L. album* accessions.

Keywords: Abiotic stress; *Linum album* Ky. ex Boiss.; Morphological properties; Phenology; Pigments; Seed yield.

1. Introduction

Linum album Ky. ex Boiss. is a perennial medicinal plant belonging to the Linaceae family. This plant known as an endemic plant of Iran and grows in northwest, west, and center of Iran. Flowering and maturing of this plant last from May to July [1,2]. *L. album* contains important lignan compounds such as podophyllotoxin and 6-methoxy podophyllotoxin that have antiviral and antitumoral properties [3]. These substances have great importance today as raw materials for some anticancer drugs. Podophyllotoxin is the most important aryltetralin lignan for human health [4]. The compound is used to produce the semi-synthetic anticancer drugs like Etoposide, Teniposide and Etopophos, which are used to curing lung, ovarian and brain cancer [5]. *L. album* seeds also accumulate fatty acid compounds like palmitic, stearic, oleic, linoleic, and linolenic acid [6].

Plants are confronted with lots of biotic (such as fungi, viruses, and insects) and abiotic (such as drought and salinity) stresses in the environment. Environmental stresses confine crops yield and originate many changes in their molecular processes like variation in metabolite profile [7]. Water deficit stress is a condition of insufficient water availability, caused by intermittent to continuous periods without irrigation [8]. In Iran, more than 75% of the arid and semiarid regions have been classified as water deficient regions [9]. The outcome of water deficient is limiting the distribution and survival of plants in arid and semi-arid regions [10-12]. Water deficit condition as the most important stress in plants conducts to an unusual increase in reactive oxygen species (ROS) production [13]. ROS can damage cells membrane and increase production of malondialdehyde

 doi:10.20944/preprints202010.0110.v1

(MDA) content [14]. However, to fight the produced ROS, plants use enzymatic and non-enzymatic systems. Plants employ both enzymatic and non-enzymatic systems to cope with produced ROS [13]. The enzymatic antioxidant processes involved the activity of enzymes like catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX), and polyphenol oxidase (PPO) [13,15,16].

Water deficit stress also affects the rate of plant growth and development [17,18]. Under water stress conditions, plants completes their life cycle faster than under normal conditions, consequently, crop growth stages will have a short duration, with fewer days to accumulate assimilates during life cycle, and in consequence the production of biomass is reduced [19,20]. Crops have a definite temperature requirement before they attain certain phenological stages. The accumulative heat units and system was adopted for determining the dates to flowering and maturity of different field crops [18,21]. However, different phenological stages vary in their sensitivity to drought, and this depends on plant species and genotype as there are wide inter- and intra-specific variations [22,23].

Abscisic acid (ABA) is one of the most important hormonal moderators of abiotic and biotic signals, and as such, ABA acts as the interpreter of the environment, regulating key physiological processes such as germination, stomatal movements, dormancy, and plant-microbe interactions [24]. Accordingly it is urgent to assess the variations in biochemical mechanisms induced by the application of water deficit conditions by analyzing the role of abscisic acid [25] and also proline accumulations [26,27]. In fact, ABA is known as a key stress-signaling hormone, acting in the regulation of stomatal closure, synthesis of compatible osmolytes, and in the upregulation of genes leading to adaptive responses [25]. Proline, as an active molecule between osmolytes, is formed as a result of oxidative stress by free radicals stimulating. [26,27]. Proline accumulation acts as a protective osmolyte for plants in the face of different environmental stresses [28,29].

The focus of most researches on *L. album* has been to enhance lignan compounds in in vitro culture. Different techniques such as optimization of culture medium [30], use of elicitors [31] and inducing polyploidy [32] have been effective in increasing the accumulation of lignans in in vitro culture of *L. album*. However, insufficient research has been done to increase lignans under greenhouse or field conditions

In short, the purpose of this study is to investigate and understanding morphological, phenological, and physiological responses of six *L. album* accessions to water deficit stress conditions.

2. Results

2.1. Morphological properties

The response of *L. album* accessions to different irrigation levels showed a significant difference in most traits including seed width, weight of 1000 seeds, inflorescence length, main branch length, plant height, leaf length, flower diameter, root length, fresh and dry weight of shoots and roots (Table 3 and S1).

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Table 3. Effect of water deficit stresses on morphological traits of *L. album* accessions.

Accession/ Irrigation	Number of seeds per capsule	Seed yield per plant (mg)	Seed width (mm)	Weight of 1000 seeds (mg)	Number of flowers in inflorescence	Number of mature capsules	Inflorescence length (cm)	Main branch length (cm)	Plant height (cm)
UTLA1									
100% AW	8.7±0.15	1857.67±33.58	2.35±0.03	3124.33±102.63	7.33±0.51	6.76±0.57	13.24±0.38	14.83±0.44	27.5±0.76
75% AW	8.31±0.18	613±36.37	2.29±0.06	3031.22±140.7	11.67±0.67	11±1	13.33±1.2	13.33±0.67	23.83±0.6
50% AW	8.5±0.69	599±58.69	2.25±0.03	3124.22±162.93	10.08±1.08	9.42±1.39	15.11±0.49	11±0.58	24.67±2.19
25% AW	7.56±0.34	705±56	2.31±0.01	3362.22±163.57	8.08±0.65	6.25±0.8	17.75±0.43	13.5±1.04	25±0.5
UTLA6									
100% AW	7.55±0.62	1340.33±157.99	2.43±0.06	3415.11±157.26	11.39±1.52	10.56±1.44	15.37±1.81	15.5±1.32	29.33±1.45
75% AW	7.92±0.52	959.33±85.23	2.36±0.04	3506±124.67	9.33±0.95	8.83±0.98	17.49±1.5	17±1.04	34.67±0.73
50% AW	7.04±0.61	1014±96.44	2.48±0.06	3871.78±127.82	12.18±0.84	11.16±0.63	15.48±1.58	14.67±0.73	30.17±1.64
25% AW	9.06±0.48	1333.33±93.98	2.55±0.01	3697.11±115.25	9.94±1.06	8.44±0.29	12.59±0.6	15.83±0.6	27.83±1.3
UTLA7									
100% AW	8.49±0.18	1465.33±147.89	2.46±0.02	3567.11±154.27	8.94±0.78	8.49±0.7	16.45±0.78	17.33±0.6	31.5±1.53

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75% AW	9.23±0.31	2785.67±76.36	2.4±0.07	3426.67±160.81	8.78±0.91	7.89±1.02	18.78±0.94	21±0.58	36.17±0.73
50% AW	8.59±0.33	1241.67±127.91	2.48±0.06	3656.89±82.32	8.87±0.38	7.88±0.06	15±0.86	19.33±1.09	31.83±1.17
25% AW	7.44±0.06	546.33±71.52	2.49±0.07	3394.89±140.83	7.21±0.74	6.07±0.97	12.88±0.74	17.67±0.17	28.33±0.33
UTLA9									
100% AW	8.96±0.13	1434.33±130.24	2.31±0.05	3383.11±116.11	12.81±0.78	11.72±0.64	23.25±0.59	17.33±0.88	36.5±1.32
75% AW	8.74±0.41	2267±184.46	2.54±0.07	3835.33±108.74	10.74±0.9	10.3±0.71	21.66±1.28	18.5±0.29	36.17±1.88
50% AW	6.92±0.36	1159.67±137.19	2.42±0.09	3714.22±44.73	10.92±0.84	9.19±0.91	19.87±0.13	16.5±1.04	34.67±0.67
25% AW	8.96±0.14	1907.67±15.3	2.18±0.05	2976±60.48	8.56±0.29	6.73±0.91	15.67±1.18	14±0.5	30.5±1.76
UTLA10									
100% AW	8±0.19	1186.67±79.96	2.37±0.06	3670.89±39.88	9±0.96	8.89±0.87	17.2±0.31	18.17±0.88	31.5±1
75% AW	8.83±0.33	1320±48.05	2.46±0.08	3667.22±84.54	10.97±0.51	9.72±0.36	17.63±0.59	18.83±0.93	33.17±0.93
50% AW	8.44±0.48	1312±134.61	2.39±0.04	3494±94.56	8.78±1.61	7.69±0.54	14±0.51	20.43±0.74	30.17±1.48
25% AW	9.05±0.22	1484.67±62.6	2.42±0.01	3860.89±155.74	7.78±0.22	6.41±0.13	14.72±1.07	17.83±0.44	29.67±1.3
UTLA12									
100% AW	8.39±0.29	1240.67±104.22	2.39±0.04	3807.78±147.78	9.58±1.05	9.4±0.97	18.97±0.8	19.67±0.88	36.17±0.73

 doi:10.20944/preprints202010.0110.v1

75% AW	8.72±0.24	1384.33±286.45	2.38±0.07	3925.78±128.13	10.22±0.67	9.44±0.73	14.17±0.73	13.33±0.6	22.17±1.01
50% AW	8.86±0.56	452.33±63.3	2.46±0.08	3913.22±82.71	7.42±0.3	6.83±0.17	16.92±0.92	15.33±0.88	28±1.53
25% AW	8.34±0.6	587±71.67	2.41±0.07	3677.78±70.63	7.02±0.39	6.11±0.36	13.88±0.51	15.83±0.6	25.83±1.42
LSD (1%)	1.5	441.85	0.2	465.77	3.05	2.95	2.68	2.96	4.67

Continued Table 3.

Accession/ Irrigation	Leaf length (mm)	Number of inflorescences per plant	Number of chlorosis leaves	Flower diameter (mm)	Root length (cm)	Shoot fresh weight (mg)	Shoot dry weight (mg)	Root fresh weight (mg)	Root dry weight (mg)
UTLA1									
100% AW	16.07±1.27	5.33±0.33	1.44±0.29	34±0.58	26.6±0.81	21.85±1.47	5.92±0.42	24.11±3.2	3.44±0.32
75% AW	19.08±0.58	3±0.58	1.56±0.06	32.33±0.67	23.5±0.87	27.4±0.35	7.68±0.19	46.05±2.42	5.19±0.67
50% AW	20.07±0.23	2.33±0.33	2.67±0.17	34.33±0.6	27.5±0.87	17.38±0.36	4.46±0.86	21.17±4.2	2.32±0.62
25% AW	19.98±0.59	2±0.58	4.22±0.4	30.17±0.44	32±0.58	10±1.15	3.7±0.17	20.61±0.8	3.05±0.03
UTLA6									
100% AW	18.45±1.12	5.83±0.44	1.39±0.46	32.78±0.62	25±0.58	28.19±0.73	10.41±0.43	56.9±5.12	6.33±0.88

75% AW	21.97±0.38	4.67±0.88	1.22±0.22	34.56±0.68	27.05±1.18	39.95±1.7	14.05±1.18	48.35±5.98	8.1±0.64
50% AW	24.33±0.45	4±0.58	3±0.58	31±0.76	23.55±0.89	26.6±0.58	9.62±0.07	48.06±6.35	8.37±0.21
25% AW	21.07±0.52	3.67±0.88	5.28±0.31	33.45±0.48	26.57±1.26	18.7±0.81	7.78±0.05	38±4.09	5.93±0.16
UTLA7									
100% AW	21.19±0.74	6.33±0.67	1.11±0.4	36.87±1.04	30.05±1.18	56.8±1.27	19.44±1	62.47±10.91	10.56±1.17
75% AW	23.78±1.01	6.5±0.5	1.78±0.22	34.11±0.67	29±2	32.33±1.59	13.63±0.89	87.05±26.03	9.36±2.6
50% AW	22.08±1.29	4.67±0.67	3.56±0.29	33.17±1.09	24.88±1.07	32.8±2.19	10.45±0.2	56.66±0.81	7.91±1.12
25% AW	21.36±0.5	3.83±0.73	4.67±0.38	32.72±1.07	35.15±0.61	23.2±1.33	7.85±0.78	28.8±0.8	5.4±0.29
UTLA9									
100% AW	22.32±0.22	4.5±0.29	1.11±0.11	41±0.76	33.5±1.44	42.03±1.6	15.47±1.63	65.69±5.31	9.03±0.56
75% AW	20.9±0.57	7.67±0.33	1.89±0.11	36.08±1.23	28.05±0.66	44.15±0.66	15.91±0.17	68.84±5.45	11.26±0.86
50% AW	20.67±1.15	4.67±0.33	2.66±0.33	38.07±0.95	26.55±1.82	19.15±2.17	6.51±1.03	27.23±0.77	3.46±0.31
25% AW	20.57±1.16	4.67±0.33	3.78±0.4	32.61±0.81	30.88±1.77	19.6±2.66	6.45±0.72	27.57±7.34	3.79±0.79
UTLA10									
100% AW	22.37±0.9	3.67±0.67	1.22±0.4	32.72±0.64	32.5±0.87	43.76±1.25	15.02±1.58	91.24±19.24	11.02±1.63

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75% AW	23.48±0.67	6.33±0.67	1.39±0.06	30.43±0.74	31±1.15	37.28±0.85	10.86±0.42	56.65±0.4	8.02±0.09
50% AW	20±0.75	5±0.58	2.67±0.44	35.28±0.55	27.25±0.43	14.55±0.32	4.24±0.14	28.45±1.47	3.78±0.15
25% AW	24.77±1.13	5.67±0.67	4.17±0.25	34.83±0.88	25.75±1.24	28.62±2.08	9.9±0.44	58.89±6.21	8.92±0.72
UTLA12									
100% AW	20.98±0.28	4.67±0.67	3.11±0.49	34.5±1.04	27.1±0.52	25.78±0.85	9.9±0.76	38.02±2.21	4.15±0.24
75% AW	21.68±0.41	4.33±0.67	3.22±0.28	33.39±0.56	26±0.58	13.2±0.64	4.66±0.32	35.93±3.89	4.53±0.48
50% AW	21.83±0.93	1.67±0.33	2.78±0.29	31.5±0.76	31.2±0.69	13.65±0.95	5.13±0.38	37.85±3.38	4.25±0.37
25% AW	22.97±0.88	2.67±0.33	4±0.51	33.55±0.78	28.55±0.32	30.72±1.36	11.31±0.25	51.37±1.11	6.62±0.73
LSD (1%)	3.12	2.12	1.29	3.07	4.08	4.51	2.81	30.58	3.18

Values are given as mean ± SE

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By reducing irrigation level, the number of seeds per capsule decreased (in UTLA1 and UTLA7 accessions), increased (UTLA10) or was observed without significant change (UTLA6, UTLA9, and UTLA12). Seed yield per plant in UTLA1, UTLA7 and UTLA12 accessions under water deficit stress were significantly decreased. Seed width was not affected by water stress (Table S1). The maximum and minimum seed width was observed in UTLA7 and UTLA1 accessions. The weight of 1000 seeds and the number of flowers in the inflorescence showed a significant decrease only in UTLA9 accession. Water deficit led to significantly decrease of the mature capsules number in accessions of UTLA9 and UTLA12. The inflorescence length decreased in all accessions except UTLA1 with increasing stress level, in UTLA1 a significant increase was observed. The length of the main branch in accessions UTLA7 and UTLA9 and the height of the plant in UTLA6, UTLA7 and UTLA9 showed a significant decrease by decreasing irrigation level. While other accessions were not particularly affected by water deficit stress, leaf length in UTLA1 showed a significant increase. By increasing water deficit stress level, the number of inflorescences per plant in accessions of UTLA1, UTLA6, UTLA7, and UTLA12 showed a significant decrease. The number of chlorosis leaves in all accessions increased significantly with increasing stress levels. The highest flower diameter was observed in accession of UTLA9, although a significant decrease in flower diameter occurred with decreasing irrigation. As the stress level increased, the root length decreased in accession UTLA10 and increased significantly in UTLA1. By reducing irrigation level, shoot fresh weight in all accessions except UTLA12 decreased. Also, the shoot dry weight showed a significant decrease in UTLA6, UTLA7, UTLA9, and UTLA10. Root fresh and dry weight in UTLA7 and UTLA9 showed a significant decrease by increasing stress levels (Table 3).

Capsule diameter and length of the seeds and the leaves under water deficit stress did not show significant changes (Table S1). However, there is a significant difference between accessions. The largest capsule diameter was observed in UTLA12 (5.86 mm) and the lowest in UTLA1 (5.41) (Figure 1). Minimum and maximum seed and leaf lengths were observed in UTLA1 and UTLA10 accessions, respectively (Figure 1).

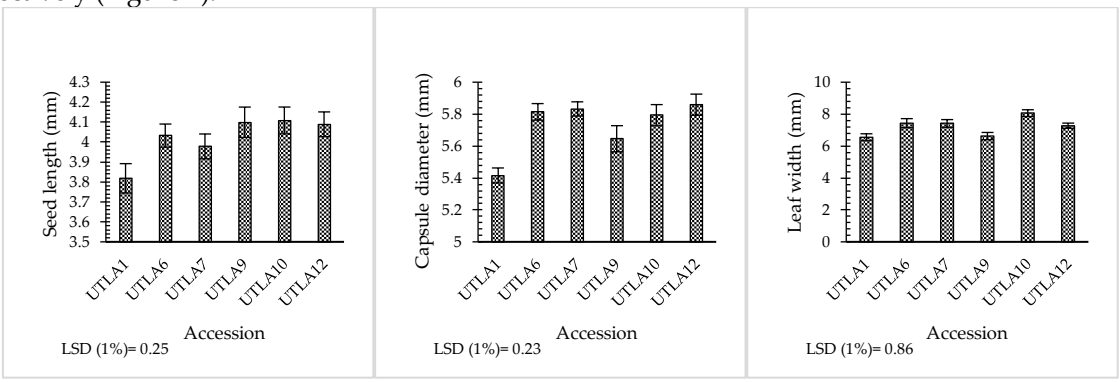

Figure 1. Mean of morphological traits in *L. album* Accessions. Values are given as mean ± SE

The capsule to flower ratio was affected by water deficit stress, while the response of the accessions did not show a significant difference (Table S1). The lowest percentage of flower to capsule conversion with a significant difference compared with other levels was observed at 25%AW treatment (Figure 2).

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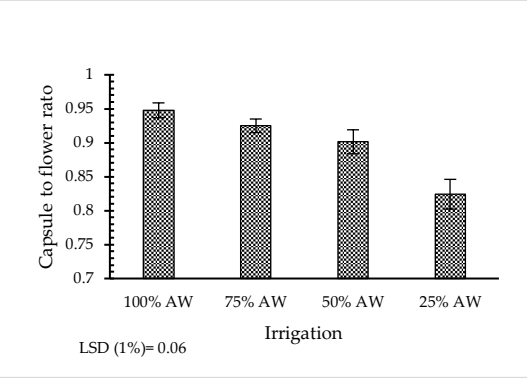
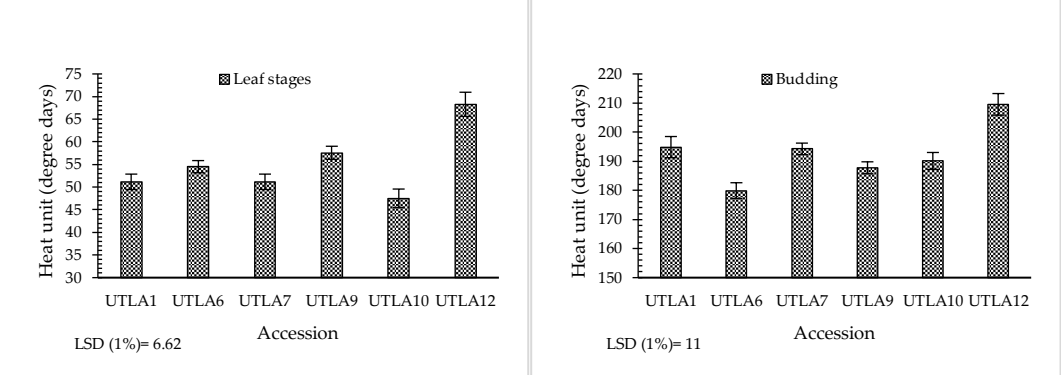


Figure 2. Effect of water deficit stresses on capsule to flower ratio of *L. album*. Values are given as mean ± SE

2.2. Phenological stages

The occurrence of different phenological stages in *L. album* accessions showed a significant difference (Table S2). The growth of UTLA10 plants was started earlier than other accessions, but the maturation occurred later. Plants of UTLA6 accession entered the bud and flowering stages earlier than other accessions (Figures 3 and 4). Among the recorded phenological stages, only maturity was affected by water deficit stress (Table S2). Plants under 25% AW treatment matured significantly sooner than other irrigation levels (Figure 5).

Figure 3. Phenological stages in *L. album.* Starting leaf stage (a), budding (b), flowering (c) and maturity (d).



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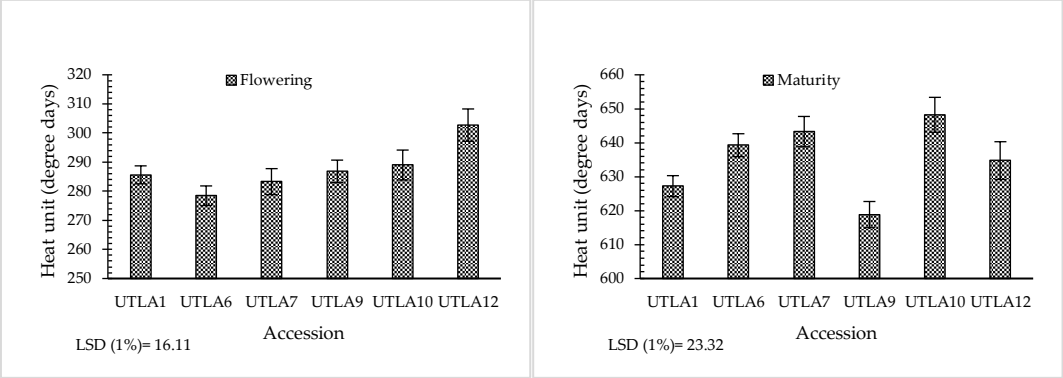

Figure 4. Phenological stages of *L. album* accessions. Values are given as mean ± SE

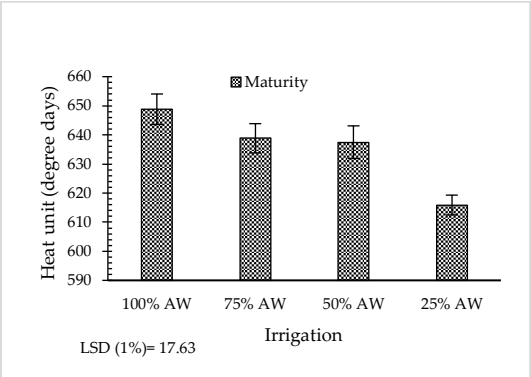

Figure 5. Effect of water deficit stresses on maturity of *L. album*. Values are given as mean ± SE

2.3. RWC, Chlorophyll and carotenoid contents

Different accessions showed different physiological responses to irrigation levels (Table S3). By increasing stress level, the RWC decreased in accessions of UTLA6, UTLA7 and UTLA10, increased in UTLA1 and UTLA12 and was observed in UTLA9 without significant change compared with control (Table 4). Chlorophyll a and b and carotenoids showed a significant decrease with increasing stress level in all accessions except accessions UTLA6 and UTLA12. Accession UTLA1 showed the lowest amount of pigments and accession UTLA7 showed the highest amount (Table 4).

 doi:10.20944/preprints202010.0110.v1

Table 4. Effect of water deficit stresses on physiological traits of *L. album* accessions.

Accession/ Irrigation	RWC (%)	Chlorophyll a (µg/ml)	Chlorophyll b (µg/ml)	Carotenoids (µg/ml)	Proline (µmol/g FW)	Glycine betaine (µmol/g DW)	Electrolyte leakage (%)	MDA (µmol/g FW)	ABA (pmol/g DW)
UTLA1									
100% AW	77.05±1.98	19.3±0.6	7.22±0.25	5.45±0.15	2.64±0.04	228.75±7.27	55.55±3.71	0.102±0.023	150.89±3.45
75% AW	80.74±0.91	16.47±1.48	5.51±0.53	4.99±0.35	5.62±0.3	166.48±5.82	65.7±1.08	0.113±0.0143	177.75±11.72
50% AW	80.67±2.14	17.5±0.19	5.55±0.46	5.19±0.12	3.67±0.23	189.09±10.66	78.21±2.56	0.136±0.0053	134.8±13.83
25% AW	85.11±1.11	13.2±1.82	4.72±0.51	3.87±0.41	5.78±0.15	130.19±5.25	65.59±2.56	0.149±0.005	340.01±26.85
UTLA6									
100% AW	84.61±0.54	20.38±0.27	7.43±0.1	5.47±0.09	4.99±0.22	169.59±4.04	62.54±0.83	0.175±0.0021	101.59±5.86
75% AW	84.07±0.73	19.3±0.19	7.01±0.11	5.37±0.05	5.07±0.21	122.69±6.7	75.82±0.64	0.151±0.0088	89.62±5.55
50% AW	80.43±0.62	19.18±0.33	6.93±0.15	5.37±0.08	4.06±0.32	141.62±1.76	67.9±1.06	0.155±0.0109	135.71±13.3
25% AW	74.37±1.16	20.44±0.63	7.59±0.11	5.72±0.19	4.19±0.05	168.04±0.14	72.66±1.16	0.154±0.0108	112.73±1.54
UTLA7									
100% AW	84.95±0.92	22.68±0.74	9.11±0.05	5.97±0.19	5.28±0.2	199.09±6.55	64.52±1.89	0.184±0.0085	136.87±15.44

 doi:10.20944/preprints202010.0110.v1

75% AW	88.36±0.94	20.88±0.46	7.76±0.19	6.12±0.14	5.22±0.12	164.51±5.83	74.67±1.28	0.12±0.0101	137.79±15.53
50% AW	86.01±1.1	20.15±0.77	7±0.32	6.47±0.21	4.44±0.18	152.06±13.22	63.27±1.55	0.174±0.0102	109.44±5.3
25% AW	77.38±1.23	18.88±0.3	6.86±0.36	5.76±0.04	6.01±0.13	209.33±5.82	60.9±2.78	0.128±0.0099	113.19±3.21
UTLA9									
100% AW	76.81±0.42	21.19±0.1	7.75±0.29	6.13±0.1	5.15±0.08	227.11±11.27	57.45±1.65	0.157±0.0099	103.23±6.11
75% AW	83.33±1.44	21.62±1	7.92±0.19	5.96±0.09	5.04±0.14	193.48±2.17	75.89±3.42	0.139±0.0079	90.38±5.29
50% AW	82.63±1.25	18.99±0.3	6.79±0.2	5.28±0.07	4.38±0.21	171.31±8.41	71.27±1.53	0.208±0.008	115.82±4.89
25% AW	75.82±1.2	17.53±0.33	5.81±0.59	5.01±0.17	5.4±0.15	180.33±4.68	67.71±1.27	0.203±0.014	139.39±6.16
UTLA10									
100% AW	83.43±0.52	21.44±0.56	8.03±0.19	5.5±0.15	4.49±0.14	182.79±7.82	67.52±1.11	0.112±0.0006	101.87±1.88
75% AW	84.92±0.18	20.42±0.61	7.43±0.43	5.64±0.29	5.23±0.17	185.98±2.01	73.89±1.4	0.085±0.0073	122.38±4.71
50% AW	84.18±1.06	17.58±0.47	5.73±0.28	5.1±0.05	5.44±0.16	190.73±6.47	41.19±3.54	0.18±0.0039	117.18±6.21
25% AW	78.57±0.42	16.82±0.13	6.25±0.35	5.44±0.16	5.93±0.15	249.64±4.68	58.36±0.9	0.182±0.0066	155.81±11.55
UTLA12									
100% AW	83.59±2.92	17.58±0.79	6.1±0.32	5.25±0.23	4.5±0.1	168.78±4	60±1.85	0.131±0.0079	117.36±9.29

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75% AW	85.46±0.92	17.14±0.67	6.17±0.09	4.64±0.17	3.21±0.05	215.52±7.89	67.95±1.57	0.122±0.009	150.82±11.34
50% AW	87.5±1.24	15.28±0.65	5.58±0.21	4.69±0.19	5.72±0.16	215.93±8.4	80.17±0.35	0.146±0.0067	204.98±10.91
25% AW	82.18±1.32	18.8±0.48	6.72±0.1	5.87±0.14	5.46±0.08	195.9±7.14	85.14±3.61	0.14±0.0068	174.2±7.06
LSD (1%)	4.536	2.011	1.156	0.562	0.658	25.7	7.768	0.0375	37.79

Values are given as mean ± SE

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2.4. Proline and glycine betaine

A significant increase in proline levels was observed in all accessions in the 25% AW treatment compared with the control. The highest amount of glycine betaine was observed in accession UTLA10 under treatment 25% AW, while with increasing stress level, the amount of glycine betaine in accessions UTLA1 and UTLA9 showed a significant decrease (Table 4).

2.5. Electrolyte leakage and malondialdehyde

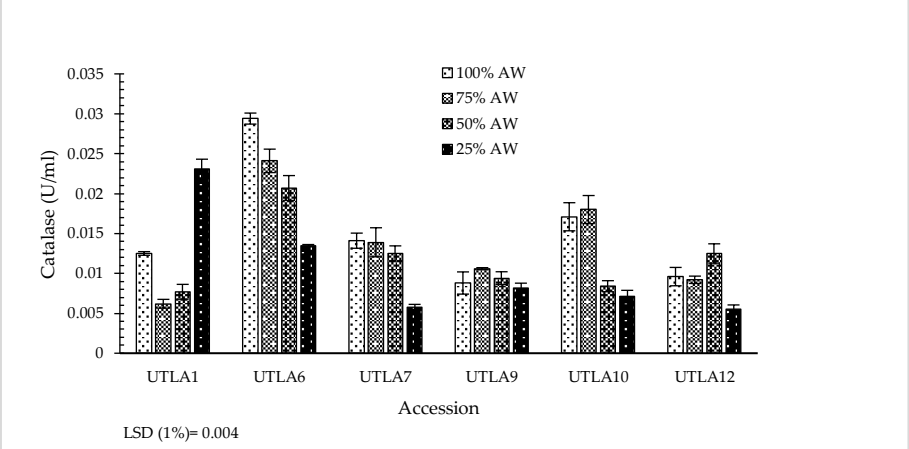
Water deficit stress in accessions of UTLA1, UTLA6, UTLA9, and UTLA12 caused a significant increase in ion leakage, while in accession of UTLA10 the ion leakage decreased and accession of UTLA7 did not show any significant change. In accessions of UTLA1, UTLA9, and UTLA10, exacerbation of dehydration caused a significant increase in MDA. Accessions of UTLA6 and UTLA12 did not show significant changes (Table 4).

2.6. Abscisic acid

The highest ABA content was observed in accession of UTLA1 and the lowest in UTLA2. Abscisic acid content in accession of UTLA1 showed a significant increase in treatment 25% AW, while accessions of UTLA2 and UTLA3 did not show significant differences. In accessions of UTLA4, UTLA5, and UTLA6, the ABA content increased significantly with increasing stress levels (Table 4).

2.7. Enzymatic antioxidant activity

With decreasing irrigation level, the activity of catalase enzyme increased in accession of UTLA1 and showed a significant decrease in accessions of UTLA6, UTLA7, and UTLA10 (Figure 6). The activity of guaiacol peroxidase enzyme in *L. album* accessions showed a significant difference (Table S3). Accession of UTLA1 showed the highest and UTLA7 showed the lowest activity of this enzyme. The activity of this enzyme showed a significant increase with increasing stress levels in accessions of UTLA6 and UTLA10. The response of different accessions based on the activity of the ascorbate peroxidase enzyme showed a significant difference. Decreases were observed in accessions of UTLA7 and UTLA10 and significant increases were observed in accessions of UTLA9 and UTLA12 (Figure 6).



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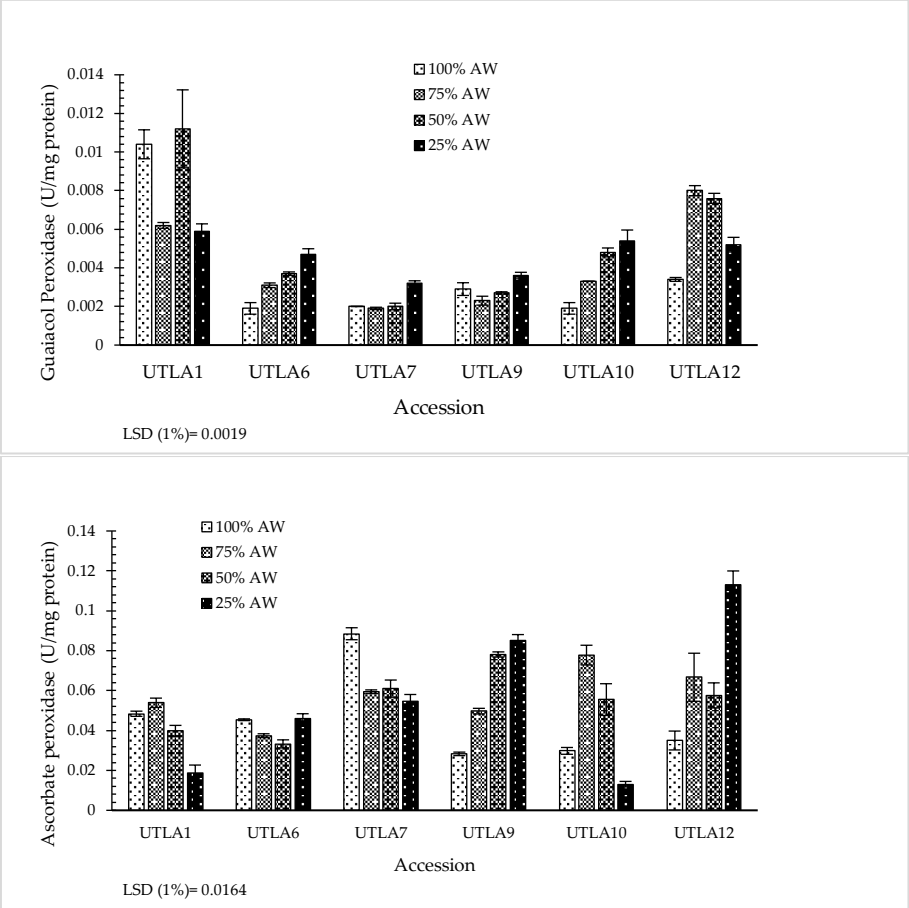

Figure 6. Effect of water deficit stresses on enzymatic antioxidant activity of *L. album* accessions. Values are given as mean ± SE

2.8. Correlations between traits

Significant correlations were found between the studied traits. Positive correlation coefficient of the number of inflorescences, with seed yield (r = 0.81), the number of mature capsules (r = 0.96), seed length with leaf length (r = 0.65), and weight of 1000 seeds (r = 0.72), also associations of shoot dry weight with root dry weight (r = 0.90), chlorophyll a and chlorophyll b with the number of inflorescences (r = 0.65 and 0.64), shoot dry weight (r = 0.79 and 0.83), and root dry weight (r = 0.72 and 0.77) were observed. Guaiacol peroxidase activity with plant height (r = -0.72) and shoot dry weight (r = -0.70), ABA content with chlorophyll a, chlorophyll b, and carotenoids (r = -0.75, 0-.60, and -0.69) were negatively correlated (Table S4).

 doi:10.20944/preprints202010.0110.v1

3. Discussion

In this study, the effect of water deficit stress on six accessions of *L. album* was examined based on various traits. The results showed that there was a significant difference between accessions in all studied traits. However, water deficit, as an important factor in plant growth and development, has provoked different responses in different accessions .Based on the results of the analysis of morphological traits, high genetic diversity of samples can be considered as the main cause of significant differences, as in previous studies, the existence of diversity among *L. album* accessions has been reported [6,33,34].

Growth is complete through cell division, enlargement, and differentiation and depends on genetic, physiological, ecological, and morphological events and their complex interactions. The quality and quantity of plant growth depend on these events, which are affected by water deficit. Cell growth is one of the most drought-sensitive physiological processes due to the reduction in turgor pressure [35]. As the results of morphological traits present, the effect of water deficit stress on accessions was not the same and also differences were observed within each accession under different irrigation levels. For water stress, severity, duration, and timing of stress, as well as responses of plants after stress removal, and interaction between stress and other factors are extremely important [36]. Plant growth is greatly affected by water deficit. At a morphological level, the shoot and root are the most affected and both are the key components of plant adaptation to drought as in this research, they were correlated. Plants generally limit the number and area of leaves in response to drought stress just to cut down the water budget at the cost of yield loss [37]. Since roots are the only source to acquire water from soil, the root growth, its density, proliferation, and size are key responses of plants to drought stress [38].

Based on the results of this research, the capsule to flower ratio in 25% AW treatment showed a significant decrease. Decreased grain growth in wheat due to reduced sucrose synthase activity [39] and increased the frequency of kernel abortion due to water deficit during pollination in corn [40,41] has been reported. Acceleration of the final stage in seed abortion for plants subjected to this stress appears to be a survival mechanism. Nevertheless, the filling period was reduced because of earlier physiological maturity. This shorter period reduced seed growth [42].

The significant effect of genetic diversity in *L. album* accessions was observed on the occurrence of different phenological stages, while water deficit stress only affected the time of maturity. The effects of drought range from morphological to molecular levels and are evident at all phenological stages of plant growth at whatever stage the water deficit takes place. Plants that were subjected to stress during flower and pod formation had a shorter organ appearance period. This seemed to be due to an increase in the progression rates of reproductive organs under stress. Water stress applied at pre-anthesis reduced time to anthesis, while at post-anthesis it shortened the grain-filling period in triticale genotypes [43].

In summary, plants can escape water deficit stress by shortening their growth duration, and avoid the stress with the maintenance of high tissue water potential either by reducing water loss from plants or improving water uptake, or both. Some plants may reduce their surface area by leaf shedding or production of smaller leaves [44].

Drought stress reduces the relative water content of leaves and is used as a reliable method for measuring the osmotic stress status [45]. Also, RWC is commonly used for the measurement of plant water status in terms of the physiological and biochemical consequences of water deficit in plant cells [46]. It has been reported that plants with more RWC content are more resistant to water deficit stress [47]. In this study, accessions of UTLA1 and UTLA12 had the highest RWC in the lowest level of irrigation.

Decreased levels of chlorophyll a and b have been reported in flaxseed (*Linum usitatissimum*) under dehydration stress [48], as observations of this study have shown a decrease in chlorophyll a, chlorophyll b, and carotenoids content in *L. album* accessions. In most plant species, water deficit stress decreases the level of chlorophyll a, chlorophyll b, and total chlorophyll [11,49,50,]. Drought stress made changes in photosynthetic pigments and components [51], damaged photosynthetic apparatus [52], and decreased activities of Calvin cycle enzymes, which are important causes of

 doi:10.20944/preprints202010.0110.v1

reduced crop yield [53]. In this study, a positive relationship between chlorophyll a, and b content and yield-related traits (number of inflorescences, shoot dry weight, and root dry weight) was observed. Another important effect that inhibits the growth and photosynthetic abilities of plants is the loss of balance between the production of reactive oxygen species and the antioxidant defense [52,54], causing accumulation of reactive oxygen species which induces oxidative stress in proteins, membrane lipids and other cellular components [44].

The reactive oxygen species in crops are removed by a diverse of antioxidant enzymes and/or lipid-soluble and water soluble scavenging molecules [55]. The antioxidant defense system consists of non-enzymatic components, such as ascorbate, glutathione, proline, carotenoids, flavonols, α-tocopherols, glycine betaine, anthocyanin, and amino acids [13]. Proline as an important compatible solute is a general response of higher plants, algae, animals, and bacteria to low water potential [56,57] . In plants, its synthesis in leaves at low water potential is caused by a combination of increased biosynthesis and slow oxidation in mitochondria. Despite some controversy, many physiological roles have been assigned to free proline including stabilization of macromolecules, a sink for excess reductant, and a store of carbon and nitrogen for use after relief of water deficit [56]. As seen in this experiment, many experiments reported the increase of proline levels in plants under water stress conditions in different plants [48,58-61].

Glycine betaine is another important compatible solute in plants, animals, and bacteria observed aplenty in response to water deficit stress [19,62-64]. Many studies represent that glycine betaine acts an important role in improving plant tolerance under many of abiotic stresses including drought stress [65]. In addition to direct protective roles of glycine betaine either through positive effects on enzyme and membrane integrity or as a compatible solute, it can also protect cells from environmental stresses indirectly by participating in signal transduction pathways [66]. Glycine betaine is reported to accumulate in response to stress in many plants, including sugar beet (*Beta vulgaris*), spinach (*Spinacia oleracea*), barley (*Hordeum vulgare*), and wheat (*Triticum aestivum*) [67-69]. In these species, tolerant genotypes accumulate more GB than sensitive genotypes in response to stress. However, this is not general relationship and it is most likely a relationship between GB accumulation and stress tolerance is species- or even genotype-specific. As in the *L. album*, the response of accessions was different. Thus, with the increasing stress levels, the amount of GB increased in accession of UTLA10, while decreased in accessions of UTLA1 and UTLA9.

The determination of MDA concentration, as a lipid peroxidation product, is used for quantifying the level of membrane peroxidation that leads to ion leakage [70]. Under stressful conditions, unsaturated fatty acids of cell membranes are impressed by free radicals and form a chain reaction of lipid peroxidation [71]. In this study, ion leakage and MDA content were relatively low in plants under normal irrigation treatment but increased as water stress intensified in accessions of UTLA1 and UTLA9, indicating loss of cell stability and viability [72,73]. Zarrinabadi et al. [60] have reported MDA content increased in pot marigold (*Calendula officinalis*) genotypes under water deficit conditions. Stress sensitive species exhibit a sharper increase in lipid peroxidation than regular species under water deficit stress [74,75]. Also, ion leakage decreased in accession of UTLA10 by increasing stress level, while no significant decrease in MDA was observed in any of the accessions. Since MDA is the final product of lipid peroxidase, various studies have reported that lower MDA content within genotypes indicates the greater antioxidant activities alongside resistances to arid conditions [76,77].

Plants response to water deficit condition by ABA-dependent and ABA-independent pathways [44]. Abscisic acid is a stress hormone that regulates gene expression and acts as a signal for the initiation of processes involved in adaptation to water deficit and other environmental stresses [44]. When plants face with water deficit condition, ABA levels in most cases rise as a result of increased synthesis [78]. Variety in response of accessions to water deficit stress based on the amount of ABA was observed in the present study. The accessions of UTLA2 and UTLA3 did not show a significant change in ABA content under different irrigation levels, while in accessions of UTLA1, UTLA4, UTLA5, and UTLA6, ABA content increased significantly. This increase was more pronounced in accession of UTLA1 and as a result, the root length was significantly higher in 25%AW treatment.

 doi:10.20944/preprints202010.0110.v1

Abscisic acid changes the relative growth rates of various plant parts such as increasing the root-to-shoot dry weight ratio, inhibition of leaf area development and producing deeper roots [79]. In the results section, it has been reported that the amount of ABA showed a negative correlation with pigments (chlorophyll a, b, and carotenoids) content .Under water stress conditions, apoplastic pH increases resulting more retention of ABA, functioning as a signal to stomatal closure and then follow by reducing transpiration in leaves, which is an important water conservation response [24,80].

In the present study, the activity of antioxidant enzymes in the accessions and levels of available water showed differences. ROS production is affected by severity and duration of stress, species, genotype, and the developmental stage of the plant [81,82]. It is also affected by the ability of the plant to adapt to stress conditions [83]. Major enzymes of the enzymatic antioxidant defense system include superoxide dismutase, catalase, ascorbate peroxidase, glutathione peroxidase, guaiacol peroxidase, monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR) [13,84,85]. When the defense system is not able to neutralize the high levels of ROS, Oxidative stress happens. In this study, the response of accession of UTLA1 to the increase in stress level was an increase in CAT activity, while APX activity decreased in this accession. However, in accessions of UTLA6 and UTLA10, the activity of the CAT decreased and the activity of the POX increased. Interestingly, accession of UTLA12 showed a decrease in CAT and an increase in APX .Catalase is the main antioxidant enzyme that scavenges the oxidant H_2O_2 by decomposition to oxygen and water [86]. In drought-tolerant genotypes, catalase activity increased under water stress conditions, which could be an adaptive mechanism to ROS [87]. Ascorbate peroxidase is a key antioxidant enzyme in plants [88], which catalyzes the conversion of H_2O_2 into H_2O. It has been reported that under drought stress, ascorbate peroxidase activity rises alongside other enzymes [89] Askari and Ehsanzadeh [58] studied different drought treatments on fennel genotypes, and reported a higher increase of antioxidant enzymes, especially catalase, in drought-tolerant genotypes. The same effect of water deficit on the antioxidant content has been illustrated in medicinal plants like peppermint (*Mentha piperita*), Oregano (*Origanum vulgare*) and marigold genotypes [60,90,91].

4. Materials and Methods

4.1. Plant and soil materials

This study is continuation of a project on morpho–physiological variation in different populations of *Linum album* in west of Iran [6,92]. To study the effect of water deficit stress on some valuable characteristics of this species, six superior accessions were selected and subjected to the treatments (Table 1, seeds of UTLA12 accession was obtained from the seed gene bank at the Forest and Rangeland Research Institute in Tehran, Iran). Seeds were treated with 1000 ppm gibberellic acid for 24 hours to overcome seed dormancy, and germinated in a plastic germination tray containing coco-peat, in April 2018 [92]. Growing media consisted of a mixture of field soil, sand, and leaf mold in equal ratio (Table 2). Leaf mold was provided from botanic garden of University of Tehran. Sixty days after germination, uniformly sized seedlings were randomly selected and transplanted into the pots (one seedling per pot). After one year growth the dried shoots of plants were uniformly cut at one cm above the soil level in January 2019.

Table 1. *L. album* seed source locations

Accession code	Latitude (N)	Longitude (E)	Altitude (m)	Voucher number[a]
UTLA1	34°13'56"	48°57'25"	1904	6426
UTLA6	34°55'50"	48°11'34"	2176	6425
UTLA7	34°41'12"	48°38'02"	2124	6430

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UTLA9	34⁰46'11"	48⁰43'17"	1955	6427
UTLA10	34⁰22'45"	48⁰40'02"	1721	6428
UTLA12	32°54'11"	50° 4'39"	2630	-

[a]Department of Horticultural Sciences Herbarium, University of Tehran

Table 2. Edaphic parameters of soil

pH	EC (Ds/m)	Silt (%)	Clay (%)	Sand (%)	Soil class	FC (%)	PWP (%)	OC (%)	Total N (%)	Usable potassium (mg/kg)	Usable phosphorus (mg/kg)
8.1	2.8	21	11	68	Sandy loam	24.98	12.52	3.18	0.25	397	47.6

4.2. Water stress treatment

The irrigation of plants during the first year of cultivation was carried out continuously to the extent of field capacity. Water deficit stress (different levels of irrigation consist of 100 (as control), 75, 50, and 25% of plant available water (AW)) was applied in the second year of cultivation. The weight method was used and the pots were weighed every 48 h. Due to non-uniformity in growth of plants, flower bud emergence was considered as the criterion for the onset of water deficit stress. The experiment was carried out as a factorial experiment in a randomized complete block design (RCBD) with three replications, and three observations (three pots) in each replication.

4.3. Determination of phenological stages

Phenological stages of accessions were recorded individually from the beginning of the growth of the first plant in the second year of growth (March 2, 2019). The occurrence of phenological stages was reported based on growing degree days (GDD). The following formula was used to calculate the GDD [93].

GDD =∑ [(Tmax-Tmin)/2] - Tbase

Tbase = 2.67 [92]

4.4. Relative water content

Relative water content (RWC) was determined by the following procedure outlined by Turner [94]. From each sample in the water stress treatments, 15 fully extended leaves were removed from the plant stem, weighed (FW) and then floated on double distilled water for 24 h at 4°C and then turgid leaves were quickly weighed, the weight of the samples was considered as the turgidity weight (TW) and then samples oven-dried at 70°C for 48 h and reweighed for a dried leaf weight (DW). The relative water content was calculated by the following equation.

RWC (%) = [(FW-DW)/ (TW-DW)] ×100

4.5. Chlorophyll and carotenoid contents

For all physiological experiments, samples were taken from fully mature leaves, during maturing period. Samples from each replicate and treatment separately mixed, ground, and stored at −80 °C.

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To measure leaf chlorophyll content, 500 mg of frozen powdered sample was mixed with 10 ml of 95% ethanol. Homogenized sample mixture was centrifuged at 8,000 rpm for 15 min. Supernatants were read by a microplate spectrophotometer (BioTek Eon, USA) at 664 nm for chlorophyll a (Ch a), 649 for chlorophyll b (Ch b), and 470 nm for carotenoids [95]. The amounts of chlorophyll and carotenoid were calculated by following formulae.

Ch a (µg/ ml) = $13.36_{A664} - 5.19_{A649}$

Ch b (µg/ ml) = $27.43_{A649} - 8.12_{A664}$

Carotenoids (µg/ ml) = (1000A470 –2.13Ch a- – 97.63Ch b)/209

4.6. Determination of proline and glycine betaine

Proline content was determined using Bates et al. [96] method. For start 500 mg of leaves was commixed in 10 ml of sulfosalicylic acid 3%, and the mixture was centrifuged at 10,000 rpm for 10 min. Then, 2 ml of the supernatant was added to 2 ml of acid–ninhydrin solution and 2 ml of glacial acetic acid in a tube. Tubes were incubated in a bain-marie at 100 °C for 1 h. The reaction was stopped in ice. The reaction mixture was extracted with 4 ml of toluene and vortexed for 15–20 s. The tubes were allowed to stand for at least 20 min in darkness at room temperature for separation of toluene from the aqueous phase. The toluene phase was then collected into tubes, and the absorbance at 520 nm was measured with a microplate spectrophotometer (BioTek Eon, USA). The proline concentration was determined according to a standard curve of proline.

The amount of glycine betaine was evaluated according to Grieve and Grattan [97]. 250 mg of leaf powder (dried leaves) was shaken with 10 ml of deionized water for 48 h at 25 °C. The extracts were filtered using filter paper and then diluted (1:1) with H_2SO_4 (2N) and cooled in ice water for 60 min. After that, 0.2 ml of cold KI-I2 was added to the samples and softly mixed. Tubes were kept at 4 °C for 16 h and then centrifuged at 10000 rpm for 15 min at 0 °C. The supernatant was attentively discarded and periodide crystals were dissolved in 9 ml of 1,2-dichloroethane. After 2 h, the value of absorbance at 365 nm was evaluated with a spectrophotometer. The amount of glycine betaine was calculated according to a standard curve of glycine betaine.

4.7. Measurement of electrolyte leakage and malondialdehyde contents

Malondialdehyde determination was started by homogenizing 500 mg of fresh leaves in 5 ml of 10% trichloroacetic acid (TCA). In the next step samples were centrifuged at 12,000 rpm for 10 min at 4 °C. Two ml of supernatant was added to 4 ml of 0.6% thiobarbituric acid (TBA, in 10% TCA) and incubated at 100 °C in a bain-marie for 15 min. The samples were cooled in room temperature, and the absorbance of the supernatant was measured at 450, 532, and 600 nm, using a microplate spectrophotometers (BioTek Eon, USA). The MDA contents were calculated by using the following formula [98]:

MDA (µmol g^{-1} FW) = 6.45 (OD532 −OD600) − 0.56 OD450

Electrolyte leakage (EL) was estimated using a conductivity meter. Fresh leaf samples were cut into 10 equal sized pieces, each with 4.5 mm diameter. Then they were dipped in 15 ml of distilled water and shaken at 100 rpm for 24 h at room temperature. The initial electrical conductivity (EC1) of the solution was recorded. The tubes were then autoclaved at 120 °C for 20 min. After cooling of tubes to room temperature, the final EC (EC2) was recorded [99]. Finally, the percentage of ion leakage was calculated by using the following equation:

EL (%) = [EC1/ EC2] ×100

4.8. Enzymatic antioxidant activity

For enzyme assays, frozen leaf samples were ground to a fine powder with liquid nitrogen and extracted with 50 mM phosphate buffer (pH = 7.0). The extracts were centrifuged at 4 °C for 15 min at 13,000 rpm and after that supernatant was collected and used for protein content assay and enzyme activities. Protein extraction was carried out according to Bradford [100] using Bovine Serum Albumin as a standard. Catalase activity was determined using spectrophotometric method (BioTek Eon, USA)

 doi:10.20944/preprints202010.0110.v1

as described by Hadwan [101]. Total guaiacol peroxidase activity was determined as described by Plewa et al. [102]. Reaction mixture included 3,000 µl of 50 mM phosphate buffer (pH = 7), 10 µL of 30% hydrogen peroxide, three µL of 200 µM guaiacol solution and 100 µL of enzymatic extract. The addition of enzyme extract started the reaction and the increase in absorbance was recorded at 470 nm for 4 min (Perkin Elmer, UV-VIS Spectrophotometer, LAMBDA EZ201, USA). The activity of ascorbate peroxidase was measured as described by Ranieri et al. [103]. The reaction mixture contained 600 µl of 0.1 mM EDTA, 1500 µl of 50 mM phosphate buffer (pH = 7), 400 µl of 0.5 mM ascorbic acid, 400 µl of 30% hydrogen peroxide and 100 µl enzyme extract. Enzyme activity assays were recorded at 470 nm for four minutes (Perkin Elmer, UV-VIS Spectrophotometer, LAMBDA EZ201, USA).

4.9. Abscisic acid extraction and quantification

250 mg of dried leaf samples (sampling was performed at the end of flowering stages) were extracted in 2 mL of aqEtOH (80%) solvent. The extracts were sonicated by an ultrasonic bath for 60 min (USC1200TH, Prolabo, Fontenay-sous-Bois, France) with a maximal heating power of 400W (i.e., acoustic power of 1W/cm^2). The extracts supernatant was filtered through 0.45 µm nylon syringe membranes. Abscisic acid was quantified using a Phytodetek ABA ELISA kit (Agdia) and (±) *cis–trans* ABA (Sigma) as a standard.

4.10. Statistical analysis

All data were subjected to analysis of variance (ANOVA) using SAS V.9.2 software followed by an LSD test with $P< 0.01$ as the significant differences between means. The results are presented as the means ± SE (standard errors). Pearson Correlation was investigated with IBM SPSS Statistics 23.0.

5. Conclusions

Evaluation of the response of plant accessions under different environmental conditions helps to understand the growth characteristics of plants. All in all, what was seen was significant variety of *L. album* accessions, which is very important in advancing breeding programs. In most morphological traits studied, a decrease was observed due to increased water deficit stress. Accessions of UTLA7, UTLA9, and UTLA10 had higher vegetative yield and seed yield and are recommended for use in breeding research. Water deficit stress accelerates the maturation of the plant, and accession of UTLA9 completes its growth sooner, and if the duration of growth is an important factor for production, this accession is an ideal option for seed production. If the vegetative yield is considered during flowering stage, accession of UTLA6 can be recommended due to early entry into the flowering stage. Genetic variation between accessions was also confirmed by physiological responses. Examination of physiological characteristics confirms the better performance of accessions of UTLA7 and UTLA9. Overall, among the accessions, accession of UTLA1 had different characteristics and responses, while accessions UTLA7 and UTLA9 were almost similar. The use of leaf length traits and chlorophyll content to estimate seed and shoot yield can be an acceptable marker. Examining this relationship in differentiated accessions can yield significant results. Using chlorophyll content as an easy measurable trait is recommended to screen tolerate genotypes. Finally, it can be said that what affected the yield of *L. album* plants was more the genotype than the water deficit stress. It is recommended to determine the water requirements and also to study the effect of lower irrigation level in different *L. album* accessions.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, **Table S1:** Analysis of variance of morphological traits, **Table S2**: Analysis of variance of phenological traits, **Table S3**: Analysis of variance of physiological traits, **Table S4**: Correlation between traits by Pearson method

Author Contributions: Conceptualization: V.N., M.S.; Data curation: R.K.; Formal analysis: M.S. and R.K.; Funding acquisition: V.N., M.S. and C.H.; Investigation: R.K.; Methodology: V.N., M.S., C.H. and R.K.; Project administration: V.N. and M.S.; Resources: V.N., M.S., C.H. and R.K.; Software: R.K.; Supervision; V.N. and M.S.; Validation: V.N., M.S., C.H. and R.K.; Visualization: R.K.; Writing - original draft: R.K.; Writing - review & editing: V.N., M.S., C.H. and R.K.

Funding: This research was supported by grants from University of Tehran and Région Centre-Val de Loire (Acti-LIN).

Acknowledgments: R.K. acknowledges research fellowship from Ministry of Science, Research and Technology of Iran.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Rechinger, K. H. *Flora Iranica no. 106 Linaceae*. Akademische druck-u. verlag sanstalt. Graz Austria 1974.
2. Sharifnia, F. and Asadi, M. A phenetic study of the genus *Linum* L. (Linaceae) in Iran. *Iran. J. Bot* 2002, 9(2), pp.135-139.
3. Vardapetyan, H. R.; Kirakosyan, A. B.; Oganesyan, A. A.; Penesyan, A. R.; Alfermann, W. A. Effect of various elicitors on lignan biosynthesis in callus cultures of *Linum austriacum*. *Russian journal of plant physiology* 2003, 50(3), 297-300.
4. Moss, G. P. Nomenclature of lignans and neolignans (IUPAC Recommendations 2000). *Pure and applied chemistry* 2000, 72(8), 1493-1523.
5. Farkya, S.; Bisaria, V. S.; Srivastava, A. K. Biotechnological aspects of the production of the anticancer drug podophyllotoxin. *Applied microbiology and biotechnology* 2004, 65(5), 504-519.
6. Nazeri, V.; Kiani, R.; Rezaei, K.; Kalvandi, R. Diversity study of some ecological, morphological and fatty acid profile of *Linum album* Ky. ex Boiss. *Iranian Journal of Medicinal and Aromatic Plants* 2017, 33(1).
7. Akula, R.; Ravishankar, G. A. Influence of abiotic stress signals on secondary metabolites in plants. *Plant signaling & behavior* 2011, 6(11), 1720-1731.
8. Taiz, L.; Zeiger, E.; Møller, I. M.; *Murphy, A. Fisiologia e desenvolvimento vegetal*. Artmed Editora 2017.
9. Tabari, H.; Talaee, P. H. Temporal variability of precipitation over Iran: 1966–2005. *Journal of Hydrology* 2011, 396(3-4), 313-320.
10. Lu, C.; Zhang, J. Effects of water stress on photosystem II photochemistry and its thermostability in wheat plants. *Journal of Experimental Botany* 1999, 50(336), 1199-1206.
11. Liu, C.; Liu, Y.; Guo, K.; Fan, D.; Li, G.; Zheng, Y.; Yu, L. Yang, R. Effect of drought on pigments, osmotic adjustment and antioxidant enzymes in six woody plant species in karst habitats of southwestern China. *Environmental and Experimental Botany* 2011, 71(2), 174-183.
12. Tabari, H.; Abghari, H.; Hosseinzadeh Talaee, P. Temporal trends and spatial characteristics of drought and rainfall in arid and semiarid regions of Iran. *Hydrological Processes* 2012, 26(22), 3351-3361.
13. Gill, S. S.; Tuteja, N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant physiology and biochemistry* 2010, 48(12), 909-930.
14. Choudhury, F. K.; Rivero, R. M.; Blumwald, E.; Mittler, R. Reactive oxygen species, abiotic stress and stress combination. *The Plant Journal* 2017, 90(5), 856-867.
15. Talbi, S.; Romero-Puertas, M. C.; Hernández, A.; Terrón, L.; Ferchichi, A.; Sandalio, L. M. Drought tolerance in a Saharian plant Oudneya africana: role of antioxidant defences. *Environmental and Experimental Botany* 2015, 111, 114-126.
16. Dietz, K. J.; Mittler, R.; Noctor, G. Recent progress in understanding the role of reactive oxygen species in plant cell signaling. *Plant physiology* 2016, 171(3), 1535-1539.
17. Hall, A. E. *Crop responses to environment*. CRC press 2000.
18. Sikder, S. Accumulated heat unit and phenology of wheat cultivars as influenced by late sowing heat stress condition. *Journal of Agriculture & Rural Development* 2009, 59-64.
19. Wahid, A.; Gelani, S.; Ashraf, M.; Foolad, M. R. Heat tolerance in plants: an overview. *Environmental and experimental botany* 2007, 61(3), 199-223.
20. Fischer, R. A. Crop Temperature Modification and Yield Potential in a Dwarf Spring Wheat 1. *Crop Science* 1976, 16(6), 855-859.
21. Bishnoi, O. P.; Singh, S.; Niwas, R. Effect of temperature on phenological development of wheat (*Triticum aestivum*). *Indian Journal of Agricultural Sciences* (India) 1995.
22. Ashraf, M.; Harris, P. (Eds.). *Abiotic stresses: plant resistance through breeding and molecular approaches*. CRC Press 2005.

23. Wollenweber, B.; Porter, J. R.; Schellberg, J. Lack of interaction between extreme high-temperature events at vegetative and reproductive growth stages in wheat. *Journal of Agronomy and Crop* Science 2003, 189(3), 142-150.
24. Finkelstein, R. Abscisic acid synthesis and response. The Arabidopsis book/*American Society of Plant Biologists* 11, 2013.
25. Bartels, D.; Sunkar, R. Drought and salt tolerance in plants. *Critical reviews in plant sciences* 2005, 24(1), 23-58.
26. Moustakas, M.; Sperdouli, I.; Kouna, T.; Antonopoulou, C. I.; Therios, I. Exogenous proline induces soluble sugar accumulation and alleviates drought stress effects on photosystem II functioning of *Arabidopsis thaliana* leaves. *Plant Growth Regulation* 2011, 65(2), 315.
27. Zaher-Ara, T.; Boroomand, N.; Sadat-Hosseini, M. Physiological and morphological response to drought stress in seedlings of ten citrus. *Trees* 2016, 30(3), 985-993.
28. Hong, Z.; Lakkineni, K.; Zhang, Z.; Verma, D. P. S. Removal of feedback inhibition of Δ1-pyrroline-5-carboxylate synthetase results in increased proline accumulation and protection of plants from osmotic stress. *Plant physiology* 2000, 122(4), 1129-1136.
29. Deuschle, K.; Funck, D.; Hellmann, H.; Däschner, K.; Binder, S.; Frommer, W. B. A nuclear gene encoding mitochondrial Δ1-pyrroline-5-carboxylate dehydrogenase and its potential role in protection from proline toxicity. *The Plant Journal* 2001, 27(4), 345-356.
30. Ahmadian Chashmi, N.; Sharifi, M.; Behmanesh, M. Lignan enhancement in hairy root cultures of *Linum album* using coniferaldehyde and methylenedioxycinnamic acid. *Preparative Biochemistry and Biotechnology* 2016, 46(5), 454-460.
31. Javadian, N.; Karimzadeh, G.; Sharifi, M.; Moieni, A.; Behmanesh, M. In vitro polyploidy induction: changes in morphology, podophyllotoxin biosynthesis, and expression of the related genes in *Linum album* (Linaceae). *Planta* 2017, 245(6), 1165-1178.
32. Tashackori, H.; Sharifi, M.; Chashmi, N. A.; Safaie, N.; Behmanesh, M. Induced-differential changes on lignan and phenolic acid compounds in *Linum album* hairy roots by fungal extract of Piriformospora indica. *Plant Cell, Tissue and Organ Culture* 2016, 127(1), 187-194.
33. Kiani, R.; Nazeri, V.; Kalvandi, R. Investigation of genetic diversity in some populations of flax (*Linum album* Ky. ex Bioss.) using morphological traits, The 4th National Congress on Medicinal Plants, Tehran, Iran 2015.
34. Kiani, R.; Nazeri, V.; Rezaei, K.; Kalvandi, R. Evaluation of Morphological Diversity in *Linum album* Ky. ex Boiss. populations in Karaj conditions. 11th Iranian Horticultural Science, Urmia, Iran 2019.
35. Taiz, L.; Zeiger, E. *Plant Physiology*, 4th Ed. Sinauer Associates. Inc.; Sunderland, MA 2006.
36. Plaut, Z. Plant exposure to water stress during specific growth stages, *Encyclopedia of Water Science* 2003.
37. Schuppler, U.; He, P. H.; John, P. C.; Munns, R. Effect of water stress on cell division and Cdc2-like cell cycle kinase activity in wheat leaves. *Plant physiology* 1998, 117(2), 667-678.
38. Kavar, T.; Maras, M.; Kidrič, M.; Šuštar-Vozlič, J.; Meglič, V. Identification of genes involved in the response of leaves of *Phaseolus vulgaris* to drought stress. *Molecular Breeding* 2008, 21(2), 159-172.
39. Ahmadi, A.; Baker, D. A. The effect of water stress on the activities of key regulatory enzymes of the sucrose to starch pathway in wheat. *Plant Growth Regulation* 2001, 35(1), 81-91.
40. Morgan, P.W. Effects of abiotic stresses on plant hormone systems, in: Stress Responses in plants: adaptation and acclimation mechanisms, Wiley-Liss, Inc. 1990, pp. 113–146.
41. Ober, E. S.; Setter, T. L.; Madison, J. T.; Thompson, J. F.; Shapiro, P. S. Influence of water deficit on maize endosperm development: enzyme activities and RNA transcripts of starch and zein synthesis, abscisic acid, and cell division. *Plant Physiology* 1991, 97(1), 154-164.
42. Sionit, N.; Kramer, P. J. Effect of water stress during different stages of growth of soybean. *Agronomy Journal* 1977, 69(2), 274-278.
43. Estrada-Campuzano, G.; Miralles, D. J.; Slafer, G. A. Genotypic variability and response to water stress of pre-and post-anthesis phases in triticale. *European Journal of Agronomy* 2008, 28(3), 171-177.
44. Farooq, M.; Wahid, A.; Kobayashi, N.; Fujita, D. B. S. M. A.; Basra, S. M. A. *Plant drought stress: effects, mechanisms and management*. In Sustainable agriculture 2009, *Springer*, Dordrecht, pp. 153-188.
45. Uzilday, B.; Turkan, I.; Sekmen, A. H.; Ozgur, R. E. N. G. İ. N.; Karakaya, H. C. Comparison of ROS formation and antioxidant enzymes in Cleome gynandra (C4) and Cleome spinosa (C3) under drought stress. *Plant Science* 2012, 182, 59-70.

46. Barrs, H. D.; Weatherley, P. E. A re-examination of the relative turgidity technique for estimating water deficits in leaves. *Australian journal of biological sciences* 1962, 15(3), 413-428.
47. Arjenaki, F. G.; Jabbari, R.; Morshedi, A. Evaluation of drought stress on relative water content, chlorophyll content and mineral elements of wheat (*Triticum aestivum* L.) varieties. *International Journal of Agriculture and Crop Sciences* 2012, 4(11), 726-729.
48. Guo, R.; Hao, W.; Gong, D. Effects of water stress on germination and growth of linseed seedlings (*Linum usitatissimum* L), photosynthetic efficiency and accumulation of metabolites. *Journal of Agricultural Science* 2012, 4(10), 253.
49. Talebi, R.; Ensafi, M. H.; Baghebani, N.; Karami, E.; Mohammadi, K. Physiological responses of chickpea (*Cicer arietinum*) genotypes to drought stress. *Environ Exp Biol* 2013, 11, 9-15.
50. Pirzad, A.; Shakiba, M. R.; Zehtab-Salmasi, S.; Mohammadi, S. A.; Darvishzadeh, R.; Samadi, A. Effect of water stress on leaf relative water content, chlorophyll, proline and soluble carbohydrates in *Matricaria chamomilla* L., *Journal of Medicinal Plants Research* 2011, 5(12), 2483-2488.
51. Anjum, F.; Yaseen, M.; Rasul, E.; Wahid, A.; Anjum, S. Water stress in barley (*Hordeum vulgare* L.). II. Effect on chemical composition and chlorophyll contents. *Pak. J. Agric. Sci* 2003, 40, 45-49.
52. Fu, J.; Huang, B. Involvement of antioxidants and lipid peroxidation in the adaptation of two cool-season grasses to localized drought stress. *Environmental and Experimental Botany* 2001, 45(2), 105-114.
53. Monakhova, O. F.; Chernyad'ev, I. I. Protective role of kartolin-4 in wheat plants exposed to soil draught. *Applied Biochemistry and Microbiology* 2002, 38(4), 373-380.
54. Reddy, A. R.; Chaitanya, K. V.; Vivekanandan, M. Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. *Journal of plant physiology* 2004, 161(11), 1189-1202.
55. Hasegawa, P. M.; Bressan, R. A.; Zhu, J. K.; Bohnert, H. J. Plant cellular and molecular responses to high salinity. *Annual review of plant biology* 2000, 51(1), 463-499.
56. Zhu, J. K. Salt and drought stress signal transduction in plants. *Annual review of plant biology* 2002, 53(1), 247-273.
57. Wahid, A.; Close, T. J. Expression of dehydrins under heat stress and their relationship with water relations of sugarcane leaves. *Biologia Plantarum* 2007, 51(1), 104-109.
58. Askari, E.; Ehsanzadeh, P. Osmoregulation-mediated differential responses of field-grown fennel genotypes to drought. *Industrial Crops and Products* 2015, 76, 494-508.
59. Dhar, P.; Ojha, D.; Kar, C. S.; Mitra, J. Differential response of tossa jute (*Corchorus olitorius*) submitted to water deficit stress. *Industrial crops and products* 2018, 112, 141-150.
60. Zarrinabadi, I. G.; Razmjoo, J.; Mashhadi, A. A.; Boroomand, A. Physiological response and productivity of pot marigold (*Calendula officinalis*) genotypes under water deficit. *Industrial Crops and Products* 2019, 139, 111488.
61. Kaur, G.; Asthir, B. J. B. P. Proline: a key player in plant abiotic stress tolerance. *Biologia plantarum* 2015, 59(4), 609-619.
62. Venkatesan, A.; Chellappan, K. P. Accumulation of proline and glycine betaine in Ipomoea pes-caprae induced by NaCl. *Biologia Plantarum* 1998, 41(2), 271-276.
63. Mansour, M. M. F. Nitrogen containing compounds and adaptation of plants to salinity stress. *Biologia Plantarum* 2000, 43(4), 491-500.
64. Yang, W. J.; Rich, P. J.; Axtell, J. D.; Wood, K. V.; Bonham, C. C.; Ejeta, G.; Rhodes, D. Genotypic variation for glycinebetaine in sorghum. *Crop Science* 2003, 43(1), 162-169.
65. Quan, R.; Shang, M.; Zhang, H.; Zhao, Y.; Zhang, J. Improved chilling tolerance by transformation with betA gene for the enhancement of glycinebetaine synthesis in maize. *Plant Science* 2004, 166(1), 141-149.
66. Subbarao, G. V.; Wheeler, R. M.; Levine, L. H.; Stutte, G. W. Glycine betaine accumulation, ionic and water relations of red-beet at contrasting levels of sodium supply. *Journal of Plant Physiology* 2001, 158(6), 767-776.
67. Fallon, K. M.; Phillips, R. Responses to water stress in adapted and unadapted carrot cell suspension cultures. *Journal of Experimental Botany* 1989, 40(6), 681-687.
68. McCue, K. F.; Hanson, A. D. Drought and salt tolerance: towards understanding and application. *Trends in Biotechnology* 1990, 8, 358-362.
69. Rhodes, D.; Hanson, A. D. Quaternary ammonium and tertiary sulfonium compounds in higher plants. *Annual review of plant biology* 1993, 44(1), 357-384.
70. Heath, R. L.; Packer, L. Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. *Archives of biochemistry and biophysics* 1968, 125(1), 189-198.

71. Gaweł, S.; Wardas, M.; Niedworok, E.; Wardas, P. Malondialdehyde (MDA) as a lipid peroxidation marker. Wiadomosci lekarskie (Warsaw, Poland: 1960) 2004, 57(9-10), 453-455.
72. Masia, A. Physiological effects of oxidative stress in relation to ethylene in postharvest produce. *Postharvest oxidative stress in horticultural crops* 2003, 165-197.
73. Xu, Z. Z.; Zhou, G. S.; Wang, Y. L.; Han, G. X.; Li, Y. J. Changes in chlorophyll fluorescence in maize plants with imposed rapid dehydration at different leaf ages. *Journal of plant growth regulation* 2008, 27(1), 83-92.
74. Khoshro, H. H.; Taleei, A.; Bihamta, M. R.; Shahbazi, M.; Abbasi, A. Expression analysis of the genes involved in osmotic adjustment in bread wheat (*Triticum aestivum* L.) cultivars under terminal drought stress conditions. *Journal of Crop Science and Biotechnology* 2013, 16(3), 173-181.
75. Shanjani, P. S.; Izadpanah, M.; Mohamadpour, M. R. Effects of water stress on germination of yarrow populations (*Achillea* spp.) from different bioclimatic zones in Iran. *Plant Breeding and Seed Science* 2014, 68(1), 39.
76. Dhanda, S. S.; Sethi, G. S.; Behl, R. K. Indices of drought tolerance in wheat genotypes at early stages of plant growth. *Journal of agronomy and crop science* 2004, 190(1), 6-12.
77. Yousfi, N.; Slama, I.; Ghnaya, T.; Savoure, A.; Abdelly, C. Effects of water deficit stress on growth, water relations and osmolyte accumulation in *Medicago truncatula* and *M. laciniata* populations. *Comptes Rendus Biologies* 2010, 333(3), 205-213.
78. Taylor, I. B. Genetics of ABA synthesis. Abscisic Acid, Physiology and Biochemistry 1991, 23-38.
79. Sharp, R. E.; Wu, Y.; Voetberg, G. S.; Saab, I. N.; LeNoble, M. E. Confirmation that abscisic acid accumulation is required for maize primary root elongation at low water potentials. *Journal of Experimental Botany* 1994, 1743-1751.
80. Turner, N. C.; Wright, G. C.; Siddique, K. H. M. Adaptation of grain legumes (pulses) to water-limited environments. *Advances in agronomy* 2001, 71, 194-233.
81. Abedi, T.; Pakniyat, H. Antioxidant enzymes changes in response to drought stress in ten cultivars of oilseed rape (*Brassica napus* L.). *Czech Journal of Genetics and Plant Breeding* 2010, 46(1), 27-34.
82. Caverzan, A.; Casassola, A.; Brammer, S. P. Antioxidant responses of wheat plants under stress. *Genetics and molecular biology* 2016, 39(1), 1-6.
83. Miller, G. A. D.; Suzuki, N.; Ciftci-Yilmaz, S. U. L. T. A. N.; Mittler, R. O. N. Reactive oxygen species homeostasis and signalling during drought and salinity stresses. *Plant, cell & environment* 2010, 33(4), 453-467.
84. Mittler, R.; Vanderauwera, S.; Gollery, M.; Van Breusegem, F. Reactive oxygen gene network of plants. *Trends in plant science* 2004, 9(10), 490-498.
85. Mittler, R. ROS are good. *Trends in plant science* 2017, 22(1), 11-19.
86. Mittler, R. Oxidative stress, antioxidants and stress tolerance. *Trends in plant science* 2002, 7(9), 405-410.
87. Tunc-Ozdemir, M.; Miller, G.; Song, L.; Kim, J.; Sodek, A.; Koussevitzky, S.; Shintani, D. Thiamin confers enhanced tolerance to oxidative stress in Arabidopsis. *Plant physiology* 2009, 151(1), 421-432.
88. Orvar, B. L.; Ellis, B. E. Transgenic tobacco plants expressing antisense RNA for cytosolic ascorbate peroxidase show increased susceptibility to ozone injury. *The Plant Journal* 1997, 11(6), 1297-1305.
89. Shigeoka, S.; Ishikawa, T.; Tamoi, M.; Miyagawa, Y.; Takeda, T.; Yabuta, Y.; Yoshimura, K. Regulation and function of ascorbate peroxidase isoenzymes. *Journal of experimental botany* 2002, 53(372), 1305-1319.
90. Rahimi, Y.; Taleei, A.; Ranjbar, M. Long-term water deficit modulates antioxidant capacity of peppermint (*Mentha piperita* L.). *Scientia Horticulturae* 2018, 237, 36-43.
91. Morshedloo, M. R.; Salami, S. A.; Nazeri, V.; Craker, L. E. Prolonged Water Stress on Growth and Constituency of Iranian of Oregano (*Origanum vulgare* L.). *Journal of Medicinally Active Plants* 2017, 5(2), 7-19.
92. Kiani, R. Evaluation of morphological and phytochemical diversity of *Linum album* Ky. ex Bioss.; an endemic medicinal plant in Iran. Master of Science Thesis. Department of Horticultural Sciences College of Agriculture and Natural Resources, University of Tehran 2016.
93. Miller, P.; Lanier, W.; Brandt, S. Using growing degree days to predict plant stages. Ag/Extension Communications Coordinator, Communications Services, Montana State University-Bozeman, Bozeman, MO 2001, 59717(406), 994-2721.
94. Turner, N. C. Crop water deficits: a decade of progress. *In Advances in agronomy* (Vol. 39, pp. 1-51), Academic Press 1986.

 doi:10.20944/preprints202010.0110.v1

95. Sumanta, N.; Haque, C. I.; Nishika, J.; Suprakash, R. Spectrophotometric analysis of chlorophylls and carotenoids from commonly grown fern species by using various extracting solvents. *Research Journal of Chemical Sciences* ISSN 2014, 2231, 606X.
96. Bates, L. S.; Waldren, R. P.; Teare, I. D. Rapid determination of free proline for water-stress studies. *Plant and soil* 1973, 39(1), 205-207.
97. Grieve, C. M.; Grattan, S. R. Rapid assay for determination of water soluble quaternary ammonium compounds. *Plant and soil* 1983, 70(2), 303-307
98. Hodges, D. M.; DeLong, J. M.; Forney, C. F.; Prange, R. K. Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. *Planta* 1999, 207(4), 604-611.
99. Nayyar, H.; Walia, D. P. Water stress induced proline accumulation in contrasting wheat genotypes as affected by calcium and abscisic acid. *Biologia Plantarum* 2003, 46(2), 275-279.
100. Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical biochemistry* 1976, 72(1-2), 248-254.
101. Hadwan, M. H. Simple spectrophotometric assay for measuring catalase activity in biological tissues. *BMC biochemistry* 2018, 19(1), 7.
102. Plewa, M. J.; Smith, S. R.; Wagner, E. D. Diethyldithiocarbamate suppresses the plant activation of aromatic amines into mutagens by inhibiting tobacco cell peroxidase. *Mutation research/fundamental and molecular mechanisms of mutagenesis* 1991, 247(1), 57-64.
103. Ranieri, A.; D'urso, G.; Nali, C.; Lorenzini, G.; Soldatini, G. F. Ozone stimulates apoplastic antioxidant systems in pumpkin leaves. *Physiologia Plantarum* 1996, 97(2), 381-387.