

## ABSCISIC ACID ACCUMULATION AND PHYSIOLOGICAL INDICES IN RESPONSES TO DROUGHT STRESS IN WHEAT GENOTYPES

S. A. Qadir<sup>1</sup>M. Q. Khursheed<sup>2</sup>T. S. Rashid<sup>3</sup>H. K. Awla<sup>4</sup>

Lecturer

Prof.

Lecturer

Lecturer

<sup>1</sup>Dept.of Fores., Coll. of Agric, Salahaddin University . Erbil, Iraq. <sup>2</sup>Dept.of Biol.Coll. of Educat. Salahaddin University, Erbil, Iraq. <sup>3</sup>Dept.of Plant Prot. Coll. of Agric. Salahaddin University, Erbil

<sup>4</sup>Department of Plant Protection, Khabat Instit.. Erbil Polytechnic University

Email: hayman.k.a@epu.edu.krd

### ABSTRACT

The aim of this study to determine how tolerant wheat genotypes to drought stress. Three drought tolerant *Triticum aestivum* L. genotypes; Rizgary, Sham-6 and Abu-Ghraib compared with three drought-susceptible; IPA95, Tammuz-2 and IPA99 under water deficit 20% WHC (Water Holding Capacity). ABA level, drought related parameters; shoot and root dry weights, root and shoot ratio. As well as Leaf relative water content (LRWC %), membrane stability index (MSI %) and proline content determined in the leaves. A considerable increase of ABA noted in drought tolerant as compared to susceptible genotypes under drought stress condition. That is led to reduction in shoot dry weight in Rizgary, Sham-6 and Abu-ghreb; 2.71, 2.70 and 2.62 g/plant respectively. In addition they adapted to proliferate larger root system with 2.63, 2.16 and 1.71 g/plant respectively. Consequently the ratio of root: shoot increased; 0.98, 0.80 and 0.66. Rizgary, Sham-6 and Abu-ghreb retained a significantly higher LRWCs %; 79.81, 77.17 and 78.53 % respectively at 20 % WHC as compared to susceptible genotypes. Membrane Stability Index (MSI %) decreased significantly at lower rate in tolerant genotypes; 65.69, 67.28 and 67.18 % respectively. As well as proline increased as an osmoticum to reduce the harmful effect of drought on plant cell in resistant genotypes; Rizgary, Sham-6 and Abu-ghreb; 0.71, 0.72 and 0.61 mg g<sup>-1</sup> D. in sequence as compared to sensitive genotypes. The tolerant genotypes have stronger adaptability mechanisms to cope water scarcity as compared to susceptible genotypes.

**Key words:** ABA, root: shoot ratio, LRWC%, MSI%, proline.

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تراكم حامض الابسيسك و دراسة الصفات الفسيولوجية المتعلقة بالجفاف في القمح الناعم تحت ظروف الشد المائي

هيمن كاة خان عولا<sup>4</sup>

تافكه سليمان رشيد<sup>3</sup>

محمد قادر خورشيد<sup>2</sup>

سروه انور قادر<sup>1</sup>

مدرس

مدرس

أستاذ

مدرس

معهد خابات

كلية الزراعة

كلية التربية

كلية الزراعة

جامعة بوليتكنيك - اربيل

جامعة صلاح الدين - اربيل

جامعة صلاح الدين - اربيل

المستخلص

الهدف من هذه الدراسة هو تحديد مدى كيفية الأنماط الوراثية المقاومة للمح للجهود المائي الناتج عن الجفاف. تمت مقارنة ثلاثة تراكيب جينية مقاومة من *Triticum aestivum* L. رزطاري وشام -6 وأبو غريب مع ثلاثة تراكيب وراثية حساسة للجفاف ؛ اباء - 95 ، تموز - 2 و اباء - 99 تحت مستوى 20 % للسعة الحقلية (WHC%). تم تقدير نسبة هورمون الأبسيسك اسيد ABA ، البارامترات المتعلقة بالجفاف ؛ الأوزان الجافة للمجموع الخضري و الجذري ونسبة المجموعة الجذرية الى الخضرية . بالإضافة إلى محتوى الماء النسبي للورقة (LRWC % ) ، ودليل ثبات الغشاء الخلوي (MSI %) ومحتوى البرولين في الأوراق. لوحظت زيادة كبيرة في تركيز ABA في الأنماط الجينية المقاومة بالمقارنة مع الأنماط الجينية الحساسة تحت ظروف الإجهاد المائي. حيث أدى ذلك إلى انخفاض الوزن الجاف في رزطاري وشام -6 وأبو غريب 2.71 و 2.70 و 2.62 غم / نبات على التوالي. بالإضافة إلى تكيفها لتكاثر نظام الجذري الأكبر مع 2.63 ، 2.16 و 1.71 جم / نبات على التوالي. ونتيجة لذلك زادت نسبة الجذر: المجموعة الخضرية 0.98 و 0.80 و 0.66. احتفظ كل من رزطاري ، شام -6 و أبو غريب بنسبة أكبر من LRWCs % ؛ 79.81 و 77.17 و 78.53 / على التوالي عند 20% من ال WHC مقارنة بالأنماط الوراثية الحساسة. كذلك انخفض (MSI %) بشكل ملحوظ في معدل أقل في الأنماط الوراثية المقاومة 65.69 و 67.28 و 67.18 % على التوالي. بالإضافة إلى زيادة البرولين كمنتج أسموزي لتقليل التأثير الضار للجفاف على الخلايا النباتية في الأصناف المقاومة. رزطاري ، شام - 6 وأبو غريب ؛ 0.71 و 0.72 و 0.61 ملجم / نبات على التوالي بالمقارنة مع الأنماط الوراثية الحساسة. تتمتع الأنماط الوراثية المقاومة بآليات أقوى للتكيف لمواجهة ندرة المياه مقارنة بالأنماط الوراثية حساسة التأثر.

كلمات مفتاحية: الانماط الوراثية، تراكيب وراثية، هورمانات، البروتين، الجذر، الاوراق.

## INTRODUCTION

Water resources reduction due to climatic changes around the world thought is one of the most essential environmental stresses in arid and semi-arid regions. Significantly cause yield loss in several crops mainly in wheat bread *Triticum aestivum* L. (6). Phytohormones create an ability in plants to conform to abiotic stresses via mediating a huge range of adaptive responses (26). Decrease in soil moisture can impose osmotic pressure on the plant at metabolic, physiological and morphological ranges, and secondary indicators, e.g. abscisic acid (ABA) will arouse (41). At water deficit conditions, the buildup of ABA hormone plays a wonderful role in response and tolerance to dehydration. Closure of stomata and induction of the expression of more than one genes involved in protection towards the drought stress are functions of ABA (14). The amount of ABA in the xylem sap will increase drastically, and this results in elevated ABA concentration in different leaf compartments (38). Drought is firstly sensed by roots; then, root-to-leaf signaling through the transpiration movement triggers stomata closure, which is an essential adaptation mechanism to water scarcity (4). Vital drought stress responses consist of; root length and behavior. Root elongations maintain even at excessive drought stress and complete inhibition of shoot growth in few plant species, (31). Fan et al., (13) showed that root/shoot dry weight ratio will increase as water availability decreases because of relative reductions in shoot dry weight and leaf area. Shrinkage cells with low water content can bring about decrease in relative water content material (RWC). It regarded as important indicator to examine the sensitivity of genotypes to water deficit (32). At biochemical level the reactive oxygen species liberated due to lipid peroxidation decreases membrane stability (MSI %) (23). Thus under water stress cell membrane integrity and stability award the degree of drought resistance of the plant species (39). Osmotic adjustment consisting of proline accumulation can partially protect the plant against slight drought stress (3). It does not war with ordinary biochemical reactions however grant the plant to continue to exist

under drought condition (21). Bhaskara et al. (8) pronounced that the accumulation of proline became dominant within the tolerant than inside the sensitive plant. This implied that proline able to support the plant to recover after water stress. The aim of the present study to determine endogenous ABA content and physiological behavior of two sets of bread wheat *Triticum aestivum* L. differing in their degree resistance for moisture tension condition.

## MATERIALS AND METHODS

### Treatment and experimental design

Six (*Triticum aestivum* L.) bread wheat genotypes, the drought-tolerant genotypes; Sham-6, Rizgary, Abu-ghreb and the drought-sensitive genotypes; Tammuz-2, Ebba-95 and Ebaa-99 as their genetic diversity for drought tolerance previously investigated by (27) using a valuable diagnostic tool Random Amplified Polymorphic DNA (RAPD) technique. Grains were grown under glasshouse conditions. Four seeds had been randomly sowed in plastic pot, each containing 6 kg of soil. Thinning was done after the 5th day of seedling emergence. The primary dose of recommended fertilizer 120 kg ha<sup>-1</sup> DAP (N=18% and P=46%) brought prior to seed sowing and the second dose was Urea (N= 46%) after 60 days from sowing. For drought treatment, units of pots had been kept at 90% water holding capacity (WHC) of soil (control), and 20% WHC (extreme stress). The WHC was decided by saturating the soil within the pot, protecting the tops with aluminum foil and weighing day by day till there has been no weight loss within 24, 48 and 72 hours period. 450 and 100 mL water had been determined respectively as 90% and 20% WHC (22). After (80-90) days from sowing (late the stem elongation before heading stage) the observations used to determine the following measurements:

### Biomass characteristics

**Shoot and root dry weights:** The whole plant become uprooted through pouring water into the plant's pot; roots had been cautiously wiped clean with tap water and later washed with distilled water then separated into shoot and root. The shoot and root have been dried in an oven at (70°C) for 48hrs, then shoot and root dry weight had been measured and the ratio of shoot dry weight (g plant<sup>-1</sup>)/ root dry

weight (g plant<sup>-1</sup>) was calculated (7). Then Root: shoot dry weight ratio plant<sup>-1</sup> was calculated according to the following formula:

$$\text{Root: shoot ratio} = \frac{(\text{root dry weight})}{(\text{shoot dry weight})}$$

Leaf relative water content (RWC %):

Leaf relative water content was measured by sampling two similar fully expanded leaves per pot. Leaf samples had been sealed in plastic bag, placed above ice and transported to the lab to obtain the fresh weight. Leaves had been then floated on water for twenty-four hours to saturate and then weighted to obtain the turgid weight. The turgid leaves dried at 60° C for 48 hours until constant weight obtained. RWC were calculated as described by Shivakrishna et al. (36).=

$$\text{RWC}(\%) = \frac{(\text{Fresh weight} - \text{dry weight})}{(\text{turgid weight} - \text{dry weight})} \times 100$$

Membrane stability index (MSI %):

Membrane stability index (MSI) of leaf cells determined according to the method of Singh et al. (37). Leaf discs (a 100 mg) were thoroughly washed in walking tap water, twice washed with double distilled water and thereafter the discs were heated in 10 mL of double distilled water at 40° C for 30 minutes. Then electric conductivity (C1) recorded by EC meter. Subsequently the same samples were placed in a boiling water bath (100 °C) for 10 minutes and their electric conductivity become additionally recorded (C2).The MSI was calculated according to the following formula:

$$\text{Membrane stability index} = \left[ 1 - \left( \frac{C1}{C2} \right) \right] \times 100$$

Proline (mg g<sup>-1</sup> D. W):

Proline content of leaves became predicted following the approach of Hofmann et al. (17). Fresh plant material (0.1 g) homogenized with four mL sulfosalicylic acid (3 %) in mortar and kept overnight at 5 °C. Suspension subjected to centrifugation at room temperature to 3000 rpm for five minutes. Supernatant mixed with 4 ml acidic ninhydrin reagent. Reaction kept under automatically shaker; the contents in the tubes were heated in boiling water bath for 1 hour. Thereafter, the tube content was cooled and proline extracted with 4 ml of toluene in a separating funnel. The absorbance of toluene layer turned into record at 520 nm. The concentration of the

unknown sample was calculated with regards to the standard curve.

#### ABA extraction and quantification

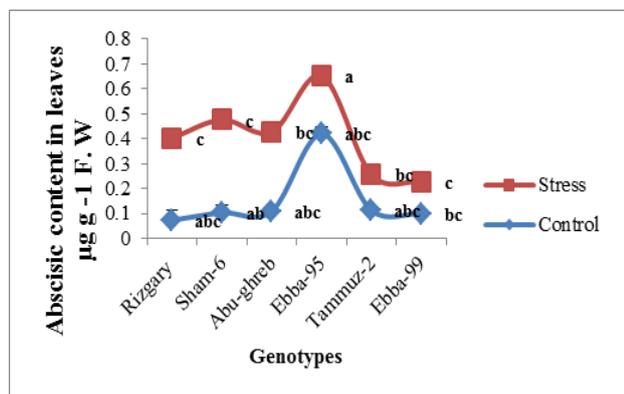
Ten fresh leaves have been at once frozen and stored at -70 °C till ABA evaluation. Samples approximately 1 g had been ground in liquid nitrogen homogenized and then extracted overnight with 30 ml 80% cold aqueous methanol (< 0) in darkness at 4 °C. The extract becomes centrifuged at 5000 r/min and 4 °C for 15 min and the supernatant collected. Then cold methanol added, extracted three instances with aforementioned methods. The whole methanolic extract dried in rotary evaporator and dissolved in 10 ml methanol. The ABA contents were determined using high-performance liquid chromatography (Waters, USA) with photodiode array (PDA) detection at 254 nm. ABA was measured by the injection of the extract into a reverse-phase HPLC, with a methanol gradient in 0.6 % acetic acid (11).

#### Statistical analyses

The experiment set in a factorial with a Complete Randomized Design (CRD) with two watering level; (90% and 20% WHC) and six bread wheat genotypes replicated three instances. Analysis of variance (ANOVA) of the data computed using the Statistical package for the Social Sciences (SPSS) model 22. The Duncan's test used to check the variations among the mean values of studied parameters. 5 % level of possibility for greenhouse measurements and 1 % degree for the laboratory measurements.

#### RESULTS AND DISCUSSION

The important phytohormone that cope environmental abiotic stress mostly is ABA in a wide range of crops (34). The accumulation of the hormone ABA is a key reaction to water deficiency in plant life. At water deficit conditions it increased appreciably in tolerant genotypes; Rizgary, Sham-6 and Abu-ghreb compared to susceptible genotypes; Ebba- 95, Tammuz-2 and Ebba- 99 as shown in figure (1). Such increase formerly had been determined by Abdalla (1) and Sarafrz-Ardakani et al., (33). Inspiring stress-tolerance effects to help the plant to adapt and continue its life cycle in stressed environment (24).



**Fig 1. Abscisic content in leaves of tolerant and sensitive bread wheat genotypes under drought stressed 20% field capacity as compared to control condition**

Under drought stress, ABA hormone becomes suggested to act as a long-distance signal among roots and shoots (25). The first consequences of ABA activity under moisture tension vital for water uptake in drying soil, conserves plant hydration through decreased shoot growth, inhibition of leaf area and decreased stomatal conductance (9). Promotes root growth to provide the plant water from deeper soil depth, because as root density increases the contact point between root and soil increases (29). The high level of ABA in tolerant genotypes alters the plant growth

under water stress. As table 1 show, there is a decrease in shoot dry weight. Vice versa an increase observed in root dry weight and root: shoot ratio under 90% WHC. The tolerant genotypes; Rizgary, Sham-6 and Abu-ghreb generally tend to have significantly the higher shoots dry weight; 2.71, 2.70 and a 2.62 g plant<sup>-1</sup> respectively, due to the fact they adapted to proliferate larger root system with 2.63, 2.16 and 1.71 g plant<sup>-1</sup>. Therefore the ratio of root: shoot; 0.98, 0.80 and 0.66 was increased respectively. This has major effect on the crop tolerance and the maintenance of crop water status whilst drought stress developed (40). While the sensitive genotypes; Ebba- 95, Tammuz-2 and Ebba- 99 recorded the lower means for shoot dry weight; 2.10, 1.97 and a 2.07 g plant<sup>-1</sup>, due to the fact they failed to adapt to water shortage because of their smaller root system with; 1.03, 1.13 and 1.10 g plant<sup>-1</sup>. As a result their root: shoot ratio; 0.49, 0.57 and 0.51 reduced in comparison to the tolerant genotypes. This finding reflects the findings of different authors Boutraa et al. (10) and Hussein and khursheed (18).

**Table 1 Shoot and root dry weight with root: shoot ratio of tolerant and sensitive bread wheat genotypes.**

Wheat Genotypes	Treatments	Shoot dry weight g plant <sup>-1</sup>	Root dry weight g plant <sup>-1</sup>	Root: shoot ratio
Rizgary	Co	2.99 ± 0.065 a	2.06 ± 0.033 b	0.69 ± 0.013 c
	S	2.71 ± 0.220 ab	2.63 ± 0.102 a	0.98 ± 0.050 a
Sham-6	Co	2.79 ± 0.015 ab	1.72 ± 0.074 c	0.62 ± 0.027 cd
	S	2.70 ± 0.0240 ab	2.16 ± 0.055 b	0.80 ± 0.023 b
Abu-ghreb	Co	2.98 ± 0.062 a	1.15 ± 0.005 d	0.39 ± 0.010 h
	S	2.62 ± 0.113 b	1.71 ± 0.023 c	0.66 ± 0.027 c
Ebba-95	Co	2.63 ± 0.099 b	1.15 ± 0.046 d	0.44 ± 0.015 gh
	S	2.10 ± 0.033 cd	1.03 ± 0.015 d	0.49 ± 0.009 fg
Tammuz-2	Co	2.68 ± 0.050 b	1.12 ± 0.055 d	0.42 ± 0.018 h
	S	1.97 ± 0.073 d	1.13 ± 0.057 d	0.57 ± 0.015 de
Ebba-99	Co	2.32 ± 0.068 c	1.15 ± 0.018 d	0.50 ± 0.012 efg
	S	2.07 ± 0.087 cd	1.10 ± 0.019 d	0.51 ± 0.024 ef

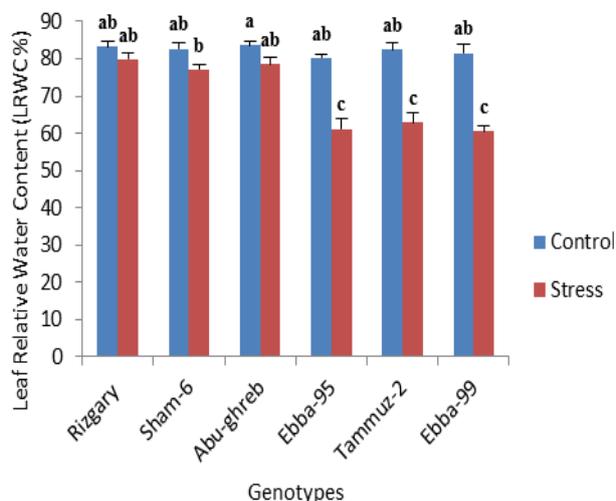
\*Co: Control; S: Stress

\*The treatments with the different letters are significantly different

Water potential and relative water content of leaves appeared as important markers of water status in crop plants (19). A big reduction recorded in LRWC% due to water stress in all genotypes (figure 2). Resistant genotypes; Rizgary, Sham-6 and Abu-ghreb retained a substantially better LRWCs %; 79, 81, 77.17 and 78.53 % under 20 % WHC as compared to

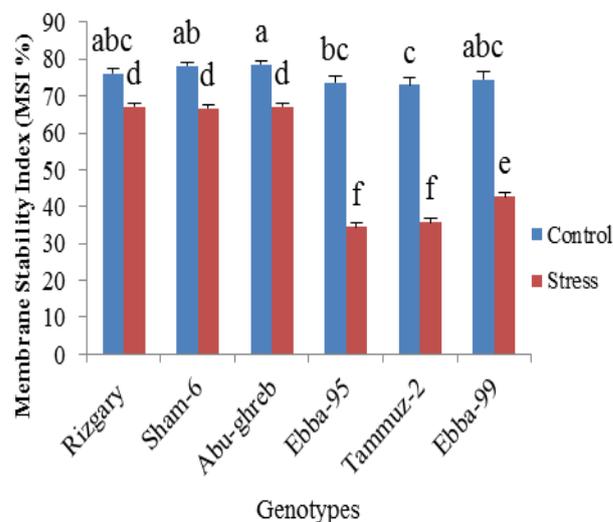
susceptible genotypes; Ebba- 95, Tammuz-2 and Ebba- 99, they recorded decrease means; 61.18, 62.957 and 60.59 % respectively under the same WHC. The RWC is typically higher in plants, which might be adapted low soil moisture content, and comparable observations had in advance been recorded by Arjenaki et al. (5) and Hasheminasab et al. (16). This is may be due differences in their capacity to absorb more water from the soil due to their bigger root system. As well as smaller shoot

system (i.e.; lesser number of stomata per plant area which lead to lower transpiration rate). As well as high RWC is the end result of higher osmotic regulation non enzymatic defense system or much less elasticity of the cell wall (30).



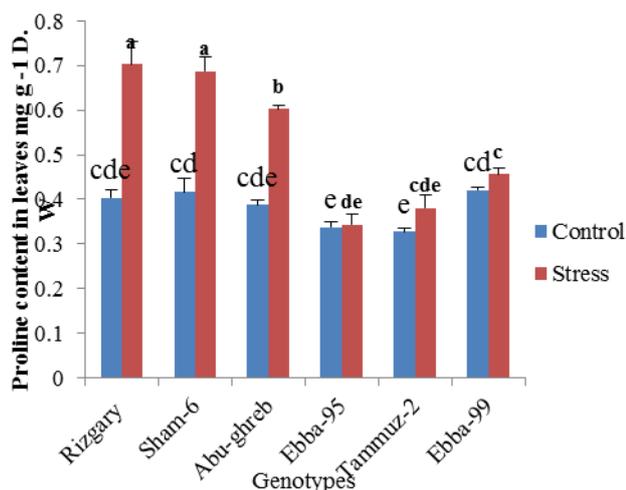
**Figure 2. Leaf relative water content (LRWC %) under drought stressed 20% field capacity of tolerant and sensitive bread wheat genotypes as compared to control condition**

Membrane harm is the foremost parameter to estimate lipid destruction due to stress condition which causes cellular membrane harm and leakage of electrolytes and damage down its balance (20). MSI% reduced extensively at drought stress condition also confirmed more decline in susceptible genotypes. It was determined that the tolerant genotypes; Rizgary, Sham-6 and Abu-ghreb had been recorded considerably higher MSI%; 65.69, 67.28 and 67.18 % respectively as compared to susceptible genotypes; Ebba- 95, Tammuz-2 and Ebba- 99; 34.52, 34.44 and 45.46 % respectively at 20 % WHC (figure 3). The results are much like those found by Ahmadzadeh et al. (2); Razzaq et al. (28) and Sharifi et al. (35).



**Fig 3. Membrane stability index (MSI %) of tolerant and sensitive bread wheat genotypes under drought stressed 20% field capacity as compared to control condition**

Results in figure (4) indicate that water stress induced accumulation of proline in wheat leaves in regard to drought stress circumstance. Its content increased appreciably in all genotypes at drought stress condition that's according with the findings of Anjum et al. (4) and Hussein and khursheed (18). Drought resistant wheat genotypes; Rizgary, Sham-6 and Abu-ghreb indicated higher accumulation of proline; 0.71, 0.72 and 0.61 mg g<sup>-1</sup> D. W compared to sensitive genotypes; Ebba- 95, Tammuz-2 and Ebba-99; 0.39, 0.42 and 0.47 mg g<sup>-1</sup> D. W this is comparable with Hasheminasab et al. (16) findings; that proline accumulation indicates the tolerance ability in wheat genotypes. Proline act as an osmolyte, accumulates under moisture tension situation. That can guard the cell via increasing the osmotic content than outside environment to enhance water movement according to its gradual potential (15), as it acts as an effective non- enzymatic antioxidant mechanism inside the cell against reactive oxygen species (ROS) (12).



**Fig 4. Proline content in leaves under drought stressed 20% field capacity of tolerant and sensitive bread wheat genotypes.**

It is elucidated that ABA performs a vital function in wheat's response and resistance to drought stress. ABA accumulation during water stress may often function to help maintain suitable shoot size in accordance to root growth, rather than to inhibit growth as is commonly believed. As well as plant use other adaptation mechanism like cell shrinkage to decreased water content. Cell membrane stability index and increased proline content of the leaves more remarkably differ in tolerant genotypes as compared to susceptible one

**Conflict of interest:** The authors have no conflict of interest to declare.

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#### REFERENCES

1. Abdalla, M. M. 2015. Morphological and physiological changes in two *Triticum aestivum* cultivars differing in water stress tolerance. *Journal of Agricultural Technology* 11(1). 143-164.
2. Ahmadizadeh, M., A. H. Nori, Shahbazi, and S. Aharizad. 2011. Correlated response of morpho-physiological traits of grain yield in durum wheat under normal irrigation and drought stress conditions in greenhouse. *African Journal of Biotechnology* 10(85). 19771-19779
3. Alexieva, V., I. Sergiev, S. Mapelli, and E. Karanov. 2001. The effect of drought and ultraviolet radiation on growth and stress

markers in pea and wheat. *Plant Cell and Environment* 24(12). 1337-1344

4. Anjum, S. A., X. Y. Xie, L. C. Wang, M. F. Saleem, C. Man, and W. Lei. 2011. Morphological, physiological and biochemical responses of plants to drought stress. *African journal of agricultural research* 6(9): 2026-2032

5. Arjenaki, F. G., R. Jabbari, and A. Morshedi. 2012. 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* 4(11). 726-729

6. Babu, R. C., J. Zhang, A. Blum, T. H. D. Ho, R. Wu, and H. T. Nguyen. 2004. HVA1, a LEA gene from barley confers dehydration tolerance in transgenic rice (*Oryza sativa* L.) via cell membrane protection. *Plant Science* 166(4). 855-862.

7. BAĞCI, S. A., H. Ekiz, and A. Yilmaz. 2003. Determination of the salt tolerance of some barley genotypes and the characteristics affecting tolerance. *Turkish Journal of Agriculture and Forestry* 27(5). 253-260

8. Bhaskara, G. B., T. H. Yang, and P. E. Verslues. 2015. Dynamic proline metabolism: importance and regulation in water limited environments. *Frontiers in Plant Science* 6. 484

9. Blum, A. 2011. Drought resistance—is it really a complex trait? *Function Plant Biology* 38(10). 753-757

10. Boutraa, T., A. Akhka, A. A. Al-Shoabi, and A. M. Alhejeli. 2010. Effect of water stress on growth and water use efficiency (WUE) of some wheat cultivars (*Triticum durum*) grown in Saudi Arabia. *Journal of Taibah University Medical Sciences* 3(1). 39-48

11. Chen, Y. P., and W. Y. Yang. 2005. Determination of GA~ 3, IAA, ABA and ZT in dormant buds of *Allium ovalifolium* by HPLC. *Sichuan Nongye Daxue Xueba* 23 (4). 498

12. Demiral, T., and I. Türkan. 2005. Comparative lipid peroxidation, antioxidant defense systems and proline content in roots of two rice cultivars differing in salt tolerance. *Environmental and Experimental Botany* 53(3). 247-257

13. Fan, L., R. Linker, S. Gepstein, E. Tanimoto, R. Yamamoto, and P. M. Neumann. 2006. Progressive inhibition by water deficit of cell wall extensibility and growth along the elongation zone of maize roots is related to increased lignin metabolism and progressive stelar accumulation of wall phenolics. *Plant physiology* 140(2). 603-612
14. Farooq, M., A. Wahid, N. Kobayashi, D. Fujita, and S. M. A. Basra. 2009. Plant drought stress: effects, mechanisms and management. In *Sustainable Agriculture* (pp. 153-188). Springer, Dordrecht
15. Geravandi, M., E. Farshadfar, and D. Kahrizi. 2011. Evaluation of some physiological traits as indicators of drought tolerance in bread wheat genotypes. *Russian Journal of Plant Physiology* 58(1). 69-75.
16. Hasheminasab, H., Assad, M. T., Aliakbari, A., and Sahhafi, S. R. 2012. Influence of drought stress on oxidative damage and antioxidant defense systems in tolerant and susceptible wheat genotypes. *The Journal of Agricultural Science* 4(8).20
17. Hofmann, R. W., B. D. Campbell, S. J. Bloor, E. E. Swinny, K. R. Markham, K. G. Ryan, and D. W. Fountain. 2003. Responses to UV-B radiation in *Trifolium repens* L.–physiological links to plant productivity and water availability. *Plant, Cell & Environment* 26(4). 603-612
18. Hussein, Z. K., and M. Q. Khursheed. 2014. Effect of foliar application of ascorbic acid on growth, yield components and some chemical constituents of wheat under water stress conditions. *Jordan Journal of Agricultural Sciences* 10. 1-15.
19. Jongdee, B., S. Fukai, and M. Cooper. 2002. Leaf water potential and osmotic adjustment as physiological traits to improve drought tolerance in rice. *Field Crops Research* 76(2-3). 153-163
20. Khayatnezhad, M., and Gholamin, R. 2012. The effect of drought stress on leaf chlorophyll content and stress resistance in maize cultivars (*Zea mays*). *African Journal of Microbiology Research* 6(12). 2844-2848
21. Kohl, D.H., E.J. Kennelly, Y. Zhu, K.R. Schubert, G. Shearer. 1991. Proline accumulation, nitrogenase (C<sub>2</sub>H<sub>2</sub> reducing) activity and activities of enzymes related to proline metabolism in drought-stressed soybean nodules. *Journal of Experimental Botany* 42(7). 831-837
22. Kramer, P. J., J. S. Boyer. 1995. *Water relations of plants and soils*. Academic press
23. Kumari A. 2012. *Physiological And Molecular Constraints Limiting Grain Growth Under Water Stress In Wheat Genotypes*. (Doctoral Dissertation, Iarqi, Division Of Plant Physiology).
24. Ng, L. M., K. Melcher, B. T. Teh, and H. E. Xu. 2014. Abscisic acid perception and signaling: structural mechanisms and applications. *Acta Pharmaceutica Sinica B* 35(5). 567.
25. Osakabe, Y., K. Yamaguchi-Shinozaki, K. Shinozaki, and L. S. P. Tran. 2014. ABA control of plant macroelement membrane transport systems in response to water deficit and high salinity. *New Phytologist* 202(1). 35-49
26. Pospisilova J. 2003. Participation of phytohormones in the stomatal regulation of gas exchange during water stress. *Biologia Planta*. 46(4). 491-506
27. Qadir, S. A., M. Q. Khursheed, and F. Z. Huyop. 2017. Drought tolerance and genetic diversity among selected wheat cultivars. *ZANCO Journal of Pure Applied Science* 29(3). 110-117.
28. Razzaq, A., Q. Ali, A. Qayyum, I. Mahmood, M. Ahmad, and M. Rasheed. 2013. Physiological responses and drought resistance index of nine wheat (*Triticum aestivum* L.) cultivars under different moisture conditions. *Pakistan Journal of Botany* 45(S1). 151-155
29. Reynolds, M., and R. Tuberosa. 2008. Translational research impacting on crop productivity in drought-prone environments. *Current Opinion in Plant Biology* 11(2). 171-179
30. Ritchie, S. W., H. T. Nguyen, and A. S. Holaday. 1990. Leaf water content and gas-exchange parameters of two wheat genotypes differing in drought resistance. *Crop Science* 30(1). 105-111.
31. Salazar, C., C. Hernández, and M. T. Pino. 2015. Plant water stress: Associations between ethylene and abscisic acid response. *Chilean Journal of Agricultural Research* 75. 71-79
32. Sánchez-Rodríguez, E., M. Rubio-Wilhelmi, L. M. Cervilla, B. Blasco, J. J. Rios, M. A. Rosales,... and J. M. Ruiz. 2010.

Genotypic differences in some physiological parameters symptomatic for oxidative stress under moderate drought in tomato plants. *Plant science* 178(1). 30-40

33. Sarafraz-Ardakani, M. R., R. A. Khavari-Nejad, F. Moradi, and F. Najafi, 2014. Abscisic acid and cytokinin-induced carbohydrate and antioxidant levels regulation in drought-resistant and-susceptible wheat cultivar during grain filling under field conditions. *International Journal of Biosciences* 5(8). 11-24.

34. Schroeder, J. I., J. M. Kwak, and G. J. Allen. 2001. Guard cell abscisic acid signalling and engineering drought hardiness in plants. *Nature* 410(6826). 327

35. Sharifi, P., R. Amirnia, E. Majidi, H. Hadi, B. Nakhoda, H. M. Alipoor, and F. Moradi. 2012. Relationship between drought stress and some antioxidant enzymes with cell membrane and chlorophyll stability in wheat lines. *African Journal of Microbiology Research* 6(3). 617-623

36. Shivakrishna, P., K. A. Reddy, and D. M. Rao. 2018. Effect of PEG-6000 imposed drought stress on RNA content, relative water content (RWC), and chlorophyll content in peanut leaves and roots. *Saudi Journal of Biological Sciences* 25(2). 285-289

37. Singh, A., J., Kumar, and P. Kumar, 2008. Effects of plant growth regulators and sucrose on postharvest physiology, membrane stability and vase life of cut spikes of gladiolus. *Plant Growth Regulation* 55(3). 221

38. Sourour, A., O. Afef, R. Mounir, and B. Y. Mongi. 2017. A review: morphological, physiological, biochemical and molecular plant responses to water deficit stress. *International Journal of Engineering Science* 6(1). 2319-1805.

39. Tripathy, J. N., J. Zhang, S. Robin, T. T. Nguyen, and H. T. Nguyen. 2000. QTLs for cell-membrane stability mapped in rice (*Oryza sativa* L.) under drought stress. *Theoretical applied genetics* 100(8). 1197-1202

40. Veses, A., B. Puértolas, M. S. Callén, and T. García. 2015. Catalytic upgrading of biomass derived pyrolysis vapors over metal-loaded ZSM-5 zeolites: Effect of different metal cations on the bio-oil final properties. *Microporous and Mesoporous Materials*. 209. 189-196.

41. Xiong, L., and J. K. Zhu. 2002. Molecular and genetic aspects of plant responses to osmotic stress. *Plant Cell Environment* 25(2). 131-139