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Evaluation of the Biochemical and Antioxidant Behaviour of Four Species *Chenopodium quinoa* Willd, *Zea mays* L., *Triticosecale wittmack* and *Hordeum vulgare* L. Effected by Oxidative Stress Caused by Water Stress

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Abstract: Our study was conducted at the faculty of Sciences of the nature and life from Mentouri Brothers University of Constantine, (36°21′54″ N: 6°36′52″ E), altitude above sea level by 574 m in March 2022. Cereals and pseudo-cereals, the world's most important crop, are an important source of sustenance for humans and animals. In addition, they are distinguished by their high tolerance to abiotic stressors. Hydrological deficit is a major factor limiting agricultural production; the impact varies according to the species, the stage of development of the plant and the severity of the stress. This research aims to evaluate the impact of water stress on H_2O_2 and Malondialdehyde MDA adaptation mechanisms, as well as the quantification of proteins, glycine betaine, phenols and flavonoids that can affect drought in three local species (Triticale, Barley and Maize) and one exotic plant (Quinoa). We also recorded an induced increase in triticale, barley, maize and quinoa stressed in Hydrogen Peroxide (H_2O_2), Malondialdehyde (MDA), proteins and glycine betaine with a concentration varied from 228.75 and 404.58 µmol.g^{-1} FM, 4.92 and 20.84 µmol.g^{-1} DM, 1.35 and 3.21 mg.g^{-1} FM, 0.34 and 0.54mg.g^{-1} DM, respectively. While the concentration of flavonoids and phenols total was recorded in maize by 20.14 mg QE g^{-1} DM and 375.47 mg GAE g^{-1} DM, respectively. Moreover, it reduced in triticale, barley and quinoa, with a value of 4.26-9.9 mg. EQ g^{-1} DM and 185.97-421.31 mg. EAGg^{-1} DM. The study demonstrated the species' resistance to and effectiveness in the face of water stress.

Keywords: Cereals, quinoa, Drought tolerance, Flavonoids, Oxidative stress, Phenols.

Introduction

Additionally, the rise in The development of reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), is one of these responses to a water shortage, these build up to lower the osmotic potential, Malondialdehyde (MDA), one of the by-products of polyunsaturated fatty acid lipid peroxidation brought on by stress (Gabash *et al.,* 2024). A number of products originating from the lipoxygenase pathway, such as the creation of

alkane (ethane and pentane); have been widely employed as a trustworthy indicator of lipid peroxidation in addition to MDA (Croft *et al.,* 1993). According to Rollins & *al.* (2013), drought treatment, proteomic performance was not significantly changed.Many organisms, including those in the groups Chenopodiaceae, Amaranthaceae, Gramineae, Compositae, and Malvaceae, produce glycine betaine, an amino acid derivative (Gorham, 1996; Blunden *et al.,* 1999). It has also been demonstrated that, its role in osmoregulation, it works to sustain electron transfer in thylakoid membranes & to safeguard the functional proteins, enzymes, and lipids of the photosynthetic apparatus (Allakhverdiev *et al.,* 1996; Xing & Rajashekar, 1999).

On the other hand, it can maintain cell organelles and enhance the antioxidant defense system to combat oxidative stress brought on by stress (Demiral & Türkan, 2004). Additionally, polyphenols, which include phenolic acids, flavonoids, and tannins, are the bioactive secondary metabolites of plants that contribute to various physiological properties such as effects that offer an interesting potential in terms of nutrient capacity; antioxidant antimicrobial, anti-inflammatory, and anti-carcinogenic.

They have very important biological properties and benefit human health in the long run (Vázquez *et al.,* 2019).

In order to explore the tolerance to drought in three cereal species (triticale, barley, and corn) and in a pseudo-cereal species, quinoa, the study is designed to identify a number of physio-biochemical features. Moreover, how phenolic compounds affect &how plants react to stress.

Materials & Methods

This study was carried at the faculty of sciences of the nature and life from Mentouri Brothers University of Constantine (36 °21'54" N; 06°36'52" E) & 574m asl, Algeria) in March, 2022, it deals with the evaluation of the vegetative growth of four species, Triticale (*Triticosecale Wittmack*), Barley (*Hordeum vulgare* L.), Maize (*Zea mais* L.) and Quinoa (*Chenopodium quinoa* Willd.) from different introduced and local origins. The study aimed to describe the water status, oxidative stress characteristics (hydrogen peroxide dosage, lipid peroxidation via malondialdehyde dosage), small organic (dosage of glycine betaine & total proteins), and polyphenolic compound intervention in stress tolerance (dosage of phenols & total flavonoids) of the four species under study.

The different species under study and their origins

Triticale *triticosecale wittmack* juaonllo159 G2 Chellia

Barley *Hordeum vulgare* L. Saida183G1 Algeria

Maize *Zea mais* L. Farello R1

Quinoa *Chenopodium quinoa* Willd Amarellasacaca Perou

Experimental design

The experiment was carried out in greenhouse conditions. In two separate batches, the seeds of the four species were planted in plastic pots with a mixture of 2/3 soil sieved to 2 mm and 1/3 sand., Ten grains per pot were planted in three repetition

There is only one species per batch (batch under normal water condition and a batch with a water deficit). The four species were given the same amount of water until heading stage for triticale, barley, & maize, and start of the panicle stage for quinoa. After that, the water constraint was applied by ceasing irrigation completely

until flowering, while the controls were routinely watered twice a week.

Content of hydrogen peroxide (H_2O_2)

A 0.1g of fresh leaves and 5 ml of 1% Trichloroacetic acid (TCA). The plant solution is crushed in ice bath, thereafter the homogenate solution was centrifuged at 12000 rpm for 15 m. After centrifugation, 0.5 ml of the supernatant was mixed with 0.5 ml of regulator buffer (potassium phosphate-KOH) & 1 ml of potassium iodine (KI). The DO was read at 390 nm using a spectrophotometer (Loreto & Velikova, 2001).

Content of malonedialdehyde (MDA)

A 50 mg dry weight of leaves is homogenized in 2 ml of 1% Trichloroacetic acid (TCA). The homogenate is centrifuged at 15000 rpm for 10 m at 4°C. Then, 0.6ml of the supernatant. 1.60 ml of Thiobarbituric acid (TBA) prepared in 20% TCA. The samples were incubated at 90°C for 20 m. After stopping the reaction on ice, the samples were centrifuged at 10000 g 5 m^{-1}. The DO was read at 532 nm using a spectrophotometer (Hernandez & Almansa, 2002).

Content of total proteins

The fresh leaves (0.25 g) was homogenized in 5 ml of potassium phosphate buffer (50 mM; pH 7.8) in ice bath and centrifuged the mixture at 12 000 rpm for 20 m at 4°C. The supernatant was separated determine total soluble protein (Aziz *et al*., 2018). The total soluble protein estimated according to the method of Bradford, (1976). An aliquot of 2 ml of Bradford's reagent and 100 ml of samples were added and kept in room temperature for 20 m and absorbance read at 595 nm.

Content of glycine betaine

As described by Gieve & Grattan (1983), 0.5 g of the dry leaf was added to 20 ml of distilled water, the resulting solution was incubated for 48 h at 25°C. The samples were then filtered and then stored in the freezer until analysis. The thawed extracts were diluted 1:1 with 2N Sulfuric acid, 0.5 ml of the resulting solution was cooled in ice water for 1 h. Kl-I2 reagent (0.2 ml) was added, the mixture was gently vortexed. The samples were stored at 0 – 40°C for 16 h. Then the samples were transferred to tubes for centrifugation at 10000 rpm at 0°C. The supernatant is carefully aspirated with micropipette. The pellet is dissolved in 9 ml of 1, 2-dichloro ethane (reagent). Firstly, wash with 0.5 ml and leave for 5 m. Mix the tubes with the vortex to achieve a complete solubility in solvent. After 2h, the absorbance was measured at 365 nm with spectrophotometer.

Content of total flavonoids

Quantitative analysis of flavonoids is performed according to the method described by Kim *et al.* (2003). The flavonoid determination was performed from the leaves, 1g of the dry leaves were ground in 50 ml of 95° ethanol & centrifuged at 3000 rpm. 500 µL of supernatant was mixed with 1500 µL of distilled water, 150 µL of 5% sodium nitrate $NaNO_2$ & allowed to stand for 5 m, to this was added 150 µL of 10% aluminium trichloride $AlCl_3$. The mixture was again allowed to stand for 11 m, at the end 500 µL of NaOH (1M) soda was added. The mixture was allowed to stand for 5_10 m and the DO is read at 510 nm.

Content of total phenols

The determination of polyphenols was carried out on the leaves. 100 mg of leaves crushed in 5 ml of 95° ethanol, then centrifuged using an MLW type centrifuge at 3000 rpm. The recovered supernatant used to determination polyphenols: The determination of total polyphenols is carried out according to the method described by Chandler and Dodds,

which modified by Randhir *et al*. (2004), 1 ml of 95% ethanol, 1 ml of distilled water, and 500 µL of 50% Folin ciocalteau reagent have been added for 1mL of the supernatant. After five minutes of reaction, 1 ml of sodium carbonate solution (NaCO3) at a concentration of 5% was added. The absorbance reading was carried out at 725 nm using a spectrophotometer (UV-visible 8500) after 60 min of incubation at room temperature. The determination of the polyphenol content was made by referring to a standard curve drawn up from a series of standard solutions of gallic acid at 1 $mg.ml^{-1}$.

Statistical analysis

ANOVA is a statistical tool used to detect differences between experimental groups means (Frih, 2021) for analysis of variance randomization design. Fisher's LSD multiple ranges test was employed for the mean comparisons.

Results

The various physio-biochemical tests carried out on the four species made it possible to determine the presence or absence of mechanisms of resistance to water stress. A mechanism can be strong, weak or medium for each species studied. Since then, each mechanism has received a score for each species. In table (1), the total concentrations are displayed.

Statistical analysis shows that there is a significant difference among species ($p \leq 0.001$) under optimal conditions; this proves that there is a varietal response even in the absence of stress. The accumulation of H_2O_2 in the species tested increased significantly ($p \leq 0.001$) during water stress ranging from 8,091_4,57 µmol g^{-1}/FM, compared to species without water deficit which ranged from 6,357 to 2,83µmol. g^{-1}/FM, and a maximum increase was observed in quinoa leaf dryness with a value of 8,091µmol g^{-1}.

The MDA content increased high significantly ($p \leq 0.001$) when subjected to a water deficit in Triticale (0. 521µmol g^{-1} /DM) and Quinoa (0,388 µmol.g^{-1}/DM), compared to the control (0.233 µmol.g^{-1} /DM and 0,0096 µmol g^{-1} /DM), respectively (Fig. 2); and an average increase in the total soluble protein content at the level of dryness between 1.68 and 0,066 mg g^{-1} /FM compared to the control group which varied between 0.01 and 1,01 mg g^{-1} /FM ($p \leq 0.001$, Fig. 3). On the a concentration varying between 0.0143 and 0.008 mg g^{-1} /DM, even the 4 species reacted positively. On other hand, all the species reacted differently towards the accumulation by accumulating glycine betaine with

Accumulation of glycine betaine with a low water deficit oscillating between 0.0052 and 0.083 mg.g^{-1} /DM ($p \leq 0.001$).

Figs. (4, 5 and 6) showed the results of the total flavonoids and phenols assay for both conditions. Under irrigated conditions, leaves show significantly high contents ($p \leq 0.001$) of total flavonoids and phenols in barley with a concentration of 2,74-4,69 mg EQ g^{-1} /DM, respectively, followed by quinoa and triticale with a concentration of 2,44–2,24 mg EQ g^{-1} /DM and 1,54 – 3,58 mg EAG g^{-1} /DM, respectively, compared to maize, which register values of 1,31 mg EQ g^{-1} /DM and 1,45 mg EAG g^{-1} /DM. Water stress induces a significant increase ($p \leq 0.001$) in maize under stressed condition with a value of 2,02 mg EQ g^{-1} /DM and 2,88 mg EAG g^{-1} /DM.

Table (1): Effect of water stress application on antioxidant and mechanisms of action in Triticale, Barley, Maize and Quinoa.

Species	Glycine betaine (mg g^{-1} /DM)		Total flavonoid (mg EQ g^{-1} /DM)		Total phenols (mg EAG g^{-1} /DM))	
	Control	**Drought**	**Control**	**Drought**	**Control**	**Drought**
Triticale	0.0083±0.004 a	0.0143±0.0003a	1.542±0.021c	0.39±0.026c	3.58±0.01b	1.65±0,097c
Barley	0.0084±0.0004a	0.0121±0,0003b	2.74±0.045 a	0,806±0,031b	4.69±0.01a	3.28±0,032a
Maize	0.0037±0.0002c	0.008±0.0005 c	1.313±0.89c	2.021±0.026a	1.45±0.01d	2.88±0.015b
Quinoa	0.0052±0.0003b	0.0114±0.0003b	2.448±0.031b	0.136±0.063d	2.24±0.02c	1.22±0.019d
p≤0.001	***	***	***	***	***	***
LSD5%	6.92 $x10^{-4}$	7.41 x 10^{-4}	0.0672	0.075	0.0316	0.099
Species	**H_2O_2** ($\mu molg^{-1}$/FM)		**MDA (µmol g^{-1} /DM)**		**Protein (mg g^{-1} /FM)**	
	Control	**Drought**	**Control**	**Drought**	**Control**	**Drought**
Triticale	6.357±0.004 a	6.925±0.004a	0.233±0.012a	0.521±0.005a	0.0134±0.00d	0.066±0.003d
Barley	3.665±0.003c	4.575±0.013b	0.080±0.004c	0.123±0.002d	0.1086±0.003a	0.159±0.003a
Maize	2.83± 0.06d	4,591 ± 0.06 b	0.172±0.003b	0.22±0.0007c	0.0237±0.004c	0.099±0.003b
Quinoa	4.8 ±0.139b	8.09±0.076a	0.080±0.009c	0.38±0.005b	0.048±0.01b	0.08±0.05c
P ≤0.001	***	***	***	***	***	***
LSD5%	0.0515	0.0382	0.0157	0.010	0.0056	00055

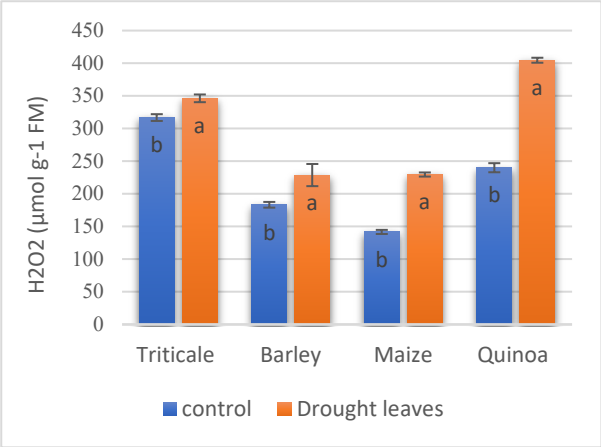

Fig. (1): H_2O_2 content in plants grown without and under stress. (Each bar represents the mean ± SE tress triplicates).

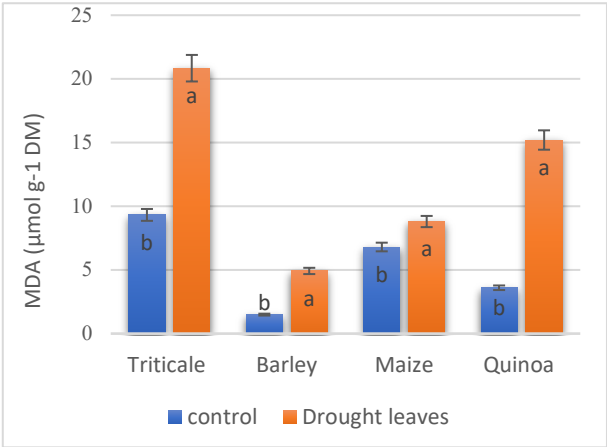

Fig. (2): MDAcontent in plants grown without and under stress. (Each bar represents the mean ±SE tress triplicates).

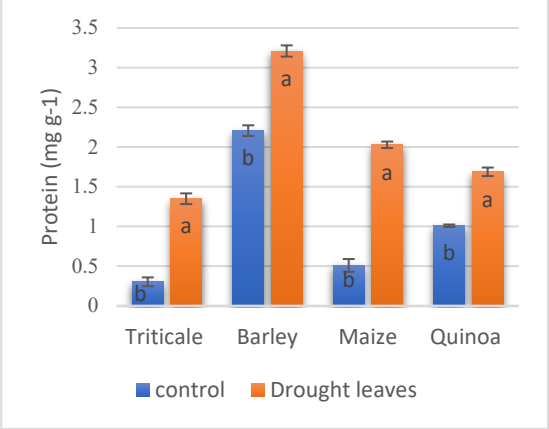

Fig. (3): Protein content in plants grown without and under stress. (Each bar represents the mean ± SE tress triplicates).

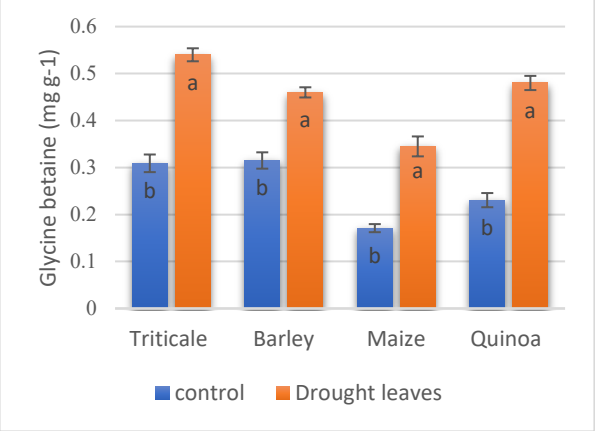

Fig. (4): Glycine betaine content in plants grown without and under stress. (Each bar represents the mean ±SE tress triplicates).

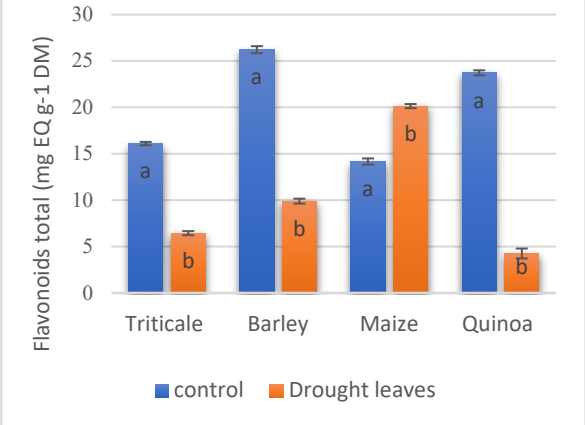

Fig. (5): Flavonoids content in plants grown without and under stress. (Each bar represents the mean ± SE tress triplicates).

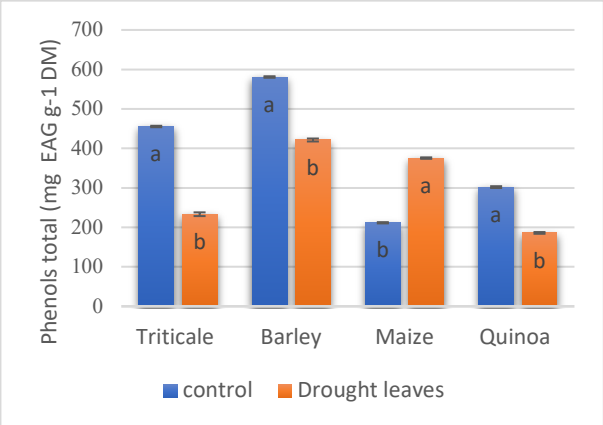

Fig. (6): Phenols content in plants grown without and under stress. (Each bar represents the mean ±SE tress triplicates).

In addition, the results allowed us to obtain a significant reduction of these same compounds in barley, triticale and quinoa between 0,136 and 1.22 mg EQ g^{-1} /DM and 0.39-1.65 mg EAG g^{-1} /DM.

Discussion

According to the results obtained, an increase in the importance quantitative characteristics of oxidative stress and organic osmolytes in water deficit conditions can be noted. The application of water stress caused a significant increase in H_2O_2 and MDA, which were higher in triticale and quinoa. H_2O_2 and MDA increased in osmotically stressed (Figs. 1 and 2), compared to well-watered for barley (Abdelaal *et al.,* 2018), H_2O_2 and MDA content increased under water stress in wheat (Chakraborty & Pradhan, 2012), maize (Moussa & Abdel-Aziz, 2008) and quinoa (Aziz *et al.,* 2018). Water deficit induces oxidative stress due to the inhibition of photosynthetic activity caused by an imbalance between light capture and utilization (Foyer & Noctor, 2000). MDA functions as a signalling molecule (Shafiq *et al.,* 2015). Lipid peroxidation products associated with stress serve as biological cues that do not require gene activation, and generate a general reaction to many environmental conditions (Bhattacharjee, 2012).

According to our results, under water stress condition, a high increase in protein content and leaf glycine betaine was recorded compared to the control condition (Figs. 3 and 4). Similar results were observed in different species such as maize (Anjum *et al.,* 2011), grape (Zonouri *et al.,* 2014) and quinoa (Aziz *et al.,* 2018). Malik *et al*. (2015) reported an increase in glycine betaine content of wheat plants under water deficit stress.

The drought condition induces disturbances in the metabolism of amino acids and carbohydrates such as glycine betaine, the latter contribute to the osmotic adjustment (Huang *et al*., 2000). Another element of stress tolerance is the creation and build-up of certain proteins. Some researchers claim that glycine betaine synthesis plays a crucial role in osmotic adjustments and is typically elevated in plants that can tolerate dryness (Husain et. *al*., 2009). Additionally, it can interact with cellular macromolecules like enzymes to stabilize their structure and function while protecting them from the damaging effects of stress and protecting membranes (Smirnoff & Cumbes, 1989, Ashraf & Iram, 2005).

The polyphenolic components (phenols and total flavonoids) in maize are distinguished by their potent concentration and favourable response to water stress. The results are consistent with Nicholas *et al.* (2015) and Al-Hussine & Alyousuf (2021) which they find that stress-induced flavonoid accumulation provides drought protection in White clover. Contrary the other species studied, lack of water affected phenol and flavonoid content. The results affirm with Aziz et *al*. (2018) that total flavonoids decreased in drought stressed quinoa plants.

The concentration of hydrogen peroxide compared to other stressed species is lower than that of the controls but specifically noted in the stressed quinoa plant .H_2O_2 plays the role of a signal molecule that alerts the cell to the presence of environmental stress (Maksymiec, 2007). According to H_2O_2 can function as a secondary messenger at low concentrations, but it becomes toxic at high concentrations. H_2O_2 can come from the disproportionation reaction of the superoxide anion by SOD (Cakmak, 2000). These results may be due to a species-specific effect or may

be related to the severity of drought stress in cells or tissues.

Conclusion

All four species were subjected to varying degrees of water stress, and this water stress had a substantial impact on the physio-biochemical parameters examined. The concentration in stressed triticale, barley, and quinoa was revealed lower under stressful conditions than that in control plants based on the findings of the assessment of total phenols and flavonoids. It is concluded that, each of the species used uses different strategies to cope with water stress, the plant must align with the others to cope with drought.

Finally, according to the results of our study, we conclude that the species *Chenopodium quinoa* Willd is more tolerant than other species against water constraints.

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Contributions of authors

B.R.: conceived the original idea, carried out the experiment and wrote the manuscript

B.A.: support the BR and SRN in writing the manuscript

S.R.N.: carried out the experiment and wrote the manuscript

G.A.: supervised the project.

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Conflicts of interest

The authors declare no conflict of interest.

Ethical approval

Ethical approval for the study was obtained from the relevant local ethics committees

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تاثير الاجهاد التاكسدي النتائج عن الاجهاد المائي في السلوك الكيموحيوي ومضادات الأكسدة لأربعة محاصيل: الكينوا *Chenopodium quinoa* Willd والذرة الصفراء *Zea mays L.* والترتيكال *Triticosecale wittmack* والشعير *Hordeum vulgare* L.

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المستخلص:أجريت الدراسة في كلية علوم الطبيعة والحياة بجامعة الاخوة منتوري بالقسنطينة خط العرض: 36°21′54″ شمالاً خط الطول: 6°36′52″ شرقاً، الارتفاع عن سطح البحر: 574 م في شهر اذار 2022 . تعتبر الحبوب وشبيهة الحبوب من أهم المحاصيل في العالم، فهي تعد مصدرًا مهمًا لغذاء الإنسان والحيوان. وبالإضافة إلى ذلك، فهي تتميز بقدرتها العالية على تحمل الاجهادات اللاحيوية. يشكل العجز المائي عاملاً رئيسياً يحد من الإنتاج الزراعي؛ ويختلف التأثير حسب النوع ومرحلة تطور النبات وشدة الإجهاد. يهدف هذا البحث إلى تقييم تأثير الإجهاد المائي على آليات التكيف مع H_2O_2 و Malondialdehyde MDA، بالإضافة إلى تقدير كمية البروتينات والجليسين البيتين والفينولات والفلافونويدات التي يمكن أن تؤثر على الجفاف في ثلاثة محاصيل محلية هي الشعير والذرة والترتيكال، ومحصول رابع تم ادخالة حديثا هو نبات الكينوا. بينت النتائج زيادة في التريتيكال والشعير والذرة والكينوا المجهدة في بيروكسيد الهيدروجين (H_2O_2) والمالونديالدهيد (MDA) والبروتينات والبيتين الجليسين بتركيزات تتراوح بين 228.75 و404.58 ميكرومول غرام$^{-1}$FM و4.92 و20.84 ميكرومول غرام $^{-1}$ مارك ألماني، 1.35 و3.21 ملغم غم $^{-1}$ FM ، 0.34 و0.54 ملغم غم$^{-1}$ مارك ألماني، على التوالي. بينما تم تسجيل تركيز الفلافونيدات والفينولات الكلية في الذرة بـ 20.14 ملجم QE g-1 DM و 375.47 ملجم GAE g-1 DM على التوالي. علاوة على ذلك، فقد انخفض في الترتيكال والشعير والكينوا بقيمة 4.26 - 9.9 ملجم EQ g-1 DM و185.97 - 421.31 ملجم .EAG g-1 DM وأظهرت الدراسة مقاومة الأنواع وفعاليتها في مواجهة الإجهاد المائي.

الكلمات المفتاحية: الحبوب، الكينوا، تحمل الجفاف، الفلافونويدات، الإجهاد التأكسدي، الفينولات.