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Lipid Peroxidation, Proline Content and Soluble Sugars as indicators of Oxidative Stress Tolerance in Some Advanced Durum Wheat Lines (*Triticum durum* Desf.).

Peroxidación lipídica, contenido en prolina libre y azúcares solubles como indicadores de tolerancia al estrés oxidativo en algunas líneas avanzadas de trigo duro (*Triticum durum* Desf.).

Peroxidação lipídica, teor de prolina livre e açúcares solúveis como indicadores de tolerância ao estresse oxidativo em algumas linhagens avançadas de trigo-duro (*Triticum durum* Desf.).

Abdelmalek Oulmi[1]
Sarah Benkadja[2*]
Ali Guendouz[3]
Benalia Frih[1]
Amor Mehanni[4]
Samir Selloum[4]
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[1]Department of Biology and Plant Ecology, Valorization of Natural Biological Resources Laboratory, Farhat Abbas University Setif 1, Algeria.
[2]Department of Agronomy, Valorization of Natural Biological Resources Laboratory, Farhat Abbas University of Setif, Algeria.
[3]National Institute of Agronomic Research of Algeria (INRAA), Setif Unit, Algeria.
[4]Technical Institute of Field Crops, Setif, Algeria.

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Abstract

Oxidative stress induced by glyphosate is a complex phenomenon caused by an imbalance between reactive oxygen species (ROS) and antioxidants in plants cells. The present research was carried out at the field crops institute, Agricultural Experimental Station of Setif (ITGC-AES), to assess the response of some durum wheat (*Triticum Durum* Desf.) lines exposed to oxidative stress induced by glyphosate herbicide. In the heading stage, a solution of 5 Mm of glyphosate was sprayed on flag leaves, and each measurement was taken 48 hours after the glyphosate application. Lipid peroxidation, free proline and soluble sugars were determined. The results indicated that oxidative stress increased the content of lipid peroxidation, proline, and soluble sugars in flag leaves. Analysis of variance revealed significant differences among the genotypes tested, the increase in the level of lipid peroxidation is much higher in advanced lines G5 and G3, in which lipid peroxidation and membrane damage are greater. Oxidative damage also increased the proline content in lines G3 and G4, and soluble sugars in line G5, which were showing a high tolerance to the oxidative stress induced.

*Corresponding author: sarahbenkadja8@gmail.com

Resumen

El estrés oxidativo inducido por el glifosato es un fenómeno complejo causado por un desequilibrio entre las especies de oxígeno reactivo (ROS) y los antioxidantes en las células de las plantas. La presente investigación se llevó a cabo en el instituto de cultivos de campo, Estación Experimental Agrícola de Setif (ITGC-AES), para evaluar la respuesta de lineas de trigo duro (*Triticum Durum* Desf.) expuestas al estrés oxidativo inducido por el herbicida glifosato. En la etapa de encabezamiento, se pulverizó una solución de 5 Mm de glifosato en las hojas bandera, y cada medición se tomó 48 horas después de la aplicación del glifosado. Se determinó la peroxidación de lípidos, prolina libre y azúcares solubles. Los resultados indicaron que el estrés oxidativo aumentó el contenido de Peroxidación lipídica, prolina y azúcares solubles en las hojas bandera. El análisis de la varianza reveló diferencias significativas entre los genotipos probados, el aumento del nivel de Peroxidación lipídica es mucho mayor en las líneas avanzadas G5 y G3, en las que la peroxidación de lípidos y el daño de la membrana son mayores. Los daños oxidativos también aumentaron el contenido de prolina en las líneas G3 y G4, y azúcares solubles en la línea G5, que mostraban una alta tolerancia al estrés oxidativo inducido.

Palabras clave: daño de membrana, glifosato, especies de oxígeno reactivo, tolerancia.

Resumo

O estresse oxidativo induzido pelo glifosato é um fenômeno complexo causado por um desequilíbrio entre espécies reativas de oxigênio (ROS) e antioxidantes nas células vegetais. A presente pesquisa foi realizada no instituto de culturas de campo, Estação Experimental Agrícola de Setif (ITGC-AES), para avaliar a resposta de algum trigo duro (*Triticum Durum* Desf.) linhas expostas ao estresse oxidativo induzido pelo herbicida glifosato. Na fase de pontuação, uma solução de 5 mm de glifosato foi pulverizada sobre as folhas de bandeira, e cada medição foi tomada 48 horas após a aplicação do glyphosate. A peroxidação lipídica, a prolina livre e os açúcares solúveis foram determinados. Os resultados indicaram que o estresse oxidativo aumentou o teor de Peroxidação lipídica, prolina e açúcares solúveis nas folhas de bandeira. A análise da variância revelou diferenças significativas entre os genótipos testados, o aumento do nível de Peroxidação lipídica é muito maior nas linhas avançadas G5 e G3, nas quais a peroxidação lipídica e danos à membrana são maiores. Os danos oxidativos também aumentaram o teor de prolina nas linhas G3 e G4, e açúcares solúveis na linha G5, que estavam mostrando uma alta tolerância ao estresse oxidativo induzido.

Palavras-chave: danos à membrana, glifosato, espécies reativas de oxigênio, tolerância

Introduction

Cereal cultivation is very ancient in Algeria due to its utilization as human and animal food (Ladoui *et al*., 2020). Among cereals, durum wheat (*Triticum durum* Desf.) is an important cereal crop in the Mediterranean basin that has been cultivated for centuries under widely varying climatic conditions (Ben M'Barek *et al*., 2022). It is cultivated worldwide over almost 17 million ha, with a global production of 38.1 million tons in 2019 (Xynias *et al*., 2020). Glyphosate (N-(phosphonomethyl)-glycine) is one of the most extensively used herbicide substances in modern agriculture because of its broad spectrum of weed control (Sergiev *et al*., 2020). It affects not only weeds but crop plants as well, leading to oxidative stress and disturbed cellular homeostasis in plants (Gomes *et al*., 2014; Zhao *et al*., 2020). Thus, glyphosate might stimulate the development of reactive oxygen species (ROS), resulting in oxidative stress (Spormann *et al*., 2019). Chaki *et al*. (2020) stated that high concentrations of reactive species in plants disturb redox homeostasis, which could trigger damage to membrane lipids, proteins, and nucleic acids. Lipid peroxidation can be described generally as a process under which oxidants such as free radicals or non-radical species attack lipids containing carbon-carbon double bonds (Ayala *et al*., 2014). The degree of lipid peroxidation is evaluated by the malondialdehyde content. It is one of the final products of lipid peroxidation and is frequently used as an indicator of oxidative stress since it reflects the degree of oxidative degradation of membranes (Sharma *et al*., 2016; Spormann *et al*., 2019). Similarly, Singh and Rathore (2017) noticed that the accumulation of malondialdehyde content in pea plants revealed lipid peroxidation. Plants evolved mechanisms to deal with oxidative stress caused by the accumulation of reactive oxygen species (ROS). Hence, plants accumulate certain solutes known as osmolytes to limit cellular damage and maintain the osmotic differences between the cell's surrounding membrane and the cytosol (Sharma *et al*., 2019). Among the frequent osmolytes that play an important role in osmoregulation are proline and sugar. Proline is an amino acid that plays a beneficial role in plants exposed to stressful conditions, i.e., as a metal chelator, an antioxidative defense molecule and a signaling molecule. Furthermore, a positive correlation between the accumulation of proline and improving stress tolerance in plants has been revealed (Elewa *et al.*, 2017; Hosseinifard *et al*., 2022). According to Rajametov *et al*. (2021), Proline protects the cell from damage caused by lipid peroxidation and detoxifies the membrane due to reactive oxygen species. In addition, several studies have reported that proline plays various roles during stressful conditions, which can improve protein stability and protect membrane integrity by binding to hydrogen bonds. Furthermore, proline may protect cells by increasing water uptake potential and facilitating the activation of enzymes Hosseinifard *et al*., 2022). Similar, to proline, soluble sugars serve as an osmoprotectant, aid in maintaining cell homeostasis, and reactive oxygen species detoxification, and act as a signaling molecule under stressful conditions (Chauhan *et al.*, 2022). Furthermore, sugars play an active role in the regulation of photosynthesis, osmotic homeostasis, and membrane stabilization. The present research aimed to determine the oxidative stress tolerance of some durum wheat lines by assessing the degree of lipid peroxidation, proline content, and soluble sugars accumulation.

Material and methods

Plant material and growth conditions

This study was carried out during the 2021–2022 cropping season at the Agricultural Experimental Station of Setif (Algeria), (ITGC-AES, 36° 12'N and 05° 24'E and 1.081 masl), Algeria. Six advanced lines and four varieties are included in the genetic material, three of which are local varieties (table 1). All genotypes were sown in randomized complete block design with three replications, each plot consisted of six row with 2.5 m long, spaced 20 cm between rows. At the heading stage, in each plot, we sprayed four leaves from each genotype with a 5 mM glyphosate solution. All measurements were performed 48 hours after glyphosate treatments.

Table 1. The pedigrees of the durum wheat genotypes tested.

Genotype	Pedigrees
G1	RASCON_37/GREEN_2/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/…
G2	MINIMUS_6/PLATA_16//IMMER/3/SOOTY_9/RASCON_37/9/…
G3	CMH77.774/CORM//SOOTY-9/RASCON-37/3/SOMAT-4
G4	CNDO/PRIMADUR//HAI-OU-17/3/SNITAN/4/SOMAT-3/
G5	CNDO/VEE//CELTA/3/PATA_2/6/ARAM_7//CREX/ALLA/5/ENTE/...
G6	SILVER 14/MOEWE//BISU_1/PATKA_3/3/PORRON_4/YUAN_1/9/...
Jupare C 2001	STINKPOT//ALTAR-84/ALONDRA
Bousselam	Heider/Martes/Huevos de Oro. ICD-414
Boutaleb	GTA dur /Ofanto
Oued el bared	Hedba3/Ofanto

Lipid peroxidation

Oxidative damage to lipids was evaluated by quantifying the content of malondialdehyde (MDA) in leaf samples. Leaf samples (200 mg) were ground to fine powder in liquid nitrogen using a mortar and pestle and homogenized with 3 ml of a 50 mM potassium phosphate buffer (pH 7.5). An equivalent volume of 0.5 % thiobarbituric acid was added, the mixture was placed in a boiling water bath for 30 min and centrifuged at 3000 × g for 10 min, and the absorbance was measured at 532 and 600 nm (Zhang *et al.,* 2013). The MDA content was calculated as described by Bao *et al*. (2009): [MDA] (nmol.g^{-1} FW) = [(Abs532 – Abs600) x Vt /ε x FW] x 1000.

Proline content and soluble sugars

For the quantification of proline content (PC) in fresh leaves, the method given by Monneveux and Nemmar (1986) was used. The proline is extracted at 85 °C with methanol and stained by ninhydrin in the presence of acetic acid, orthophosphoric acid, and toluene. The measurement of the red color obtained is carried out on a spectrophotometer at 528 nm. Soluble Sugars were quantified via the anthrone reagent according to Staub (1963).

Results and discussion

Lipid peroxidation assay

Malondialdehyde (MDA) level is a product of lipid peroxidation; it's commonly used as a biomarker of oxidative stress (Morales and Munné-Boschb, 2019). The data presented in table 2 showed a significant increase in levels of MDA content after glyphosate treatments. The maximum increase in MDA was observed in line G5 (65.8 nmol.g^{-1} FW), followed by line G3 (52.83 nmol.g^{-1} FW). The significant increase in MDA content indicates a strong imbalance of the biomembrane lipid peroxidation in the herbicide-treated plants (Shopova *et al.,* 2021). However, we recorded a slight increase of MDA content in genotypes Jupare C 2001, Boutaleb, and Oued el bared with 21.09, 26.32, and 26.96 nmol.g^{-1}FW respectively, with an average of 35.24; genotypes with a lower level of lipid peroxidation are considered more tolerant to oxidative damage. Both lines G5 and G3 show a high positive deviation of MDA levels above the average (figure 1), indicating a high degradation of lipids in the cell membrane, which is an indicator of severe oxidative stress. While genotypes with a negative deviation have low oxidative damage and stable cell membranes. Gomes *et al*. (2017) stated that oxidative stress caused by herbicides increases lipid peroxidation (MDA concentration) in plants. However, Shopova *et al*. (2021) observed that glyphosate caused typical adverse alterations in wheat growth; glyphosate-suppressed plant growth is a consequence of the accumulation of ROS, which induce degradation in cellular biomembranes as evidenced by the increased amount of MDA. It's also known that lipid peroxidation could have damaged the chloroplast by inhibiting the synthesis of chlorophyll and thus photosynthesis (Langaro *et al*., 2020). Membrane lipid peroxidation has often been used as a tool to determine the degree of plant sensitivity to oxidative damage. Tulkova and Kabashnikova (2021) found that lipid peroxidation refers to a series of free radical reactions in unsaturated fatty acids and that an increase in lipid peroxidation activity under prolonged stress indicates a decline in the scavenging ability within plant cells. Karabulut and Canakcı (2021) analyzed the effects of the herbicide glyphosate in maize and wheat varieties and observed that plants produce antioxidant defense systems, including enzymatic and non-enzymatic methods, as a result of ROS accumulation. Thus, as the amount of ROS increases, the level of MDA begins to accumulate. Furthermore, lipid peroxidation products induce a loss of membrane integrity that ultimately leads to unadorned cytotoxicity, and could result in unrestrained cellular growth or even apoptosis. Our findings support several reports that also found an increased amount of MDA content after glyphosate treatment in barley (Spormann *et al*., 2019), tomato (Soares *et al.*, 2019), and peas (Sergiev *et al.*, 2020). Similarly, Bouchemal *et al*. (2016) revealed that the oxidative stress mediated by paraquat herbicide increased the level of lipid peroxidation in wheat genotypes.

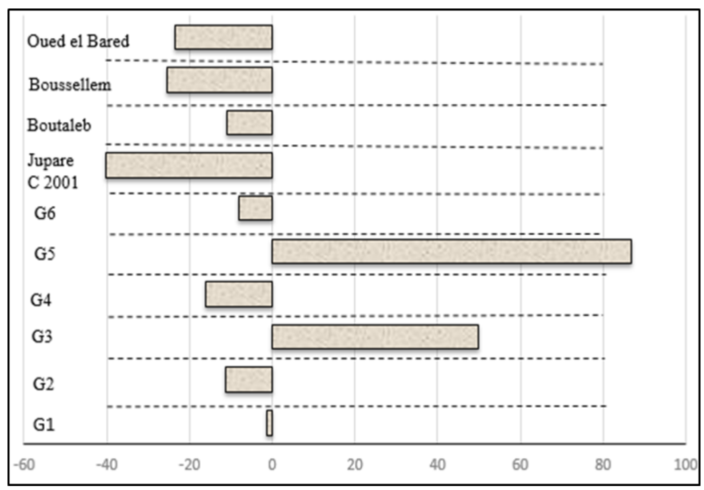

Figure 1. Deviation from the mean values of malondialdehyde under oxidative stress in genotypes tested.

Proline content and soluble sugars accumulation

Based on the results in table 2, an increase in the accumulation of free proline was observed in leaves treated with glyphosate; the highest accumulations were observed for the lines G3 (54.17 µmol.g^{-1} FW), and G4 (42.81 µmol.g^{-1} FW). While, line G6 showed the lowest value (19 µmol.g^{-1} FW), with an overall mean of 33.61. It is believed that this increase is due to oxidative damage induced by glyphosate. Furthermore, Sergiev *et al*. (2020) reported that oxidative damage caused by glyphosate application increased significantly the proline content in pea plants; a similar response was also observed in wheat (Shopova *et al*., 2021). Rapid accumulation of free proline

Table 2. Change in Malondialdehyde content, proline content and soluble sugar in genotypes tested.

Genotypes	Non-glyphosate conditions			Glyphosate application		
	MDA nmol.g^{-1} FW	PC µmol.g^{-1} FW	SS ug.g^{-1}	MDA nmol.g^{-1} FW	PC µmol g-^{1}FW	SS µg·g-^{1}
G1	6^{c}	10.10^{bc}	63.24 ^{bcd}	34.77 ^{bc}	27.2 ^{bc}	100.6 ^{bc}
G2	5.74^{c}	7.81 ^{bcde}	77.86^{abc}	31.22^{bc}	25.85 ^{bc}	134.81^{abc}
G3	10.83^{bc}	14.48^{a}	66.48^{bc}	52.83 ^{ab}	54.17 ^{a}	127.3^{abc}
G4	8.12^{bc}	5.64^{de}	25.91^{e}	29.54 ^{bc}	42.81 ^{ab}	39.44^{d}
G5	20.9^{a}	10.65 ^{ab}	104.84^{a}	65.8 ^{a}	30.58 ^{bc}	178.81^{a}
G6	13.54^{abc}	4.07^{e}	69.94^{bc}	32.45 ^{bc}	19^{c}	103.59^{bc}
Jupare C 2001	5.29^{c}	11.28^{ab}	86.29^{ab}	21.09 ^{c}	39.78 ^{ab}	152.45^{ab}
Boussalam	11.22^{bc}	10.36^{bc}	76.54^{bc}	31.41 ^{bc}	35.25 ^{bc}	132.86^{abc}
Boutaleb	15.03^{ab}	6.54	39.31^{de}	26.32 ^{c}	27.79 ^{bc}	98.49^{bc}
Oued el bared	12.83^{abc}	9.07^{bcd}	56.51^{cd}	26.96 ^{c}	33.69 ^{bc}	79.84^{cd}
Mean	10.95	9	25.29	35.24	33.61	114.82
Min	5.29	4.07	25.91	21.09	19	39,44
Max	20.9	14.48	104.84	65.8	54.17	178.81
LSD	8.53	4.03	20.05	23.45	18.26	55.7
Effect genotypes	*	**	**	*	**	**

MDA: Malondialdehyde, PC: Proline content, SS: Soluble sugars, (*/ **) significant differences at 0.05 and 0.01, respectively.

is a response to oxidative stress, with lines G4 and G3 showing a high positive deviation from the average, which are considered the most tolerant under oxidative stress (figure 2). Proline serves as an important molecule in oxidative stress resistance (Kishor *et al.*, 2022). Thus, it plays a role against oxidative damage due to its ability to eliminate ROS from the cell or activate an antioxidant defense mechanism (Langaro *et al.*,2020). According to Gomes *et al*. (2017), increased cellular ROS concentrations commonly stimulate proline biosynthesis. Proline can also act as a mediator of osmotic adjustment and protection of the plasma membrane as a source of carbon and nitrogen (Hemaprabha *et al*., 2013). Many studies have supported the idea that proline plays a diverse role during oxidative stress, including improving photosynthesis and interact with several molecules of signaling, such as nitric oxide and phytohormones, to activate the stress signaling molecules (Hanif *et al*., 2021; Rajametov*et al*., 2021). Like the proline content, the level of soluble sugars was also significantly affected by glyphosate treatments. Values of soluble sugars varied from 39.44 ug.g^{-1} for line G4 to 178.04 ug.g^{-1} for line G5, with an overall average of 114.82. The most stressed line, G5, has responded by increasing the total amount of sugars in their cells, which is an indicator of adaptation to oxidative damage (figure 3). Soluble sugars are an important osmolytes, which limit cellular damage due to oxidative stress; levels of sugar might also accumulate due to starch degradation under stress conditions (Sharma *et al*., 2019). Our findings are in agreement with Fernández-Escalada *et al*. (2019), who showed that soluble sugars increased in Palmer amaranth treated with glyphosate. Soluble sugar aids in maintaining the cellular redox homeostasis, reactive oxygen species detoxification, and protect photosynthesis systems (Chauhan *et al.*, 2022).

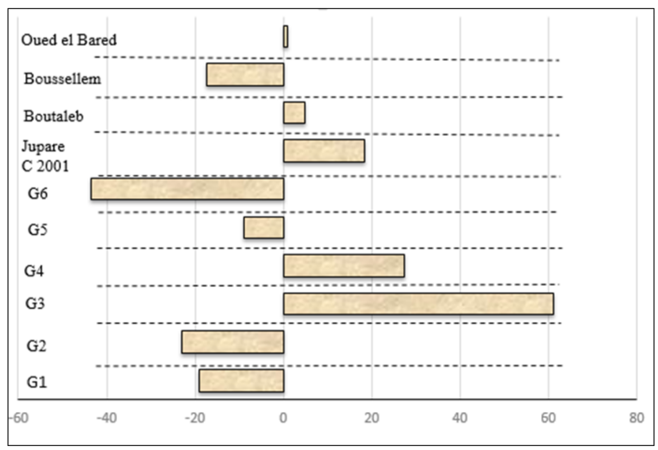

Figure 2. Deviation from the mean values of free proline under oxidative stress in genotypes tested.

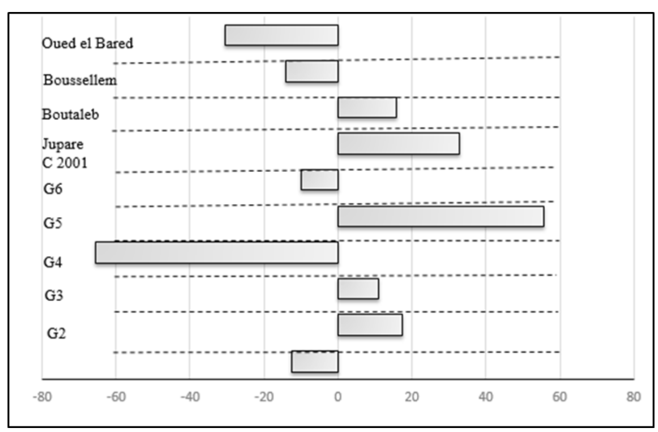

Figure 3. Deviation from the mean values of soluble sugars under oxidative stress in genotypes tested.

ROS accumulation is directly correlated with sugar accumulation to adapt to the ill effects of oxidative stress. In addition, sugar accumulation prevents the oxidation of cell membranes under water deficiency, maintains the turgidity of leaves, and prevents the dehydration of membranes and proteins (Sami *et al*., 2016). Sensitive genotypes of crop plants adapt fewer osmoprotectants with low concentrations than tolerant genotypes under stress.

Conclusion

Oxidative stress induced by glyphosate is a complex phenomenon that negatively affects plant growth. Our findings revealed a variable response to oxidative stress in the genotypes tested. The lipid peroxidation assay revealed that both lines G3 and G5 recorded the highest MDA content, which are the susceptible lines, and genotype Jupare C 2001 is the most tolerant one. It also revealed that the genotypes tested responded to oxidative damage by accumulating proline and soluble sugars. Lines G3, G4, and G5 accumulated more proline and sugars, which suggests they are the most adapted and stable lines to oxidative stress.

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