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Evaluation of chlorophyll content and membrane stability under oxidative stress induced by glyphosate herbicide as indicators of drought tolerance in some advanced durum wheat (*Triticum durum* L.) lines: *in vitro* study

Evaluación del contenido de clorofila y la estabilidad de la membrana bajo estrés oxidativo inducido por el herbicida glifosato como indicadores de tolerancia a la sequía en algunas líneas avanzadas de trigo duro (*Triticum durum* L.): estudio *in vitro*

Avaliação do teor de clorofila e da estabilidade da membrana sob estresse oxidativo induzido pelo herbicida glifosato como indicadores de tolerância à seca em algumas linhagens avançadas de trigo duro (*Triticum durum* L.): estudo *in vitro*

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Rev. Fac. Agron. (LUZ). 2023, 40(1): e234007
ISSN 2477-9407
DOI: https://doi.org/10.47280/RevFacAgron(LUZ).v40.n1.07

Crop Production
Associate editor: Professor Andreina García de González
University of Zulia, Faculty of Agronomy
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Received: 16-11-2022
Accepted: 26-01-2023
Published: 04-02-2023

Keywords:
Electrolyte leakage
Chlorophyll loss
Tolerant
Sensitivity

Abstract

Oxidative stress caused by glyphosate is a complex chemical and physiological phenomenon and develops as a result of overproduction and accumulation of reactive oxygen species (ROS). This study was carried out *in vitro* at the National Institute of Agronomic Research of Algeria (INRAA) Setif, to select the most susceptible durum wheat (*Triticum durum* L.) under oxidative stress induced by glyphosate herbicide by evaluating chlorophyll content degradation and cell membrane leakage. Genotypes showed significant variations in almost all the studied traits. The chlorophyll loss ratio ranged from 26.42 % for the genotype G5 to 48.75 % for the local variety Boutaleb, glyphosate sensitivity index values were found to be between 0.65-1.2, the advanced line G5 was found to be the most tolerant under oxidative stress with the lowest chlorophyll loss ratio and lowest Glyphosate sensitivity index. Furthermore, the advanced line G4 recorded the highest electrolyte leakage (80.16 %) while G6 showed the lowest estimate (50.77 %). Therefore, advanced lines G5 and G6 appear the most suitable for the growing conditions.

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Resumen

El estrés oxidativo causado por el glifosato es un fenómeno químico y fisiológico complejo y se desarrolla como resultado de la sobre producción y acumulación de especies reactivas del oxígeno (ROS). Este estudio se llevó a cabo *in vitro* en el Instituto Nacional de Investigación Agronómica de Argelia (INRAA) Setif, para seleccionar el trigo duro (*Triticum durum* L.) más susceptible bajo estrés oxidativo inducido por el herbicida glifosato mediante la evaluación de la degradación del contenido de clorofila y la fuga de la membrana celular. Los genotipos mostraron variaciones significativas en casi todos los rasgos estudiados. El índice de pérdida de clorofila osciló entre el 26,42 % del genotipo G5 y el 48,75 % de la variedad local Boutaleb, y los valores del índice de sensibilidad al glifosato se situaron entre 0,65 y 1,2. La línea avanzada G5 resultó ser la más tolerante al estrés oxidativo, con el menor índice de pérdida de clorofila y el menor índice de sensibilidad al glifosato. Además, la línea avanzada G4 registró la mayor pérdida de electrolitos (80,16 %), mientras que G6 mostró la estimación más baja (50,77 %). Por lo tanto, las líneas avanzadas G5 y G6 parecen las más adecuadas para las condiciones de crecimiento.

Palabras clave: fuga de electrolitos, pérdida de clorofila, tolerancia, sensibilidad.

Resumo

Estresse oxidativo causado pelo glifosato é um fenômeno químico e fisiológico complexo e se desenvolve como resultado da superprodução e acúmulo de espécies reativas de oxigênio (ROS). Este estudo foi realizado *in vitro* no Instituto Nacional de Pesquisas Agronômicas da Argélia (INRAA) Setif, para selecionar o trigo duro mais suscetível (*Triticum durum* L.) sob estresse oxidativo induzido pelo herbicida glifosato, avaliando a degradação do teor de clorofila e o vazamento da membrana celular. Os genótipos mostraram variações significativas em quase todos os traços estudados. A taxa de perda de clorofila variou de 26,42 % para o genótipo G5 a 48,75 % para a variedade local Boutaleb, os valores do índice de sensibilidade ao glifosato foram encontrados entre 0,65-1,2, a linha avançada G5 foi encontrada como a mais tolerante sob estresse oxidativo com a menor taxa de perda de clorofila e o menor índice de sensibilidade ao glifosato. Além disso, a linha avançada G4 registrou o maior vazamento de eletrólitos (80,16%), enquanto G6 apresentou a estimativa mais baixa (50,77 %). Portanto, as linhas avançadas G5 e G6 parecem ser as mais adequadas para as condições de crescimento.

Palabras-chave: vazamento de eletrólitos, perda de clorofila, tolerante, sensibilidade.

Introduction

During the last 50 years, increased global population and varied consumption preference have led to the elevated demand for wheat commodities worldwide, also considering that wheat is a staple food for more than 35 % of the world population (FAO, 2020). Durum wheat often experiences drought stress conditions during crop cycle. Thus, improvement of durum wheat tolerant to drought is a major objective in plant breeding programs for arid and semi-arid regions (Oulmi and Aissaoui, 2022; Singh *et al*., 2022; Thakur *et al*., 2022). Glyphosate (N-[phosphonomethyl]-glycine) is the most commonly used non-selective herbicide worldwide (Soares *et al*., 2019) since its commercialisation by Monsanto in the 1970. Glyphosate has been shown to affect plant physiological mechanisms such as photosynthesis, mineral nutrition and oxidative events (Rivas-Garcia *et al*., 2022; Singh *et al*., 2020; Gomes *et al*., 2014). Along with the inhibition of specific target sites, glyphosate action also leads to oxidative stress in plants, which is most probably a secondary effect of the blocked shikimate pathway (Freitas-Silva *et al*., 2017; Gomes *et al*., 2014). Oxidative stress in wheat and maize was observed in plants exposed to glyphosate as a consequence of reactive oxygen species (ROS) accumulation. Plants are able to avoid ROS adverse effects by detoxify them through the action of both enzymatic and non-enzymatic antioxidants. Additionally, increasing ROS production may adversely affect photosynthetic processes, i.e. by decreasing the amount of chlorophyll and the photochemical efficiency, which reduces plant growth. Indeed, previous studies have shown that plants exposed to glyphosate application have lower chlorophyll content. These findings have been attributed to either increased chlorophyll degradation or decreased chlorophyll synthesis (Ibrahim *et al*., 2022; Gomes *et al*., 2017). Photosynthesis plays a vital role in the synthesis and accumulation of organic matter, plant growth, nutrient absorption and response to abiotic or biotic stress. The aim of this research is to evaluate the behaviour of some advanced lines of durum wheat under the effects of oxidative stress induced by glyphosate, based on the estimation of chlorophyll degradation and electrolyte leakage from injured cells in order to select the adapted advanced lines.

Materials and methods

Plant material

This study was conducted during the 2021/2022 cropping seasons at National Institute of Agronomic Research of Algeria – INRAA - Setif. The genetic material used in this study consisted of 6 advanced lines and 4 genotypes, 3 out of them were local varieties used as control to evaluate their performance under oxidative stress (table 1).

Table 1. The pedigrees of the genotypes lines tested.

Genotype/Lines	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_l/PATKA_3/3/PORRON_4/YUAN_l/9/...
Jupare C 2001	STINKPOT//ALTAR-84/ALONDRA
Bousselem	Heider/Martes/Huevos de Oro. ICD-414
Boutaleb	GTA dur /Ofanto
Oued el bared	Hedba3/Ofanto

Experimental details

The genotypes tested were sown on December 14th, 2021 with sowing density adjusted to 300 grains.m^{-2} in a random block design with three replications, each plot consisted of 6 lines of 10 m long spaced of 0.2 m which make 12 m^2 as plot area. At heading stage, three flag leaves of each genotype were cut and dipped in 10 mL of 5 mM glyphosate solution (dissolved in distilled water). Daily, the chlorophyll contents (CC) of each flag leaf were measured using digital chlorophyll meter (CCM) with (cci) units; this device allows to measure the absorbance of light in the leaf. Electrolyte leakage of leaf tissues were measured using the method developed by Baji *et al.* (2001) with minor modifications: two leaves were randomly collected for each genotypes, washed with tap water then with distilled water and cut into 1 cm length segments. The segments were placed in tubes with 10 mL of distilled water and incubated for 24 h at the ambient temperature of the laboratory. Subsequently, the first reading (EC1) was carried out. The final conductivity (EC2) was measured after adding 0.02 mL of pure glyphosate to each tube. The relative electrolyte leakage (REL %) was calculated as follows: REL (%) = (EC1/EC2) × 100. Glyphosate sensitivity index (GSI) was calculated to determine the genotypes sensitivity to oxidative stress induced by glyphosate herbicide, GSI = chlorophyll loss for each genotype / Mean.

Results and discussion

Degradation of Chlorophyll content

Chlorophyll content of leaf before and after 6 days of treatment with glyphosate is presented in table 2. Chlorophyll content values before treatment ranged from 31.85 cci for the advanced line G5 to 53.7 cci for the introduced genotype Jupare C 2001 with an average of 45.76 cci over all genotypes, while after glyphosate application during six days the chlorophyll content decreased and the values varied between 4.35 to 14.32 cci, the highest value was obtained from the advanced line (G1) while the variety Boutaleb registered the lowest value. The decrease in total chlorophyll amount correlates with the increase in the number of days after glyphosate application (figure 1). Ahsan

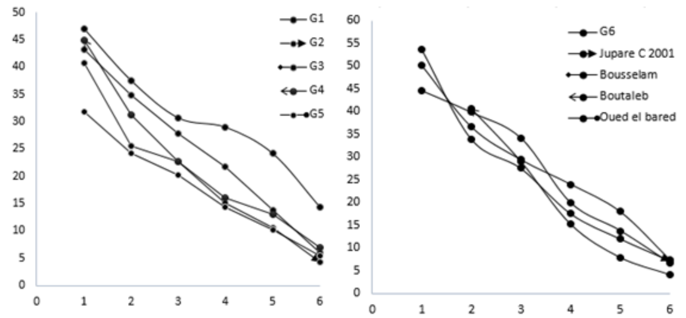

Figure 1. Degradation of chlorophyll content for durum wheat genotypes after glyphosate application.

et al. (2008) found that using glyphosate herbicide causes plants to experience oxidative stress. Our finding agree with Malalgoda *et al*. (2020) who reported that glyphosate herbicide could reduce the synthesis of chlorophyll content, which might lead to less starch accumulating. Furthermore, Bali and Sidhu (2019) reported that a significant reduction in yield might be due to the reduced rates of net photosynthesis, stomatal closure, and oxidative damage to chloroplast. Chlorophyll losses due to oxidative stress induced by glyphosate application and its velocity are shown in Table 2. ANOVA showed that genotypes effect was highly significant ($P<0.05$) with all parameters calculated. Chlorophyll loss ratio has been used to detect the genotypic differences in response to glyphosate application. The local variety Boutaleb showed the highest loss ratio (48.75 %), and the highest value of Velocity of degradation of chlorophyll (9.23). On the contrary, the lowest chlorophyll loss ratio (26.42 %) was registered by the line G5, with the lowest Velocity of chlorophyll degradation (4.64). Gomez *et al*. (2017) studied the response of willow to oxidative stress induced by various glyphosate concentrations and found that the decrease in chlorophyll concentration may also be due to its

Table 2. Chlorophyll content of leaf before and after glyphosate application, chlorophyll loss (%), index of sensitivity to glyphosate, and velocity of chlorophyll degradation for genotypes tested.

Genotypes	CC before Gly application (cci)	CC after Gly application (6 Days after) (cci)	Chlorophyll loss ratio(%)	Velocity of chlorophyll degradation	Gly sensitivity index (GSI)	Score
G1	51.67^{bc}	14.32^{a}	37.35^{de}	6.16^{e}	0.93^{de}	4
G2	40.8^{e}	4.35^{fg}	36.45^{de}	6.44^{de}	0.9^{de}	3
G3	43.27^{d}	6.02^{de}	37.25^{de}	6.41^{de}	0.96	5
G4	44.97^{d}	6.82^{bcd}	38.15^{d}	6.79^{d}	0.95^{d}	7
G5	31.85^{f}	5.42^{ef}	26.42^{f}	4.64^{g}	0.65^{f}	1
G6	50.02^{c}	7.47^{bc}	42.55^{c}	7.35^{c}	1.06^{C}	8
Jupare C 2001	53.7^{a}	7.2^{bcd}	46.5^{b}	8.65^{b}	1.15^{b}	9
Bousselem	44.62^{d}	6.6^{cde}	38.02^{d}	6.64^{d}	0.94^{d}	6
Boutaleb	52.87ab	4.12^{g}	48.75^{a}	9.23^{a}	1.21^{a}	10
Oued el Bared	43.87^{d}	7.9^{b}	35.97^{e}	5.59^{f}	0.89^{e}	2
Mean	45.76	7.02	40.11	6.79	0.96	
Min	31.85	4.12	26.42	4.64	0.65	
Max	53.7	14.32	48.75	9.23	1.21	
LSD (5%)	1.95	1.25	1.85	0.48	0.04	
Effect genotype	***	***	***	***	***	

***: highly significant effect at 5%, CC: Chlorophyll content, Gly: glyphosate, GSI: Glyphosate sensitivity index.

degradation by increased ROS content. Chlorophyll loss was shown to be accompanied by the damage of the mesophyll chloroplasts, which led to a lower photosynthetic rate (Khalilzadeh *et al*., 2016). The study reported by Zobiole *et al*. (2011) demonstrated that Glyphosate significantly decreased chlorophyll content in soybean compared with the non-glyphosate control. This decrease could be due to direct damage of the chloroplast in the presence of glyphosate, as plants from all maturity groups exposed to a single or sequential application of glyphosate frequently had chlorophyll concentrations lower than plants that were not exposed to this herbicide. It's also well known that a decrease in the chlorophyll content could be due to a decrease in the stomata aperture, aimed at limiting water losses by evaporation and by increased resistance to the entry of atmospheric CO_2 necessary for photosynthesis (Zhu *et al*., 2016; Enneb *et al.*, 2020). Karabulut and Çanakcı (2021) showed that the oxidative stress created by glyphosate treatment caused decrease in chlorophyll (a+b). As well, Caglar *et al*. (2011) analyzed the effects of the herbicide paraquat on chlorophyll content, observing that bread wheat varieties after paraquat herbicide treatment decreased chlorophyll content. Glyphosate sensitivity index (GSI) for the examined durum wheat varied between 0.65-1.21, the differences among all genotypes tested were highly significant (table 2). The variety Boutaleb was observed as the most sensitive to oxidative stress followed by Jupare C 2001. However, advanced line G5 recorded the lowest GSI thus appeared as the most tolerant to oxidative stress. Based on the ranking for traits illustrated in table 2, genotypes G5 and Oued el bared were the best performing under oxidative stress.

Electrolyte leakage

Plant membranes are subject to changes often associated with increases in permeability and loss of integrity under environmental stresses (Masoumi *et al*., 2010). Evaluation of cell damage degree was accomplished for the ten genotypes using membrane stability index: Electrolyte leakage has been recommended as a useful criterion for the selection of stress-tolerant cultivars in several crop species (Slama *et al.*, 2018). Moreover, ion leakage has been used as an efficient measure for the evaluation of the damage induced by herbicide that affects the integrity of the membranes (Silva *et al.*, 2016). There was a significant difference in the amount of electrolyte leakage (REL %) from leaf tissues for the genotypes tested, with a mean of 64.8 % (table 3). Line G6 exhibited the lowest value implying that this genotype was the most resistant under oxidative stress and line G4 the most susceptible ones. The ability of cell membranes to control the rate of ion movement in and out of cells is used as a test of damage to a great range of tissues (Masoumi *et al*., 2010). Glyphosate caused oxidative damage in plants and disturbed cellular homeostasis of plants. Under oxidative stress production of ROS increased, thus oxidizing lipids of membranes, and increasing their permeability that leads to ion leakage (Sakya *et al.*, 2018).

Conclusion

Oxidative damage induced by glyphosate herbicide affects the physiological parameters of the examined durum wheat. Hence, these parameters can be used as a criterion to select adapted genotypes to oxidative stress. Our study revealed significant variations among the genotypes ($P<0.05$). The genotypes with the lowest velocity of chlorophyll degradation and the lowest chlorophyll ratio yielded the lowest sensitivity to oxidative stress. The advanced line G5 was recorded as the most tolerant to oxidative stress. Based on our findings, the highest value of injured cells was observed in advanced line G4, while line G6 recorded the lowest, indicating that it could maintain high membrane integrity during oxidative stress. We can conclude that both advanced lines G5 and G6 are very suitable to the growing conditions.

Table 3. Changes in relative electrolyte Leakage (%) of ten durum wheat genotypes under Oxidative stress.

Genotypes	REL%
G1	61.86[bcd]
G2	64.29[bc]
G3	66.67[bc]
G4	80.16[a]
G5	72.57[ab]
G6	50.77[d]
Jupare C 2001	61.73[bcd]
Bousselem	58.2[cd]
Boutaleb	69.42[abc]
Oued el bared	62.35[bcd]
Mean	64.8
Max	80.16
Min	50.77
CV	13
LSD	11,64
Effect genotype	**

****: significant effect at 5 %, REL%: Electrolyte leakage.**

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