Effect of phosphorus stress on antioxidant enzyme activities in wheat seedlings (*Triticum durum* Desf.) under in vitro culture

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Abstract

The study investigates the role of root glutathione (GSH) content and glutathione S-transferase (GST) activity in the response of hard wheat (*Triticum durum* Desf. variety Carioca) induced by different doses of phosphorus KH_2PO_4 and their relation to growth inhibition. Four doses were evaluated: control (0), 85, 170, and 340 mg/l. The experiment was carried out under *in vitro* culture conditions, during seven days. Results showed that P addition had a significant effect on growth parameters, root water content, and the amount of GSH and GST activity, but no significant changes were observed in chlorophyll contents. Treatment of seeds with KH_2PO_4 increased the GST activity from hard wheat roots and decreased GSH content. The GSH level in control was 2-fold greater than phosphorus 340 mg/l treatment. The highest induction of root GST activity compared to control was observed in 170 mg/l treatment. Phosphorus levels closely correlated with GSH (r^2= -0.996***) and GST (r^2= 0.991***).

Keywords: durum wheat; GSH; GST; phosphorus; stress

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Introduction

In both animals and plants, glutathione S-transferases (GST) are induced by diverse environmental stimuli, with increased GST levels used to maintain cellular redox homeostasis and protect organisms against oxidative stress. GST was proposed to afford protection under various stress conditions by detoxifying endogenous plant toxins that accumulate as a consequence of increased oxidative stress (Marrs, 1996). It is obvious that GST is also stimulated by various stresses, such as pathogen infection, herbicide application, heavy metals, dehydration, senescence, hypoxic stress, and salt (Marrs, 1996; Moon, 2003)

Glutathione (GSH) is an essential thiol antioxidant as well as a scavenger of reactive electrophilic compounds, functioning with GST to detoxify a range of herbicides (Marrs and Walbot, 1997; Edwards et al., 2000) by tagging electrophilic compounds for removal during oxidative stress. Booth et al. (2000) revealed that in field experiments, those growth rates were influenced by all environmental parameters and

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these effects were interrelated. Soil type also influenced GST activity and this influence was dependent on moisture content.

Liu et al. (2009) evaluated the response of tree species to varying soil acidity. They found the highest chlorophyll content under soil pH 5.5 and significantly lower chlorophyll content under soil pH 7.5 and 8. Application of fertilizers N, P, and K to the substrate significantly influenced the growth of *Brassica oleracea* seedlings (Zhang et al., 2017), but when applied in high doses, they negatively influence plant growth. On the other hand, water and N supply led to a significant increase in the growth and biomass production of seedlings (Wang et al., 2012). Under N stress, the net assimilation rate decreased, which resulted in a decrease in total biomass, and the root/shoot ration (Wu et al., 2004a).

The present study is part of a research program on the rational fertilization of hard wheat in the arid area of southeastern Algeria. We were interested in investigating the role of roots glutathione (GSH) content and glutathione S-transferase (GST) activity in the response of plants hard wheat and their relation to growth inhibition, induced by different doses of phosphorus KH_2PO_4.

Materials and Methods

Seeds of wheat (*Triticum durum* Desf. carioca) were surface sterilized in 20% (v/v) sodium hypochlorite for 20 min, followed by three washes with sterile distilled water under aseptic condition. Explants were grown *in vitro* on MS (Murashige and Skooge, 1962) medium, containing four increasing concentrations of KH_2PO_4 (control, 85, 170, and 340 mgL^{-1}) under controlled conditions (light/dark 16h/8h, 25 °C).

Samples of treatments and control were collected on day 7 of growth. Plants (stems + roots) were harvested from each treatment and washed thoroughly in distilled water. Morphological parameters such as root and shoot length were measured manually using a graduated scale and recorded. The average water content of plants was calculated using the formula recommended by Xu et al. (2006).

Chlorophyll estimation was done according to Rao and Le Blanc (1965). Five hundred mg fresh tissue was homogenized with 80% acetone. The extract was centrifuged for 5 min. the process was repeated until the pellet became colorless. The absorbance of the extract was red at 663 and 645 nm using a UV-visible spectrophotometer (JENWAY 6300). Total chlorophyll was determined according to Brown and Whitehead (1986). Chlorophyll a and chlorophyll b were calculated using the formula suggested by Hiscox and Israelstan (1979).

The extraction of GSH was in 4 ml of 100 mM phosphate tempone, followed by deproteinization in acid sulfo-sally-silique 0.25% after centrifugation at 2000 rpm for 10 min. The absorbance of the supernatant released at 412 nm in the presence of 25 µl of DTNB (Anderson, 1985). Determination of Glutathione S-transferase activity was achieved by the Habig et al. (1974) method.

Statistical Analysis

The obtained data were recorded and classified using Microsoft Office Excel 2010. The significance of differences between the means was determined using analysis of variance (ANOVA) at $p \leq 0.05$ with the software package Statistica 8.0.

Results

Effect of P on wheat growth, physiological, and biochemical parameters

The P addition had a significant effect on all growth parameters (Table 1). Root number ($p \leq 0.01$), root and shoot length ($p \leq 0.05$) significantly decreased as the P addition rate increased from 0 mgL^{-1} to 340 mgL^{-1}. However, the addition of a high level of P (340 mgL^{-1}) not only decreased the root number but also decreased root and shoot length. This explained that growth parameters deceased under P stress.

Root water content was significantly ($p \leq 0.05$) affected by P rate, where the control, without P recorded the best value, followed by 340, 85, and 170 mgL^{-1} respectively; but, there was no effect on relative water content and chlorophyll content in shoots (Table 1).

Table 1
Effect of phosphorus levels on morphological and physiological parameters of 7-day-old wheat seedling

Levels (mg/L)	Root length (cm)	Root number	Shoot length (cm)	Root water content (%)	Foliar water content (%)
Control	09,98 a	6,22 a	12,47 a	86,93 a	72,67
85	09,52 b	5,89 b	12,82 a	78,87 b	77,52
170	10,68 a	06,0 b	13,66 a	78,28 b	73,37
340	08,13 c	5,66 c	09,15 b	84,88 a	70,80
Averages	9,579*	5,94**	12,03*	82,24*	73,59 ns
LSD (5 %)	0,747	0,136	1,263	3,036	-

*, **, and *** Significant at p≤0.05, p≤0.01, and; p≤0.001, respectively; ns: not significant, different superscript letters in a column show significant difference.

Effect of P rate on enzymatic activity

The data in figures I and II show significant relationships between phosphorus and GSH and GST activities in all treatments. The roots of plants exhibited different levels of enzyme activity upon exposure to various phosphorus levels. However, GSH decreased with increasing P levels (Fig. I). The highest and the lowest values were observed in control and 340 mg P/l, respectively (Table 2). On the other hand, GST increased with increasing of P levels (Fig. II), where the highest activity was observed at 170 mg P/l.

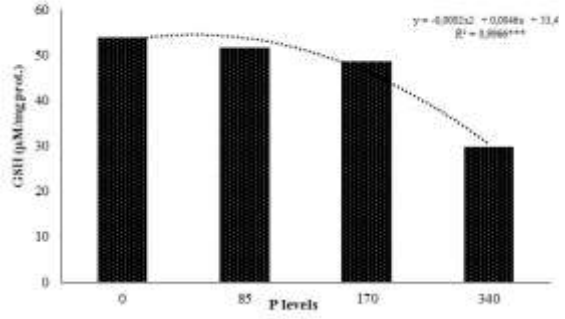

Fig. I. Effect of phosphorus on the activity of GSH in roots of wheat seedling

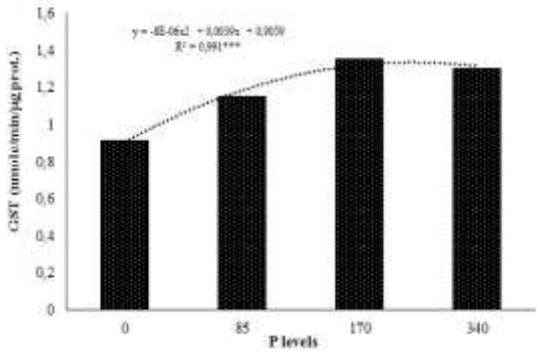

Fig. II. Effect of phosphorus on the activity of GST in roots of wheat seedling

Relationships between enzymatic activities and morphological, physiological, and biochemical parameters

The study of the relationship between the amount of GSH and the GST activity with different growth, physiological, and biochemical parameters revealed significant links. Results indicated that GSH correlated significantly and positively with root number (r^2= 0.760***) and root length (r^2= 0.642***). Similarly, GST activity had significant and negative relations with root number (r^2= -0.717***) while it positively and closely correlated with root length (r^2= 0.274*). Both parameters, GSH and GST were intimately linked to shoot length, with r^2= 0.624*** and r^2= 0.301**, respectively.

Significant relationships were established between GSH and GST on the one hand and the relative water content in root and shoot, total chlorophyll content, chla, chlb, and chla/b on the other hand. The amount of GSH was negatively linked with RW (r^2= -0.592***) and FW (r^2= -0.374**) while GST activity was linked negatively with RW (r^2= -0.597***) and positively with FW (r^2= 0.383**). Most chlorophyll parameters were closely related to GSH and GST except for chla/b which showed no significant relationship with GSH (r^2= 0.125ns).

Discussion

The results of the present study showed that wheat growth decreased with increasing P addition rate. It was also found that the mechanism of phosphorus induced oxidative stress in wheat and involved in GST and GSH.

Table 2
Effect of phosphorus levels on biochemical and enzymatic parameters of 7-day-old wheat seedlings

Levels (mg/L)	Chl*a* (µg/g FM)	Chl*b* (µg/g FM)	Chl*a+b* (µg/g FM)	Chl. a/b	GSH (µM/mg protein)	GST (nM/min/µg protein)
Control	20,67	12,20	44,2	1,736	53,79 a	0,915 c
85	18,35	10,045	38,79	1,873	49,75 a	1,152 b
170	20,37	13,15	44,19	1,604	48,56 a	1,352 a
340	19,03	9,88	39,94	1,942	27,59 b	1,303 a
Averages	19,60ns	11,32ns	41,78ns	1,788ns	44,92**	1,18***
LSD (5%)	-	-	-	-	5,712	0,074

Chl. a : chlorophyll a; chl. b : chlorophyll b; Chl. a+b: total chlorophyll; GSH: glutathionne ; GST : glutathionne S-transferases. *, **, and *** Significant at p≤0.05; p≤0.01, and; p≤0.001, respectively, ns: not significant; different superscript letters in a column shows significant difference.

Halusková et al. (2009) and Bouchelaghem et al. (2012) found a relationship between increased GST activity and root growth during P treatments. Mittova et al.(2003) demonstrated that salt increased GSH content in oxidative stress-tolerant *Lycopersicon pennellii* and induced GST. Whereas, Zhang and GE (2008) found that in rice shoots, GSH and GST activity increased with increasing Cd. According to KumarParida and BandhuDas (2005), the ability of plants to tolerate salt is determined by multiple pathways that facilitate retention and/or acquisition of water.

Zhao et al. (2014) proved that NaCl stress affected the chlorophyll content, photosynthesis, and chlorophyll fluorescence in tomato plants. When applied at high dose, this damaged the photosystem II reaction center and its reduction side in tomato (Singh et al., 2016).

Previous studies have shown that treatment with phosphorus increase chlorophyll content in maize plants compared with the plants that did not receive phosphorus (Jiang et al., 2007). The chl. a content in green algae increased with an increase of phosphorus concentration (Chen et al., 2011). While Wu et al. (2004b) found no significant change in chlorophyll a and b and carotenoid contents when the *Fraxinus mandchurica* seedlings were under phosphorus stress. These results confirm our findings that there is no significant effect of P on chl. a, chl. b and total chlorophyll contents of wheat seedling.

The present study revealed a significant relationship between GST and GSH with growth, physiological, and biochemical parameters of wheat seedlings under phosphorus treatment. A close relationship between plant growth and enzyme activities such as GST was demonstrated (Çakmakçi et al., 2007; Çakmakçi et al., 2009). Wang et al. (2012) found a positive relationship between photosynthetic capacity and leaf N content in response to water and N supply in the seedling. Under N stress, the net uptake rate of nitrogen and phosphate by the seedlings decreased, and the leaf mass ration of seedlings reduced (Wu et al., 2004a).

In conclusion, our results may reflect the protective role provided by GSH and GST extracts under P stress, due to its high antioxidant capacity and this allows us to choose the optimal phosphorus dose that promotes a better behavior of the crop in the field and avoid environmental pollution for sustainable agriculture.

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