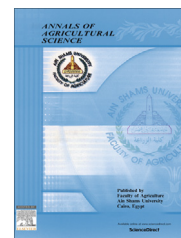




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# Enhancing antioxidant–yield relationship of pea plant under drought at different growth stages by exogenously applied glycine betaine and proline



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## KEYWORDS

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Proline

**Abstract** Pod filling stage considers as a receiver (sink), which reflects plant performance during previous growth stages. In order to study, the influence of drought imposed at different growth stages, and the impact of foliar applied glycine betaine (GB) and proline on the status of osmolytes and antioxidant defense system of pea plant during pod filling stage, a field experiment was conducted in 2012/2013 and 2013/2014 on clay loam soil. Four different irrigation regimes were applied to provide drought at different growth stages: (1) vegetative stage, (2) flowering stage, as short-term drought stress, (3) throughout the stages of vegetative + flowering growth (long-term drought stress), and (4) control (without stress). Foliar applications of GB and proline at 4 mM for each, in addition to distilled water as control, were conducted. Generally, drought applications reduced the growth and yield of pea plant. Long-term drought was more effective to reduce growth and yield than drought at flowering stage. GB increased the yield and its soluble protein concentration more than proline. Proline recorded the maximum increase in non-enzymatic antioxidant defense system under drought. Application of GB or proline enhanced the activity of SOD, APX and catalase in leaves under drought, while in seeds they increased SOD activity under long-term drought stress. APX activity in seeds under drought decreased by GB application. The maximum positive effect was for GB under unstressed condition and drought at vegetative stage, by maximizing APX activity, in addition to enhancing the production and translocation of assimilates from source to sink.

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## Introduction

Plants in their natural habitat or cultivated crops are exposed to several environmental stresses, that affecting plant growth and

productivity. Drought is the most widespread devastating environmental stress, which decreases crop productivity more than any other environmental stress (Farooq et al., 2012), for instance, continuous or frequent drought effect on up to 45% of the world agricultural lands (Ashraf and Foolad, 2007). Drought severely affects plant growth and development with consequence reductions in the rate of cell division and elongation, leaf area, root and stem growth, interrupted

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stomatal conductance, and water use efficiency, which makes photosynthesis very sensitive to drought (Farooq et al., 2009). The detrimental effects of drought on plant growth and development depend on the severity of stress and the crop growth stage. Nutrients require water for uptake and translocation. As water supply decreases, nutrient uptake does (Farooq et al., 2012).

Pea plant as other most legume crops are more sensitive to water stress during flowering and pod filling stage than during vegetative stage. Long-term drought causes destructive effects in pea plants (Karataş et al., 2014). A severe water deficit leads to a fall in the content of the proteins as well as modifying their composition (Lecœur and Guillioni, 2010). Antioxidant system in leaves of pea plant (seven weeks old) exposed to long-term salt stress (four weeks) was studied by Ozturk et al. (2012), and found that protein content was significantly decreased, while proline was accumulated with increasing in salinity level. Activity of peroxidase and superoxide dismutase (SOD) increased under salt stress, while catalase (CAT) and ascorbate peroxidase (APX) activities generally decreased in salt stressed seedlings. They suggested that increase in the activities of peroxidase and SOD/ascorbate–glutathione (AsA–GSH) cycle, improved the resistance of pea plant to oxidative stress, which enhanced salt tolerance. Moderate water stress in pea marks the beginning of the modification of the physiological status of plant tissues. Stomatal conductance falls with an increase in ABA content, reduces the size of all developing vegetative organs on the plant at the time of its occurrence, and reduces the final number of reproductive branches (Lecœur and Guillioni, 2010). At increased maturity, the greater decrease in sucrose concentration in peas was in nonstressed than drought-stressed peas (Sorensen et al., 2003).

Reactive oxygen species (ROS) are produced as a normal by-product during plant cellular metabolism with controlled amounts, and effect on the expression of a number of genes (Gill and Tuteja, 2010; Sharma et al., 2012). Exposing to abiotic stresses including drought elevates the oxidative stress with overproduction of ROS, which are highly toxic and trigger impairment to carbohydrates, proteins, lipids, and DNA, leads to deteriorate normal plant metabolism through oxidative damage, and ultimately causes cell death. Superoxide radicals ( $O_2^-$ ), single oxygen ( $^1O_2$ ), hydrogen peroxide ( $H_2O_2$ ), alkoxy radicals ( $RO^\bullet$ ), and hydroxyl radicals ( $OH^\bullet$ ) are among the major ROS generated in plants under abiotic stresses (Gill and Tuteja, 2010). The major sites for the production of  $O_2^-$  were photosystem I and II in chloroplasts, and complex I, ubiquinone and complex III of electron transport chain in mitochondria (Gill and Tuteja, 2010). Therefore, weakened activity of anabolism and catabolism essential enzymes leads to hamper the photosynthetic and respiratory activities (Farooq et al., 2012). Plants possess very efficient antioxidant defense machinery, which consists of enzymatic and non-enzymatic antioxidants. The enzymatic antioxidants consist of SOD, CAT, and AsA–GSH cycle enzymes; APX, glutathione reductase (GR), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), glutathione peroxidase (GPX), and glutathione s-transferase (GST) (Karataş et al., 2014). The non-enzymatic antioxidants such as ascorbic acid (AsA), glutathione (GSH) phenolic compounds, carotenoids, alkaloids, non-protein amino acids and  $\alpha$ -tocopherols (Sharma et al., 2012). Both of enzymatic and non-enzymatic antioxidants work in concert to operate the

ascades of uncontrolled oxidation and shield most affected plant cells components by scavenging of ROS (Gill and Tuteja, 2010).

Under environmental stresses such as drought, plants accumulate many of low molecular weight water-soluble compounds, which are known as compatible solutes, osmolytes or osmoprotectants, which decrease the cell water potential without decreasing actual water contents. The most common compatible solutes are betaines (glycine betaine, as the original betaine), soluble sugars (sucrose, trehalose, mannitol, and sorbitol), polyamines, proline and amino acids (Giri, 2011). These compatible solutes not only maintain the turgor pressure within cells, but also protect the enzymes and macromolecules from oxidation by ROS (Farooq et al., 2012).

Exogenous application of GB or proline can play an important role in enhancing plant stress tolerance. This role can be in the form of either osmoprotection or cryoprotection (Ashraf and Foolad, 2007; Giri, 2011). Proline protects cell membranes from oxidative stress by enhancing activities of various antioxidants and facilitated growth (Ashraf and Foolad, 2007). Exogenous application of proline and GB has an important role in upregulating the homeostasis in lentil under stress condition. Proline exhibited better protection than GB under drought stress, suggesting that both proline and GB provided a protective role in drought induced oxidative stress by reducing  $H_2O_2$  levels and by increasing the antioxidant defense system (Molla et al., 2014). Importantly, exogenous application of proline and GB in stressed plants further enhanced the endogenous proline content (Hasanuzzaman et al., 2014). Exogenous amino acids have been shown to promote potassium and calcium uptake. Therefore, proline and other amino acids may contribute to osmoregulation not only per se, but also by regulating the contents of inorganic solutes, which in turn may contribute to osmotolerance (Rai, 2002).

Although, the influence of water deficit on yield of pea plants has been studied previously in different viewpoints (Martin and Jamieson, 1996; Sousa-Majer et al., 2004; Duzdemir et al., 2009) without reviewing the effect of drought on antioxidant system during pod filling stage, screening of antioxidant system during pod filling stage under drought stress was the main target of the present study. Therefore, the objectives of this study revealed the following:

- The effect of drought imposed at different growth stages on yield components, plant growth and assimilates compartmentation between source and sink.
- Assessing the status of antioxidant defense system during pod filling stage under stressed and unstressed conditions.
- The effect of GB and proline application on ameliorating the adverse effects of short- and long-term drought.

## Materials and methods

The present study was conducted during the two growing seasons of 2012/2013 and 2013/2014 under open field conditions in the clay loam soil, at the experimental farm, Faculty of Agriculture, Ain Shams University, Qalyubia governorate, Egypt, in order to investigate the influence of foliar application of glycine betaine and proline under drought at different growth stages (at pre-pod filling stage) and their interaction on pea total antioxidant capacity status and assimilates

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