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Studies on role of proline, hydrogen peroxide and total antioxidant activity in Wheat (*Triticum aestivum* L.) under drought stress after anthesis

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Abstract

The investigation was conducted to evaluate the effect of drought stress on role of proline, hydrogen peroxide and total antioxidant activity (TAA) of two wheat varieties viz. WH 1105 (Drought sensitive) and WH 1025 (Drought sensitive) after anthesis. Drought was given by only pre sown irrigation. Analysis of data revealed that drought stress results an increase in proline and hydrogen peroxide (H₂O₂) contents in leaves and developing grains of both wheat varieties after anthesis, however more increase in imino amino acid proline was observed in WH 1025 which is ascribed to improved drought tolerance through osmotic adjustment of cellular contents. Drought sensitive wheat variety WH 1105 had accumulated higher hydrogen peroxide (H₂O₂) than WH 1025. Stress tolerance to plants is determined by the pool size of antioxidants and protective plant pigments particularly carotenoids. This study also revealed that the total antioxidant activity (TAA) was increased in leaves and developing grains of both wheat varieties with higher and significant increase in WH 1025 than WH 1105.

Keywords: Wheat (*Triticum aestivum* L.), drought stress, anthesis, proline, hydrogen peroxide and total antioxidant activity

Introduction

Wheat is the most widely cultivated food crop. It is eaten in various forms by more than thousand million human beings in the world. In India it is second important staple food crop after rice. Wheat is the world's leading food crop, cultivated over an area of about 215 million hectares with the production of 584 million tons of grain. Maximum area under wheat is China followed by India. Even in production also China ranks first and India ranks second. In India wheat is the main cereal crop in respect of area and production and it occupies second position which accounts 12 per cent of total wheat production of the world.

Abiotic stress such as drought, soil salinity and extreme temperatures adversely affect the productivity and quality of wheat. Drought stress is one of the major limitations to crop productivity. In India water deficit stress limits crop production in about 67 per cent of net sown area. Wheat yields are reduced by 50-90 per cent of their irrigated potential by drought on at least 60 million hectares in the developing world. Improving drought stress tolerance and productivity is one of the most difficult tasks for cereal breeders. The difficulty arises from the diverse strategies adopted by the plants themselves to combat drought stress depending on the timing, severity and stage of crop growth.

Drought stress is among the most damaging factors. Besides its direct impact on water stress osmotic stress often harmfully affects plant cell membranes. Symptoms of these adverse processes include oxidation of unsaturated fatty acids, protein degradation and the resultant loss of selective permeability of membranes. Malondialdehyde (MDA) is a harmful lipid peroxidation product and hydrogen peroxide (H₂O₂) represents a highly toxic active oxygen species that can damage many important cellular components.

Induction of proline accumulation by water deficit is well known, but little understood phenomenon in plant stress physiology. Proline accumulation is caused by primarily by increased synthesis of glutamic acid and proline accumulates in drought stressed wheat plants. Proline has been demonstrated to ameliorate dehydration induced perturbation in proteins and

exogenously supplied proline confers some osmotic tolerance to the plants (Kavi K. *et al.*, 1995) ^[11]. However the cause and effect relationship between proline and drought has not been fully established.

Materials and Methods

Seeds of two varieties of wheat *viz.* WH 1105 (Drought sensitive) and WH 1025 (Drought tolerant) were obtained from Wheat and Barley Section, Department of Genetics and Plant Breeding, College of Agriculture, CCSHAU, Hisar. Seeds were sown in micro plots in the university farm. Drought stress was created by giving pre sown irrigation only for the micro plots designated for this purpose. Normal agronomical recommended irrigations were given for other micro plots. Leaf and grain samples were collected at four stages starting from 7th day after anthesis (7, 14, 21 and 28 days). Plants samples were brought to the laboratory by keeping them in liquid nitrogen after procurement from the field. Leaf and grain extracts were prepared in suitable extraction medium and were used for quantitative estimation of proline, hydrogen peroxide and total antioxidant activity by using standard protocols.

Hydrogen peroxide

Extraction

Hydrogen peroxide was estimated by the method of Sinha (1972), where sample material of 4 g was homogenized in 5 ml of ice cold 0.01 M phosphate buffer (pH 7.0), centrifuged the content at 10,000 rpm for 10 min. and supernatant was used for estimation.

Procedure

To 0.4 ml extract, 0.6 ml of 0.1 M phosphate buffer (pH 7.0) and 3 ml mixture of 5 % (w/v) potassium dichromate and glacial acetic acid (1:3 v/v) was added. The mixture was heated for 10 min. in a boiling water bath. Colour of solution changed to green due to the formation of chromic acetate. After cooling, absorbance was recorded at 570 nm against the reagent blank without sample extract. The quantity of H₂O₂ was determined from the standard curve of H₂O₂ (10-160 μ mole).

Proline

Extraction

Proline content in samples was estimated by the method of Bates *et al.* (1973) ^[2], where the sample material of 500 mg was homogenised in 5 ml of 3 % aqueous sulfosalicylic acid using mortar and pestle, centrifuged the content at 5000 rpm for 15 min. and the supernatant was used for estimation.

Procedure

The reaction mixture consisted 2 ml of acid ninhydrin reagent (1.25 g Ninhydrin + 30 ml glacial acetic acid + 20 ml of 6M phosphoric acid), 2 ml of acetic acid and 2 ml of supernatant was boiled on water bath for 1 hour to develop colour,

reaction was stopped by placing the tubes in ice bath, shaken the content vigorously with 4 ml of toluene which led to the formation of two phases. Coloured non aqueous phase from aqueous phase was separated and its absorbance was read at 520 nm. Proline content was determined from standard curve (0.04 – 0.2 μ mole).

Total antioxidant activity

Extraction

Total antioxidant activity was estimated by the method of Prieto *et al.* (1999) ^[15] where sample material of 1 g was homogenised in 10 ml of 95 % methanol, transferred the contents into 150 ml conical flasks, sealed with parafilm, kept on shaker for 1 hour, centrifuged the contents at 10,000 rpm for 20 min. and supernatant used for estimation.

Procedure

Transferred 0.1 ml of supernatant in which 1 ml of phosphomolybdate reagent was added into polycarbon capped tubes, incubated the content on water bath at 95 °C for 90 min. cooled the content to room temperature and absorbance was read against blank at 695 nm. Total antioxidant activity was estimated from standard curve of ascorbic acid (10 – 100 μ g) and expressed in terms of ascorbic acid equivalent.

Results

Proline

As shown in Fig. 1 (A and B), proline content increased under drought stress in leaves and developing grains of both wheat varieties. Proline content varied from 33.00 to 42.50 and 34.00 to 53.00 μ g g⁻¹ f. wt. from 7th to 28th days after anthesis in leaves of WH 1105 under irrigated and drought stress conditions respectively (Fig. 1A). The per cent increase varied from 3.03 to 24.71 from 7th to 28th days after anthesis. In leaves of WH 1025 the proline content varied from 36.00 to 42.00 and 43.00 to 90.00 μ g g⁻¹ f. wt. from 7th to 28th days after anthesis under irrigated and drought stress conditions respectively. The per cent increase varied from 19.44 to 125.00 from 7th to 28th days after anthesis.

Similarly in developing grains of WH 1105, proline content varied from 30.00 to 40.00 and 34.00 to 49.00 μ g g⁻¹ f. wt. from 7th to 28th days after anthesis grown under irrigated and drought stress condition respectively indicating that proline content increased under drought stress over irrigated condition. The per cent increase varied from 13.33 to 22.50 from 7th to 28th days after anthesis. Proline content was increased by 1.68 fold at 28th day after anthesis in WH 1105 under drought stress condition. In developing grains of WH 1025, the proline content varied from 33.00 to 46.25 and 42.00 to 80.00 μ g g⁻¹ f. wt. from 7th to 28th days after anthesis grown under irrigated and drought stress conditions respectively (Fig. 1 B). The per cent increase varied from 27.27 to 72.97 from 7th to 28th days after anthesis with 2.67 fold at 28th day after anthesis in WH 1025 under drought stress condition.

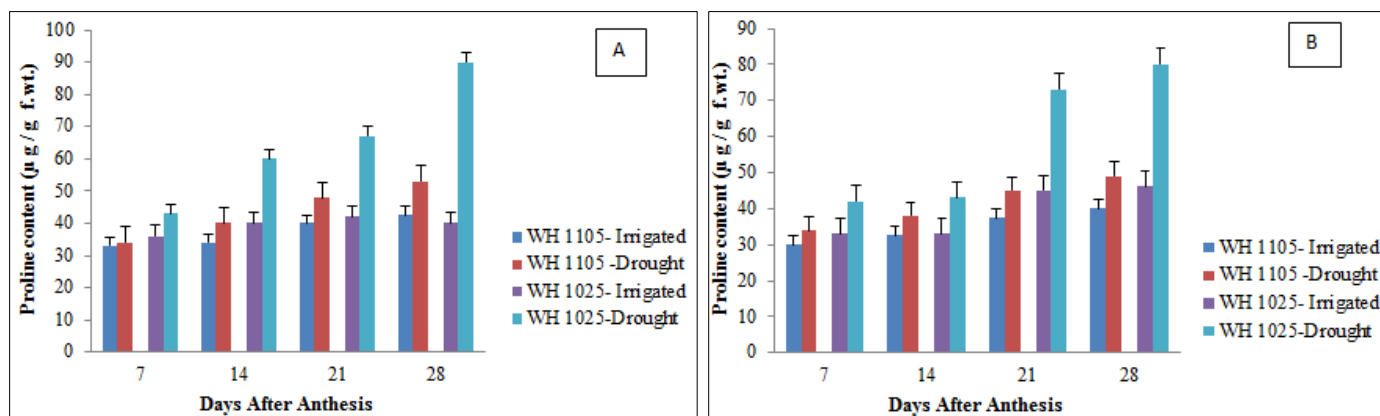


Fig 1: Effect of drought stress on proline content in leaves (A) and developing grains (B) of wheat at different days after anthesis

Hydrogen peroxide

Fig. 2 (A) shows that H_2O_2 content was significantly higher in leaves of WH 1105 than WH 1025 under drought stress condition compared to irrigated condition at different stages of grain development. In WH 1105, H_2O_2 content ranged from 165 to 229 and 194 to 330 $\mu\text{mole g}^{-1}$ f. wt. from 7th to 28th days after anthesis under irrigated and drought stress conditions. The per cent increase in H_2O_2 content ranged from 17.58 to 44.10 from 7th to 28th days after anthesis with 2.50

fold enhancement. Similarly in WH 1025, H_2O_2 content ranged from 146 to 172 and 163 to 218 $\mu\text{mole g}^{-1}$ f. wt. from 7th to 28th days after anthesis under irrigated and drought stress conditions respectively. The per cent increase in H_2O_2 content ranged from 11.64 to 26.74 from 7th to 28th days after anthesis. Though the magnitude of increase was higher in both wheat varieties under drought stress, yet H_2O_2 level in leaves of WH 1025 was significantly lower as compared to WH 1105.

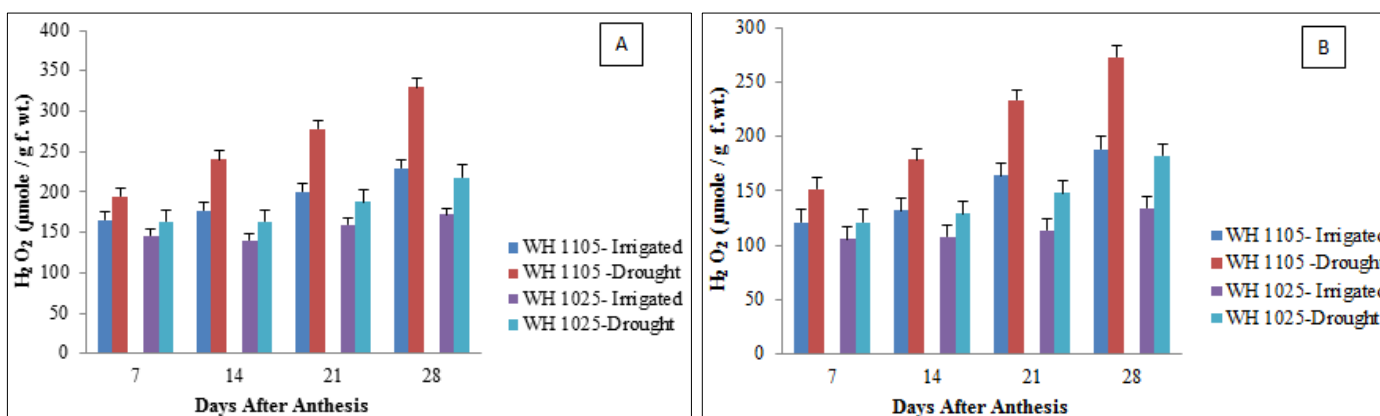


Fig 2: Effect of drought stress on H_2O_2 content in leaves (A) and developing grains (B) of wheat at different days after anthesis

Fig. 2 (B) shows that H_2O_2 content was significantly higher in developing grains of WH 1105 than WH 1025 grown under drought stress condition. In WH 1105, H_2O_2 content ranged from 121 to 188 and 152 to 273 $\mu\text{mole g}^{-1}$ f. wt. from 7th to 28th days after anthesis under irrigated and drought stress conditions respectively. The per cent increase in H_2O_2 content ranged from a 25.62 to 45.21 at 7th to 28th days after anthesis with 1.76 fold enhancement. Similarly in WH 1025, H_2O_2 content ranged from 106 to 134 and 121 to 182 $\mu\text{mole g}^{-1}$ f. wt. from 7th to 28th days after anthesis under irrigated and drought stress conditions respectively. The per cent increase in H_2O_2 content ranged from 14.15 to 35.82 from 7th to 28th days after anthesis with 2.53 fold enhancement at 28th days after anthesis. Though the magnitude of increase was higher in both wheat varieties under drought stress yet H_2O_2 level in

developing grains of WH 1025 was significantly lower as compared to WH 1105.

Total antioxidant activity

As depicted in Fig. 3 (A), total antioxidant activity in leaves of WH 1105 and WH 1025 varieties increased under drought stress compared to irrigated control. In WH 1105 the per cent increase was maximum (28.30) at 28th day after anthesis and was minimum (16.33) at 7th day after anthesis. While in WH 1025 the per cent increase was maximum (76.92) at 28th day after anthesis and was minimum (18.52) at 7th day after anthesis. Though the increase was higher in both wheat varieties under drought stress yet the level of total antioxidant activity was significantly higher in leaves of WH 1025.

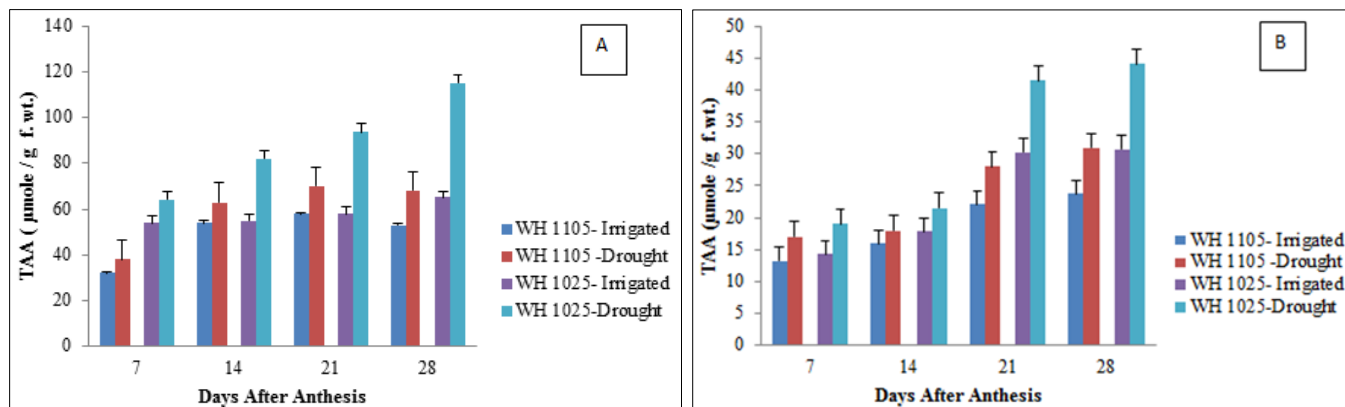


Fig 3: Effect of drought stress on total antioxidant activity in leaves (A) and developing grains (B) of wheat at different days after anthesis

Similarly results presented in Fig. 3 (B) shows that total antioxidant activity in developing grains of WH 1105 and WH 1025 varieties increased under drought stress compared to irrigated condition. The per cent increase was maximum (29.86) at 28th day after anthesis and was minimum (12.50) at 14th day after anthesis. While in WH 1025, the per cent increase was maximum (43.18) at 28th day after anthesis and was minimum (20.46) at 14th day after anthesis. Though the increase was higher in both wheat varieties under drought stress, yet the level of total antioxidant activity was significantly higher in developing grains of WH 1025.

Discussion

Proline is perhaps the most widely distributed compatible osmolyte and there is a strong correlation between increased cellular proline levels and capacity to survive both water deficit and salt stress (Asish *et al.* 2008) [1]. Proline has been reported to accumulate in higher plants up to 80 per cent of total amino acid pool under drought and salt stress as compared to mere 5 per cent under normal condition in various crop species including groundnut (Jehan *et al.* 2012) [9]. The results presented in Fig. 1 demonstrates that drought stress results an increase in proline content in leaves and developing grains at different DAA in both the wheat varieties. However, the increase in proline content was higher in WH 1025 than WH 1105. The higher levels of proline in WH 1025 could be ascribed to its improved drought resistance. The results are in agreement with the previous studies on wheat seedlings grown under drought stress (Chunmei *et al.* 2011) [6]. Similarly, Shamsi (2010) [18] reported an increase in proline content with the increase in intensity of drought stress on wheat cultivars. Similar results are also obtained by Gobinathan *et al.* (2009) [7] who observed an increase in proline accumulation in *Pennisetum* seedlings under salinity. The results are also in agreement with the previous studies on Okra where increased proline content in roots at all growth stages in all the varieties under drought stress has been reported (Beemarao *et al.* 2007) [3].

The role of H₂O₂ in stress induced damage has long been recognized but it is also accepted that H₂O₂ is an integral component of cell signaling cascades (Mittler, 2002; Vranova *et al.* 2002) [12, 21] and an indispensable second messenger in biotic and abiotic stress situations (Green & Fluhr, 1995; Pastori & Foyer, 2002) [8, 14]. The results of the present study showed that H₂O₂ content substantially increased in both wheat varieties under drought stress as compared to irrigated condition in leaves and developing grains at different DAA (Fig. 2). WH 1105 variety accumulated higher amount of

H₂O₂ than WH 1025 which shows its susceptible nature towards drought. Significant increase in H₂O₂ in winter wheat during progressive soil drying has also been reported by Zhen *et al.* (2008) [23]. Similar results have also been reported by previous workers (Chai *et al.* 2005; Celina *et al.* 2005; Zlatev & Yordanov, 2004). Sairam & Srivastava, (2002) [5, 4, 23, 17] also showed higher H₂O₂ content in salinity stress sensitive wheat cultivar HD 2687 under long term salt stress.

In response to various environmental stresses, plants produce protective cellular compounds. The capacity of the cellular antioxidative and photoprotective defence is determined by the pool size of antioxidants and protective pigments (Karin *et al.* 2002) [10]. The results of the present study indicated that total antioxidant activity increased in leaves and developing grains of wheat varieties under drought stress with higher and significant increase in WH 1025 than WH 1105 at different developmental stages (Fig.3). WH 1025 showed higher antioxidant capacity under drought suggesting its tolerance behaviour. The results of the present study are in accordance with the results of Usha & Bhumika (2012) [20] who reported that the overall total antioxidant activity of wheat varieties showed an initial increase which was still high even after nine days of stress in tolerant varieties. Ranjeet *et al.* (2013) [16] also reported maximum total antioxidant activity during milky dough and seed hardening stages in stressed wheat. Total antioxidant activity was very low in susceptible cultivar as compared to tolerant at different stages of growth. Similar results were also obtained by Mohammed & Tarpley, (2009) [13].

Conclusions

Drought stress resulted in accumulation of free proline and H₂O₂ in both leaves and developing grains. In both varieties maximum accumulation was observed at 28st day after anthesis in leaves and developing grains.

Total antioxidant activity gradually increased under drought stress with more increase was observed in WH 1025 than WH 1105. The peak value was observed at 28th day after anthesis under drought stress in WH 1025.

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