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Short Communications

Kinetics of proline accumulation in response to various stress regimes followed by recovery in different cultivars of wheat

Running Head

Proline accumulation as a biochemical stress injury indicator

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Abstract

Various abiotic stress factors provoke protein denaturation and oxidative stress in plants, which might lead to the production of certain osmolytes including different amino acids. Amino acids act as precursors and constituents of proteins playing a paramount role in plant metabolism and development. Proline amino acid is among the chief organic osmolyte accumulating in different plant species in response to stress. Although, its definite role in plant tolerance remain debatable, it is thought to have a positive effect on cell integrity along with adaptive function in mediating osmotic adjustment in plants during stress conditions. In this study, the changes in proline accumulation in response to different stress regimes (6h, 24h and 48 h) followed by re-watering were investigated in the embryo and endosperms of different wheat cultivars. The results indicated an enhancement of proline accumulation at moderate (24h) and severe (48h) stress conditions in a genotype independent manner. Based upon our results, biochemical significance of the proline accumulating during different stress intensities and recovery is discussed.

Key words: drought stress, proline, relieval, re-watering, Triticum aestivum

Introduction

Several abiotic environmental factors (salinity, drought, extremes of temperature and nutrient imbalances) are among the primary constraints to crop productivity that adversely upset yield performance, plant growth and development of a crop [1]. Different types of abiotic stress factors produce an upsurge in reactive oxygen species (ROS), thereby ensuing an oxidative stress condition in the cell due to loss of oxidant /antioxidant balance. Plants retort to stress through modifications in gene expression which lead to similar adaptive responses in the cell like the accumulation of compatible solutes, stimulation of stress proteins, and hastening up of reactive oxygen species scavenging systems [15]. It has been reported previously that under wide variety of stress conditions, plant cells achieve their osmotic adjustment by the active accumulation of some kind of compatible organic solutes to maintain both turgor and the driving gradient for water uptake and thus is claimed to be an effective stress tolerance mechanism [12]. These compatible organic solutes are highly soluble, low molecular weight osmo-protectants that are ordinarily non-toxic for the cells at high concentrations. Generally, they safeguard plants from stress by

contributing in multiple ways like: the detoxification of reactive oxygen species, cellular osmotic adjustment, protection and stabilization of cellular integrity [3]. These organic solutes include sucrose, polyols, proline, trehalose and certain quaternary ammonium compounds (QACs) [1]. Out of these, proline is considered to be the most important non-enzymatic antioxidant metabolite functioning both as a redox-buffering agent and an osmo-protectant under stress conditions due to its ROS-scavenging capability [10].

Wheat is one of the most important food crops in arid and semi-arid areas worldwide and is also sensitive to drought stress. Consequently, understanding the biochemical and physiological responses of some of the cultivated wheat plants to drought stress is helpful in selecting cultivars most adaptable and tolerant to these climates. Therefore, proline being considered as one of the potential biochemical responses to stress was evaluated in four different wheat cultivars since some aspects of its biological functions are still unclear. In the present study, endosperms and embryos were extracted from four wheat cultivars at various drought intensities (6h, 24h, 48h) followed by relieval of stress after 48h of drought stress. This study will provide documentation regarding the crucial biochemical indicator of stress, thus giving a helping hand to the plant breeding programs to improve the crop productivity by engineering of proline metabolism which could lead to stress tolerance in plants.

Materials and Methods

Seed germination and growth conditions

Seeds of commercially relevant lines of four wheat (*Triticum aestivum* L.) cultivars (HD 2851, HD 2967, PBW 550 and HD 2932) were selected for experimental purposes as all are locally grown and a comparison of their responses could give a better understanding of the susceptibility/tolerance of the different varieties to drought. Seeds were surface sterilized with 1% (w/v) mercuric chloride followed by 70 % (v/v) ethanol [11]. Seeds were thoroughly rinsed with deionized water and imbibed for 6h. After imbibition, seeds were placed in petri plates containing sterile filter sheets, moistened with water. The plates having seeds were incubated at 25 ± 1°C in a seed germinator and allowed to grow for 48h. Stress was imposed by withholding water supply and tissues (embryo and endosperm) were harvested after 6h, 24h, and 48h. After 48h of drought stress treatment, stress was relieved by re-watering the tissues and samples were collected after 48h of re-watering.

Estimation of proline content:

The proline content was determined by measuring the intensity of the red coloured formazone at 520 nm in a spectrophotometer according to [2]. Selective extraction with sulpho-salicyclic acid results in proline extraction which is made to react with acid ninhydrin in acidic medium. Acid ninhydrin was prepared by warming 1.25 g ninhydrin in 30 ml glacial acetic acid and 20 ml of 6M ortho-phosphoric acid with agitation until dissolved at 4^{o}C (reagent remains stable for 24 h). For the estimation of proline content, 400 mg endosperm and 250 mg embryo tissue was ground in 5 ml of 3% sulphosalicyclic acid followed by centrifugation at 3000 rpm for 10 min. The supernatant (2 ml) was transferred to the test tubes and to it 2 ml each of 6M ortho-phosphoric acid, acid ninhydrin, glacial acetic acid were added in same sequence. Then, the test tubes were kept in water bath (100^{o}C) for 1 h. After 1 h incubation in water bath, reaction was terminated by keeping these test tubes on ice for 10-15 min. After that, 4ml toluene was added in test tubes and the test tubes were mixed vigorously. The upper phase chromophore containing toluene was taken out gently and O.D was measured at 520 nm. The amount of proline

was calculated from the graph of a standard curve prepared using different known concentrations of proline using the formula

$$= \frac{(\mu g \text{ proline/ml}) \times \text{ml toluene}}{115.5} \times \frac{5 \text{ sample}}{g}$$

Results and Discussion:

In embryos and endosperms of all the four cultivars, there was a significant increase in the proline content under moderate (24h) and severe (48h) drought stress conditions as compared to control (0h) irrespective of the genotype. Proline, being an indispensable organic solute is known to be present widely in higher plants and accumulates in great quantities in response to harsh environmental cues [6], [9]. Proline accumulation in different plant species is generally associated with stress tolerance, with its content being much higher in stress-tolerant plants as compared to the stress-sensitive plants. Despite the existence of a strong positive correlation between proline accumulation and stress tolerance, this relationship may not be universal since it is deemed to be an indicator of stress injury rather than stress tolerance [16]. Concomitant to our findings, assessment of proline accumulation in leaves of two sorghum genotypes contrasting in salt tolerance suggested that proline accumulation was a reaction to salt stress and not a plant response associated with tolerance [4]. Increased proline content has been reported in different crop species like *Medicago sativa*, wheat and Arabidopsis [13], [7], [8] Besides being implicated as an osmoregulator for osmotic adjustment during dehydration, proline contributes to the stabilization of membranes and proteins, buffering cellular redox potential and scavenging free radicals under stress conditions. In addition, proline might function as a protein compatible hydrotrope assuaging acidosis in the cytoplasm, and sustaining proper $NADP^+$ / NADPH ratios compatible with metabolism [14], [5]. Upon relieval of stress after 48 h, there was a significant decrease in proline content in all the cultivars in both the tissues. The catabolism of proline upon relieval of stress may provide adequate reducing equivalents that support oxidative phosphorylation and ATP generation in mitochondria for repairing the stress-induced damages [5].

Conclusions

This study highlighted the importance of proline as a biochemical indicator of stress response in wheat irrespective of its genotype. Further studies are needed to determine whether the relationship between stress tolerance and accumulation of proline is genotype-specific or if it is simply a response towards the unfavourite stress effects. Keeping in view the potential of proline as a ROS scavenger, it may become a useful tool to counter the adverse effect of stressful environments thereby decreasing annual losses to agriculture. This is crucial information since the capacity to overcome and recover from water stress would aid in ascertaining adaptive response for planting wheat in areas facing changing climatic conditions.

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Legend to the figure:

Fig. 1: Proline content in embryo (A, B) and endosperm (C, D) of four wheat cultivars under control and different drought stress treatment. 0h- 0 hour drought, 6h-6hour drought, 24h-24 hour drought, 48h- 48 hour drought, 48h PS-Post Stress. Data shown are average ± SE (n=3). ^{d} indicates significant difference between C (0h) v/s 6h,24h and 48 h drought at P≤0.05. ^{e} indicates significant difference between 48h drought v/s 48 h PS at P ≤ 0.05.

Fig.1