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Effect of salt stress on proline metabolism in two high yielding genotypes of green gram

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Abstract

The effect of salt stress was studied on free proline accumulation, activities of pyrroline-5-carboxylate reductase (P-5-CR), proline oxidase and γ-glutamyl kinase, glycinebetaine levels and chlorophyll contents in two cultivars of green gram (T-44 and SML-32, salt tolerant and salt sensitive, respectively) under the conditions of absence as well as in the presence of various levels of salinity. Salt stress resulted in a significant accumulation of free proline in shoots of both the cultivars of green gram. The magnitude of increase in free proline accumulation was higher in the tolerant cultivar than in the sensitive cultivar. Salt stress in an accumulation of proline by 5.0 folds in T-44 at 200 mM NaCl (maximum salt stress) and 1.1-fold only in SML-32 at 50 mM NaCl (maximum salt stress). Pyrroline-5-carboxylate reductase and γ-glutamyl kinase activities increased in both the cultivars and the magnitude of increase was more in T-44 than in SML-32. In addition, the proline oxidase activity was inhibited under salt stress in both cultivars. Nevertheless, the reduction in the activity was more in T-44 than in SML-32. The results suggested that during salt stress, proline metabolism was significantly altered and the extent of alteration varied between the cultivars T-44 and SML-32, leading to the maintenance of the turgor by accumulating higher levels of free proline in T-44, supporting its salt tolerance. Further, salt tolerance of T-44 was evident from the higher level of glycinebetaine (GB) compared with SML-32 during salt stress. Furthermore, the salt tolerance of T-44 was indicated by lower amounts of chlorophyll degradation. The physiological significance of recorded changes are analyzed in relation to the function of these enzymes in plant metabolism under salt stress.

Keywords: Chlorophyll contents; Glycinebetaine; Osmotic potential; *Phaseolus aureus*; Proline; Salinity

1. Introduction

Salinity affects many aspects of plant metabolism and the accumulation of various organic solutes that contribute to turgor maintenance. Among them, the accumulation of low molecular weight solutes, compatible osmotytes, such as proline and glycinebetaine are thought to function as osmoprotectants [1,2]. In presence of low water potentials, the accumulation of compatible osmolytes, involved in osmoregulation allows additional water to be taken up from the environment, thus buffering the immediate effect of water shortage within the plant. Therefore, high level of proline and glycinebetaine enabled the plant to maintain low water potentials. Besides this, proline also serves as a sink for energy to regulate redox potentials [3,4], as a hydroxy radical scavenger [5], as a solute that protects macromolecules against denaturation [5], reducing the acidity in the cell [6] and acts as storage compound and nitrogen source for rapid growth after stress [7]. It has been reported that proline alleviates NaCl stress induced enhancement in oxygenase and carboxylase activities of Rubisco [8]. Recently, it has been also reported that proline accumulation protects plants against free radical induced damage by quenching of singlet oxygen [9].

Proline accumulation is one of the adaptations of plants to salinity and water deficit [10,11]. It has also been widely advocated that proline accumulation uses as parameter of selection for salt stress tolerance [12]. However, proline

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accumulation cannot be regarded as marker for salt tolerance, as it accumulates under various condition of stresses such as temperature, drought, and starvation [13], whereas in many salt stressed plants its levels decreases [14,15]. Some authors did not observe any appreciable increase in free proline content [16,17] whilst others consider enhanced proline level merely a stress effect, rather than a cause of stress to tolerance [18]. Recently, it has been reported that proline accumulation appears to be a reaction of salt stress damage and not a plant response associated with salt tolerance [19]. Hence, the role of proline accumulation and its metabolism vis-à-vis tolerance to salinity needs to be critically examined.

A quaternary ammonium compound, glycinebetaine acts as osmotic solutes. It has been reported that glycinebetaine is increased in many species under salinity stress [20]. Subcellular compartmentation of glycinebetaine biosynthesis in rice is important for increased salt tolerance [21]. NaCl stress increased the level of glycinebetaine in peanut cotyledons and embryonic axis [22]. Different approaches have been used to genetically engineered plants for enhancing stress tolerance in plants by manipulating the level of compatible solute glycinebetaine [23].

Two enzymes of proline metabolism viz., γ-glutamyl kinase and γ-glutamyl phosphate reductase are regarded as an enzyme complex called P-5-C synthetase because it catalyzes and product glutamine γ-semialdehyde (GSA) is non-enzymatically converted to pyrroline-5-carboxylate. So that, the regulation of proline biosynthesis is mainly controlled by the activity of P-5-C synthase [24]. The enzyme proline oxidase also influences the level of proline accumulation as it degrades proline to glutamate. In presence of salinity the activity of proline oxidase was reduced in vitro in a NaCl tolerant *Brassica juncea* [25]. Such reduction in proline oxidase activity and concomitant increase in proline accumulation also found in presence of low temperature and drought stress [26]. Proline synthesis from glutamate and its utilization for protein synthesis was decreased by 50% in barley under salinity stress [27].

The higher accumulation of proline could be due to enhanced activities of ornithine aminotransferase (OAT) and pyrroline-5-carboxylate reductase (P-5-CR), the enzyme involved in proline biosynthesis [28] as well as due to inhibition of proline oxidase and proline dehydrogenase (PDH), proline catabolizing enzymes [29]. Studying the effects of salt stress on enzyme activities involved in proline metabolism could provide valuable information on the physiological significance of its accumulation. Indeed, proline biosynthesis occupies a central crossroad between carbon and nitrogen assimilation pathways. The present study is based on the proline accumulation and proline metabolism in response to NaCl in green gram cultivars in presence of various levels of salinity.

Green gram (*Phaseolus aureus*), an important pulse crop of India is predominantly salt sensitive crop. As the variability in salt sensitivities/tolerances are encountered in different plant species and even in different cultivar varieties of same species. Realizing the importance of the problem, we have initiated a thorough study of salt tolerance aspects in green gram. In this context, our earlier report point to the salt tolerance of T-44, which was associated with low levels of Na^+ and high levels of K^+ accumulation and high water content while just reverse was found in salt sensitive cultivar SML-32 during salt stress conditions [30]. In order to gain further insight into the stress tolerance aspects of green gram, in this paper, we are reporting comparative analysis of tolerance potentials based on osmolyte accumulation and metabolism of proline in two cultivars of green gram differing in salt tolerance.

2. Material and methods

2.1. Plant material and stress treatments

Seeds of green gram (*P. aureus*, Family *Leguminosae*) cultivar namely, T-44 (salt tolerant) and SML-32 (salt sensitive) were surface sterilized with 1% sodium hypochlorite and germinated as described by Misra and Dwivedi [30]. Three concentrations of NaCl namely, 50, 100 and 200 mM, were used for tolerant cultivar T-44 while three concentrations of NaCl namely, 1, 10 and 50 mM were used for sensitive cultivar SML-32. Starting with 4 h soaked seeds (zero hour of seed germination) the germinated seeds were taken out at 24 h intervals up to 5 days, root and shoot (along with cotyledons) were separated from the seeds. The experiments were repeated thrice with three replicates of each treatment.

2.2. Osmotic potential

The osmotic potentials of leaf samples removed from both the cultivars on day 5th of plant growth and was determined following their freezing and thawing with a vapor pressure osmometer 5500 VIESCOR (USA).

The osmotic potential of the solutes was calculated from the van't Hoff equation, $\pi = cRT$, where c is concentration in mol/l, and RT is approximately 2.5 MPa/l/mol.

2.3. Solute determination

2.3.1. Proline

Proline accumulation was determined as described by Bates et al. [31] with slight modification. Powdered frozen (−70 °C) tissue (50 mg) both root and shoot were weighed into 1.5 ml centrifuge tubes and then added 3% 1.2 ml of sulphosalicylic acid to precipitate protein. Samples were mixed, centrifuged at 18,000 × g for 10 min, and the filtrate transferred to a fresh 1.5 ml tube. Total 500 μl of filtrate and made up 1 ml with distilled water. Added 1 ml glacial acetic acid and 1 ml ninhydrin reagent [3% (w/v) ninhydrin in 60% (v/v) 6 M phosphoric acid] for 1 h at 90 °C. After cooling of