EFFECT OF WATER STRESS ON PROLINE METABOLISM AND LEAF RELATIVE WATER CONTENT IN TWO HIGH YIELDING GENOTYPES OF GROUNDNUT (Arachis hypogaea L.) WITH CONTRASTING DROUGHT TOLERANCE

Ranganayakulu G S1,* , Chinta Sudhakar2 and Sivakumar Reddy P3

1Department of Botany, Rayalaseema University, Kurnool-518002, India
2Department of Botany, Sri Krishnadevaraya University, Anantapur- 515 003, India
3Department of Botany Yogi Vemana University, Kadapa-516003, India

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ABSTRACT

A comparative study was carried out for two groundnut cultivars (cv K-134 and cv JL-24, drought tolerant and drought sensitive, respectively) during water stress at different soil moisture levels [100 (control), 75, 50 and 25%]. The free proline content, activities of pyrroline-5-carboxylate reductase (P-5-CR), proline oxidase, proline dehydrogenase (PDH) along with levels of quaternary ammonium compounds, leaf relative water content and chlorophyll stability were investigated. Water stress resulted in a significant accumulation of free proline content in leaves of both groundnut cultivars. However cv K-134 accumulated relatively higher amounts of proline than cv JL-24. Water stress resulted 2.5 fold higher accumulation of proline in cv K-134 and 2.0 fold in cv JL-24 on day-5 at 25% SMLs. Furthermore, the tolerant cv K-134 shows a greater activity of pyrroline-5-carboxylate reductase and lesser inhibition of proline oxidase and proline dehydrogenase than susceptible cv JL-24. The greater proline levels were due to both the higher rates of proline synthesis and lower rate of proline oxidation in cv K-134 compared to cv JL-24. Results of this study indicated that water stress altered the proline metabolism and this alternation was significantly varied between the cultivars. Further, drought tolerance of cv K-134 can be justified by the higher accumulation of quaternary ammonium compounds (glycine betaine) which helps in the maintenance of leaf relative water content and higher chlorophyll stability during water stress as compared to cv JL-24.

Abbreviations: SMLs: soil moisture levels; P-5-CR: pyrroline-5-carboxylate reductase; PDH: proline dehydrogenase; QACs: quaternary ammonium compounds; CSI: chlorophyll stability index; RWC: relative water content; DCPIP: dichloro phenol indophenol; EDTA: ethylene diamine tetra acetic acid.

* Corresponding author
E-mail: gsranaganayakulu@gmail.com (Ranganayakulu GS)

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1 Introduction

Plant growth and development as well as crop production are highly influenced and sometimes limited by environmental conditions, such as drought, salinity and temperature stresses. Among these, drought stress is the most important environmental constrains to world agricultural production (Bray et al., 2000). In response to various environmental stresses, plants have developed different physiological and biochemical mechanisms to adapt or tolerate stress (Rahnama & Ebrahimzadeh, 2005; Faical et al., 2009). Osmoregulation is one of the important biochemical phenomena in plants to cope up adverse environmental conditions. Osmotic adjustment in plants subjected to drought stress occurs by the accumulation of high concentrations of osmotically active compounds known as osmolytes such as proline, glycine betaine, soluble sugars, polyamines etc., in order to lower the osmotic potential (Rontlein et al., 2002; Jouve et al., 2004).

Increased levels of proline contribute to the maintenance of turgor cells and its accumulation is considered as a stress indicator in plants under drought stress (Sairam & Tyagi 2004; Jagesh et al., 2010). Increased levels of proline in stressed plants is due to the higher expressions of Pyrroline-5-Carboxylate reductase (P-5-CR) and ornithine-δ-aminotransferase (OAT), these enzymes are involved in proline biosynthesis (Kohl et al., 1990). The aim of the present investigation was to study the level of proline content and its metabolizing enzyme activities with response to water stress in two groundnut cultivars showing differential drought resistance.

Groundnut (Arachis hypogaea L.) is a staple food for many tropical and sub-tropical countries, providing a valuable source of edible oil, proteins, fats, energy and minerals. Traditionally it is grown under rain-fed conditions. Major groundnut producers in the world are: China, India, Nigeria, USA, Indonesia and Sudan. Among these India is one of the leading countries in groundnut production and cultivation in the world. Andhra Pradesh occupies the first place in India in groundnut cultivation, with 1.88 million hectares and the production of about 1.2 million tones (Reddy et al., 2003). Considerable research has been under taken on the physiological and molecular mechanisms involved in drought adaptation (Ishitani et al., 1996). However, there is still no comprehensive standard system for measuring drought resistance of plants. Therefore in the present investigation we screen two predominantly cultivated groundnut cultivars for their drought tolerance based on their osmolyte accumulation and proline metabolizing enzymes.

2 Materials and Methods

2.1 Plant material and water stress treatments

Seeds of groundnut (Arachis hypogaea L.) cultivars namely K-134 and JL-24 were procured from Andhra Pradesh Agricultural Experimental Station Kadiri, Anantapur district. Seeds were surface sterilized with 0.1 % (w/v) sodium hypochlorite solution for 5 min, thoroughly rinsed with distilled water and then germinated in plastic pots containing 2 kg of soil and sand (2:1) mixture and allowed to grow for sixteen days. Total twelve pots were maintained in the departmental botanical garden under natural photoperiod of 10-12 h and temperature 28 ± 4°C. Sixteen-day-old plants were then divided into four sets and three replicates arranged in randomized complete black design. One set of pots received water daily to field capacity and served as control (100 %) and other three sets of pots were exposed to water stress, it was induced by adding of water daily to 75, 50 and 25 % soil moisture levels respectively. Soil moisture level was calculated by gravimetric method.

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\text{% soil moisture} = \frac{\text{Water present in the soil medium}}{\text{Maximum water the soil can hold}} \times 100
\]

Finally leaf samples were collected on day-3 and day-5 after stress induction for analysis of various parameters.

2.2 Proline enzyme extractions and assays

Free proline content was extracted from both control and stressed leaf samples in 3% aqueous sulfosalicylic acid and estimated by using ninhydrin reagent (Bates et al., 1973). Pyrroline-5-carboxylate reductase enzyme was extracted in 100 mM sodium phosphate buffer (pH 7.4) and measures the enzyme inhibition by spectrophotometrically at 340 nm according to Rena & Splittstoesser (1975). Activity of Proline oxidase was assayed by following dichlorophenolindophenol (DCPIP) reduction in mitochondrial preparation in 1 mL reaction medium containing 50 mM DCPIP and 100 mM proline according to the method of Huang & Cavalieri (1979). Proline dehydrogenase was extracted in 100 mM phosphate buffer (pH 8.0) containing 100 mM Na₂CO₃-NaHCO₃ buffer (pH 10.3), 20 mM L-proline and 10 mM NAD⁺, according to the method of Rena & Splittstoesser (1975). Glycine betaines were extracted and estimated as Quaternary ammonium compounds from 0.5 g of both control and stressed leaf samples according to Grieve & Grattan (1983) using KI-I₂ reagent.

2.3 Relative water content

Relative water content (RWC) of leaf discs were measured in both control and stressed plants according to Barrs &Weatherley (1968). Leaf material was collected from control (100 %) and stressed plants (75, 50 and 25 % SMLs) and leaf discs of 1 cm diameter prepared.
Effect of water stress on proline metabolism and leaf relative water content in two high yielding genotypes of Groundnut (*Arachis hypogaea L.*) with...

Figure 1: Free proline content in leaves of control and water stressed groundnut cultivars (cv K-134 and cv JL-24). Values are means from five replications. Vertical bars indicate ±S.D.

Figure 2: Activity of pyrroline-5-carboxylate reductase (0.001 OD = 1U) in leaves of control and water stressed groundnut cultivars (cv K-134 and cv JL-24). Values are means from five replications. Vertical bars indicate ±S.D.

Figure 3: Proline oxidase activity in leaves of control and water stressed groundnut cultivars (cv K-134 and cv JL-24). Values are means from five replications. Vertical bars indicate ±S.D.

Figure 4: Proline dehydrogenase activity in leaves of control and water stressed groundnut cultivars (cv K-134 and cv JL-24). Values are means from five replications. Vertical bars indicate ±S.D.

Figure 5: Levels of quaternary ammonium compounds (glycine betaine equivalents) in leaves of control and water stressed groundnut cultivars (cv K-134 and cv JL-24). Values are mean from five replications. Vertical bars indicate ±S.D.

Figure 6: Leaf relative water content (%) in leaves of stressed groundnut cultivars (cv K-134 and cv JL-24). Values are mean from five replications. Vertical bars indicate ±S.D.
Fresh weight of five leaf discs, in three replicates, was recorded. The leaf discs were floated in 10 mL of distilled water for 6 h and allowed to gain turgidity. Then turgid weights were recorded and dried in oven at 80°C for 24 h and dry weight of the samples was recorded. RWC was quantified and expressed in percent using the following formula.

\[
\text{RWC} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \times 100
\]

2.4 Chlorophyll Stability Index

Chlorophyll stability index was measured in the leaves of both control and water stressed groundnut plants. Chlorophyll stability was expressed as the amount of chlorophyll estimated by the method Arnon (1949).

2.5 Statistical analysis

The data were analyzed statistically using Duncan’s multiple range (DMR) test to drive significance (Duncan, 1955).

3 Results

3.1 Free proline content

Result of the estimation of free proline content in both control and drought stressed plants on day-3 and day-5 data were presented in Figure 1. Accumulation of free proline content was increased significantly in both groundnut cultivars with increasing stress over the controls. However the percent increase was found higher in tolerant cv K-134 than susceptible cv JL-24, there was by about 3.0 fold in cv K-134 and 2.2 fold in JL-24 at 25% soil moisture level when compared to controls.

3.2 Pyrroline-5-carboxylate reductase (P-5-CR)

The activity of pyrroline-5-carboxylate reductase was assayed in the leaves of both control and stressed plants and the results are presented in Figure 2. The activity of pyrroline-5-carboxylate reductase was increased significantly with increasing stress in both cultivars. Moreover, the percent of increase in P-5-C reductase activity was more in tolerant cv. K-134 than susceptible cv. JL-24 at severe stress level on day-5.

3.3 Proline oxidase

Figure 3 represented the inhibition of proline oxidase enzyme activity in both cultivars during all stress regimes (Figure 3). However, the degree of inhibition was more in susceptible cv JL-24 than tolerant cv K-134.

3.4 Proline dehydrogenase (PDH)

Proline dehydrogenase activity was decreased significantly with increasing stress severity and duration in both cultivars over controls (Figure 4). However the percent decrease was found to be more (45.56%) in susceptible cv JL-24, where as in drought tolerant cv K-134 was found to be less (34.09%) on day-5 stressed samples at 25% SMLs when compared to their respective controls.

3.5 Glycine betaine

The pool size of glycine betaine contents were increased with increasing stress severity and duration (Figure 5). However, the magnitude of increase was more in tolerant cv K-134 than susceptible cv JL-24. The amount of glycine betaine content was increased by 8.0 fold in cv K-134 and 6.0 fold in cv JL-24 on day-5 at severe stress level when compared to respective controls.

3.6 Relative water content (RWC)

Stress tolerance in terms of the leaf relative water content (RWC) was measured in both groundnut cultivars, at different soil moisture levels and results were depicted in Figure 6. From the figure, the leaf relative water content was found to be decreased in both cultivars during all stress regimes over the controls. However, the percent decrease was comparatively more in cv JL-24 than cv K-134. It was also clear from the results that tolerant cv K-134 maintained relatively higher leaf water content than susceptible cv JL-24 at all stress levels.

3.7 Chlorophyll Stability Index

Chlorophyll stability indices were measured in control and stressed leaves of both groundnut cultivars and results were depicted in Figure 7. Chlorophyll stability indices were decreased in both cultivars with increasing stress severity and duration. However the degree of reduction was more in susceptible cv JL-24 than tolerant cv K-134.

Discussion

The major emphasis of the present investigation was to assessing the drought tolerant nature in two locally important groundnut cultivars. Plants are frequently exposed to unfavorable environmental conditions due to their sessile nature. To survive, plants have developed a number of physiological and biochemical adaptations (Bartels & Salaminii, 2001). One of the mechanisms that plants use to combat the detrimental effects of water loss is to synthesize compatible solutes, such as proline, glycine betaines, polyamines, sugars, amino acids and related compounds (Ishitsans et al., 1996; Ramanjulu & Bartels, 2002; Bartels & Sunkar, 2005).

Proline is an important parameter to measure the stress tolerance capacity of the plants (Delauney & Verma, 1993), and its accumulation is considered as an early response to drought stress (Ramanjulu & Sudhakar, 2000). Convincing evidence is still lacking as to whether accumulation of proline can provide any biochemical adaptation for plants during stress.
Direct evidence for a function of proline under osmotic stress has been provided by over expression of pyrroline-5-carboxylate synthetase in transgenic tobacco that contained elevated levels of proline and exhibited increased tolerance to osmotic stress (Kavikishor et al., 1995). Synthesis or accumulation of proline is depending on the activities of two enzymes, pyrroline-5-carboxylate synthetase (P-5-C synthetase) and pyrroline 5-carboxylate reductase from glutamate. These two enzyme activities were influenced by stress levels and species variation. Several investigators (Ramanjulu & Sudhakar, 2000; Kumar et al., 2003) have established a positive correlation between the accumulation of proline and its osmoprotective role at the whole plant level and in cell cultures. Similarly, in the present study a positive correlation between water stress and free proline accumulation in two groundnut cultivars was reported. Furthermore, a significant difference was observed in free proline accumulation in two groundnut cultivars (Figure 1).

Glycine betaine is regarded as an effective compatible solute that accumulates in the chloroplast, due to stress. Besides osmoregulation glycine betaine stabilizes the oxygen evolving activity of photosystem-II protein complexes at high concentration of NaCl. The major role of glycine betaine might be to protect membranes and macromolecules from damaging effects of stress (Sawahel, 2003). Several reports on glycine betaine accumulation and drought stress have shown that accumulation of glycine betaine under drought stress was found to be high in drought tolerant species than drought sensitive (Hitz & Hanson, 1980; Wyn Jones & Story, 1981; Rhodes et al., 1987). Here a positive correlation between glycine betaine accumulation and drought stress was observed (Figure 5).

Tissue relative water content (RWC) is reported to be reliable and less prone measurement of plant water status. Weatherley (1950) viewed leaf RWC as an important parameter, which determine the ability of a plant to absorb water under moisture stress conditions and used as one of the indices to determine drought tolerance. Under continuous water deficit, there was clear decrease of leaf water potential and osmotic potential. These results are consistence with the results of Ramanjulu & Sudhakar (1997); Basu et al. (1998); Madhusudhan et al. (2002) who observed significant reduction of leaf relative water content in stressed leaves of solanum, mulberry and groundnut plants than those of controls. The degree of proline accumulation is strongly correlated with increasing water potential and RWC. In the present study, we observed a progressive reduction in leaf relative water content in both cultivars during water stress (Figure 6). However tolerant cv K-134 maintain better leaf relative water content than susceptible cv JL-24.

The chlorophyll breakdown or destruction commence rapidly at severe temperature and this property of chlorophyll stability has been used to evaluate genotypic tolerance potentials and found to correlate well with drought tolerance (Khidse et al., 1982). In general photosynthetic apparatus is very sensitive and liable to stress. Drought stress inhibits chlorophyll biosynthesis enzymes; particularly 5-aminolevulinic acid synthetase (Bharadwaj & Singhal, 1981). Several reports have been reported the decrease in total chlorophyll content in stressed plants (Castriollio & Trujillio, 1994; Ramanjulu & Sudhakar, 2000). In present study it was observed that the chlorophyll stability indices were decreased during stress in both groundnut cultivars. However, the tolerant cv K-134 shown better chlorophyll stability than susceptible cv JL-24(Figure 7).

In summary, it has been reported that the accumulation of free proline in groundnut under water stress is an active process, which requires the activation of biosynthetic and catabolic enzymes. The present investigation further confirms the relative drought tolerance of the cv K-134 based on greater
maintenance leaf relative water content coupled with better chlorophyll stability.

References


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