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BIOCHEMICAL STUDY OF *GREWIA* SPECIES UNDER DROUGHT CONDITIONS

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ABSTRACT

A drought is a period when abnormal dry weather conditions continue for a long period of time. This is due to lack of adequate rainfall which reduces soil moisture or ground water. Scarcity of water has drastic effect on the plant productivity. Drought not only damages crops but also livestock and other activities of human. Plants survive in drought condition by acclimatizing morphologically, biochemically and physiologically. Low molecular weight osmolytes like proline and glycine betaine are necessary factors for cellular function under drought stress. Proline and Glycine betaine are known as stress metabolites and they protect plants from drought stress by osmoregulation. *Grewia* species grow well in arid conditions suggesting their drought tolerant capacity. The present study therefore focusses on biochemical estimation of proline and glycine betaine in *Grewia* species to establish their drought tolerant nature andinvestigate the plant productivity. The experiments were comparative between drought leaf samples and post rain leaf samples of *Grewia asiatica* and *Grewia tenax*. It was observed thatin drought leaf samples proline concentration was very high (9.6 mg/ml) in *Grewia tenax* in comparison to *Grewia asiatica* (1.6 mg/ml) whereas in post rain leaf samples of both the species proline concentration was equal (1.2 mg/ml). The concentration of Glycine betaine also increased in response to drought stress, but not to the level of proline. Moreover the increase was significant only in *G. tenax*

Keywords: *Grewia*, drought, post rain, stress metabolites, leaves.

Introduction

Agricultural crops are affected by abiotic stress and it is a major problem for the farmers. In arid regions, drought stress is a grave hindrance in the productivity. During drought plants have to keep potential energy of water to tolerate moisture content and turgor pressure as these are helpful in development of plants. Soil solutes or formation of compatible solutes rise up the osmotic potential of cell by this preservation process. Accumulation of osmolytes such as proline, glycine betaine, etc. helps maintaining cell water status, sub-cellular structures and protecting membranes and proteins from the denaturing effects of the osmotic stress and provide protection to cells against dehydration (Ashraf and Foolad, 2007). Drought stress is one of the major abiotic stress in arid and semi-arid areas of Rajasthan. It adversely affect growth and production of various crops and trees (Ashraf, 2010). Not only in Rajasthan, but worldwide water deficit situation is an environmental factor which severely affect the development of plant. Therefore, scarcity of water is a most serious threat to food security. However, for the survival in drought situation, plants take up various strategies like drought escape, drought avoidance, drought tolerance and embolism. (Farooq *et al.,* 2009; Vilagrosa *et al.,* 2012 and Abobatta, 2019). According to (Choat *et al.,* 2012), it was reported that drought induced embolism, is the main cause of death in woody plants due to blockage of xylem vessels by air bubble or cavity.

Under abnormal climatic conditions plants protect themselves from different types of stresses by producing compatible solutes. They provide not only protection for plants but also plays an important function to regulate protective cell membranes, cellular osmosis, enzymatic activity and stabilizing proteins and scavenging reactive oxygen species (ROS) (Gill & Tuteja, 2010). These solutes are organic in nature, small water soluble compounds which remain inactive in favorable environmental condition (Yancey *et al*., 1982; Tarczynski *et al*., 1993; Holmström *et al*., 1996). These osmolytes provide protection from a stress even when they are in minute quantity (Hayashi & Murata, 1998; Bajaj *et al*., 1999).

Glycine Betaine is synthesized not only in plants but also in microorganisms (Gorham, 1995; Sakamoto & Murata, 2000; Sakamoto & Murata, 2002; Chen & Murata, 2002, 2008) under adverse environmental conditions such as low or high temperature, salinity. Genetic engineering for synthesis of GB may provide more importance in this field of research (Sakamoto & Murata, 2000, 2001). GB have separate positive and negative groups associated with quaternary ammonium, which makes it a more organized compatible solute (Rudulier *et al*., 1984). GB also maintains a hydration shell around the protein molecule to protect it from structural degradation during stress. Glycine Betaine is known to protect PS II against photoinhibition during cold, salinity and drought stress (Deshnium *et al*., 1995; Deshnium *et al*., 1997; Allakhverdiev *et al.,* 2007). It also preserves movement of macromolecules, cell membrane integrity and protect reproductive organs against drought stress.

Proline is an important amino acid it helps to recover the loss of water in cells during stress because of its antioxidant property (Huang *et al*., 2000). Proline accumulation was firstly reported in wilted perennial rye grass (Kemble & MacPherson, 1954). Accumulation of proline is also very significant in higher plants during drought stress (Slabbert & Kruger, 2014; Tabot & Adam, 2014) and same was reported by (Abdelhamid *et al*., 2013; Semida *et al*., 2014) in salt stress. Activities of different enzymes are enhanced by proline and it also maintains the native state of protein (Sharma & Dubey, 2005; Mishra & Dubey, 2006). During drought or water deficit condition the production of proline may increase and it act as a metal chelator, an antioxidant defense molecule and a signaling molecule to prevent electrolyte leakage or oxidative burst in plants. Proline is also known to enhance growth of plants promoting the nutrient absorption, especially of K^+, Ca^+, P^+ and N^+.

Grewia species belonging to family Tiliaceae prefers hot and dry environment specially during fruiting. *Grewia tenax* is also known as white cross berry, phalsa cherry, gondni etc. It is a branched shrub or small tree up to 2 m tall. Popularly it is eaten as a fruit in India. In Jaipur it is found growing wild in rocky areas of Nahargarh. *Grewia asiatica's* common name is phalsa. Its fruits have many medicinal properties like astringent, cooling, and stomachic etc. It can very easily be grown on poor soils. Loam soils are considered best for it. It makes good growth even under scanty irrigation conditions.

As already mentioned, proline and glycine betaine are known stress metabolites, *Grewia* being tolerant to drought stress was taken in the present study to analyse the effect of drought on the levels of proline and glycine betaine. Biochemical estimations of proline and glycine betaine were conducted for both post rain and drought samples to figure the out exact the mechanism involved in combatting drought stress which may be of use to induce stress tolerance in other sensitive trees.

Materials and Methods

Estimation of Proline and Glycine betaine was done according to standard protocol given by (Bates *et al*., 1973) and (Greive& Grattan,1983) respectively. In present study leaves of *Grewia tenax* and *Grewia asiatica* were collected at mid noon, in between 11 A.M. to 1 P.M. from semi-arid forests like Jhalana and Nahargarh sanctuary, of Jaipur district, in the month of May and September which pertains to drought and post rains samples respectively. To authenticate the results the samples were collected from 50 trees separately. The study is comparative and the leaves were therefore collected post rainy seasons and during extreme dry weather conditions. Biochemical estimations for both post rain and drought samples were conducted. Glycine betaine and proline was estimated in all the four leaf samples separately i.e. *Grewia tenax* (drought and post rains samples) and *Grewia asiatica* (drought and post rains samples).

Analysis of Glycine betaine Concentration

Procedure

Glycine betaine is found naturally in several plants. It is a neutral chemical compound which act as an osmoprotectant and hence guard the plant from abiotic stress such as drought. Estimation of Glycine betaine was done by fine powder of dry leaf material. In a conical flask 20 ml deionized water is added in 0.5 gm. leaf samples (*G. tenax* and *G. asiatica*) and placed for 48 hours at 25°C in a vortex shaker. Until further investigation received testers were strained and kept in freezer. In the ratio of 1:1 defrosted extract were diluted with 2N sulphuric acid. Take 0.5 ml part of aliquot in test tube and put the test tube in ice cold water for one hour. Measure 0.2 ml cold potassium iodide-iodine reagent to add in the solution and gently mixed with the help of vortex shaker. Obtained testers were kept for 16 hours at 0-4°C. 16 hours later the testers were transferred in centrifuge at 0°C for centrifugation for 15 minutes at 10,000 rpm. Take cautiously supernatants of both the plant testers with 1 ml micropipette. Tubes should be kept cold until the periodite complex is parted from acidic medium because solubility of the periodite complexes in the acid reaction mixture increases prominently with temperature. 9ml of 1, 2- dichloro ethane was added to dissolve these periodite crystals. Samples were then again transferred to vortex shaker for complete solubility in developing solvent for 2.0 to 2.5 hours. On UV-visible spectrophotometer absorbance were measured at 365 nm. In 2N sulphuric acid reference standards of glycine-betaine (50-200 µg/ml) were prepared and followed the above process which was used for tester estimation.

Analysis of Proline Concentration

Procedure

Take 0.5 gm of leaf sample with 10 ml of 3% aqueous sulphosalicyclic acid and prepare a homogenizing solution and then filter it. 2 ml of filtrate was taken in a test tube and add 2ml of acid ninhydrin and glacial acetic acid. After mixing kept the liquid in boiling water bath for heating for 1 hour. Place the test tube in ice bath to terminate the reaction. Add 4 ml of toluene just after cooling and stir for 20-30 seconds. After stirring toluene layer was formed which was separated and place the test tube at room temperature. Red colour developed and transmission of light was measured at 520 nm by spectrophotometer. Pure proline is used to run a series of standard. Make a standard curve and find out the amount of proline in the leaf samples.

Result

Proline and glycine betaine content increased significantly in *Grewia tenax*. Increase in proline concentration was drastic to the extent of 8 times in comparison to that of post rain samples. In post rain leaf samples of *Grewia tenax*, proline concentration was 1.2 mg/ml where as in drought leaf samples it was 9.6 mg/ml. There was a raise in Glycine betaine content also in drought conditions in *Grewia tenax* but the results were not as appeasing as that of proline content. In post rain leaf samples of *Grewia tenax* Glycine betaine concentration was 5.7 mg/ml which increased to 9.8 mg/ml in drought leaf samples accounting to only 1.71 times boost.

In *Grewia asiatica*, rise in proline and glycine betaine were not significant enough. It was 1.33 times in both the cases. The results are thus indicative of the idea that *Grewia tenax* with enormous proline accumulation is greatly adapted to drought stress in comparison to *Grewia asiatica*. (Table1; Figure1)

Discussion

The improvement in drought tolerance capacity of plants is of utmost importance todo away with future threats to foods security especially in the present scenario where human activities are degrading the environment recklessly. India is more prone to these indemnity particularly drought due to erratic climatic conditions and dependence on agriculture. The plants may beexposedto drought stress at any time during their growth period (Anjum *et al*., 2012).

As already stated, in present investigation, a significant increase in proline and glycine betaine concentration in both *Grewia* cultivars under drought stress was recorded which is in accordance with many earlier findings of drought-induced accumulation of stress metabolites in both dicots and monocots. (Kemble & MacPherson, 1954; Gorham, 1995; Ramanjulu & Sudhakar 2000; Sakamoto. & Murata, 2000; Chen & Murata, 2002; Sakamoto & Murata, 2002; Khan &Gul 2006; Abdelhamid *et al*., 2013; Semida *et al*., 2014)

Proline accumulates generally occurs in cytosol during water stress and where it shows major role in cytoplasmic osmotic regulation. (Anjum *et al*., 2011). In our study, higher level of proline accumulation in *G. tenax* over *G. asiatica* would have enabled drought stressed taxa to uphold low water potentials. During low water deficit accumulation of proline helps plants to persist in the environment (Verbruggen & Hermans, 2008). It was investigated many times that promotion of photosynthesis, scavenging reactive oxygen species and upholding enzymatic action is adjust by proline because it controls activity and role of the enzymes in plant cells (Ozturk & Demir, 2002; Yamada *et al*., 2005; Jaleel *et al*., 2007). Storage of proline is an adaptation in plants which is generated in water deficit stress (Verbruggen & Hermans, 2008). It was described by many reporters that proline is an important solute which regulate the enzymatic activity & hence, it is necessary compound in metabolic reactions in plant cells in dry habitats (Ozturk & Demir, 2002). It was investigated that extra cellular application of proline also helps in regulation of mineral nutrients as well as stimulate photosynthesis and scavenging reactive oxygen species in drought environment (Jaleel *et al*., 2007). Proline shows adaptive response against adverse environmental condition, in higher plants as well as in marine invertebrate and microorganisms like eubacteria, protozoa and algae (Delauney and Verma, 1993). There were number of studies which suggested the positive relation of proline storage under stressed condition stress (Rhodes *et al*., 1986; Khol*et al*., 1991; Chiang and Dandekar, 1995; KaviKishor *et al*., 1995; Nanjo *et al*., 1999; Hong *etal*., 2000; Székely *et al*., 2008) whereas (Hanson *et al*., 1979; Bhaskaran *et al*., 1985; Chandler and Thorpe, 1987; Moftah and Michel 1987; Liu and Zhu 1997; Maggio *et al*., 2002; Mani *et al*., 2002) observed negative relationship. Studies have shown that proline act as a stabilizing enzyme and compatible osmolyte (Low, 1985) subcellular structures (Kandpal and Rao, 1985) scavenger of reactive oxygen species (ROS) which formed the stable chemical compound for the stressed plant (Floyd and Nagy, 1984; Smirnoff and Cumbes, 1989; Smirnoff, 1993) and membrane (Mansour, 1998).

It increases the ability of cells to absorb the water more efficiently (Shahid *et al*., 2014, Al-Shaheen *et al*., 2014). It act as an osmotic regulators, antioxidant, transduction molecule, increase efficiency of mitochondria and gene expression (Szabadosand Savaure, 2009). Proline act as desiccated protector for pollen and seeds during the developmental stages of plants. The accumulation of proline in various parts of the plant is controlled by biosynthesis, degradation and cellular transport pathways. Studies suggested that due to its property of selective compatible solute transporter it is used in production of abiotic resistant varieties of plants. (Armengaud *et al.,* 2004).

The accumulation or degradation of proline shows that proline is a different type of amino acid (Yu *et al.,*1983; 2014). When higher plant undergoes to drought and salinity stress, proline accumulation is a common process in them and analysis of proline is an issue for researchers from since 20 years (Stewart and Larher, 1980; Thompson, 1980; Stewart, 1981; Hanson and Hitz, 1982; Samaras *et al.,* 1995; Taylor 1996; Rhodes *et al.,* 1999). During the salinity stress accumulation of proline starts in leaves (Stewart and Lee, 1974; Briens and Larher, 1982; Treichel, 1986) where as it is accumulated in leaf tissue, shoot apical meristem, desiccating pollen and root apical region during in drought stress (Barnett & Naylor, 1966; Boggess *et al.,* 1976; Jones *et al.,* 1980). Proline act as protective layer for cell membrane and proteins during ion and heat stress (Pollard and Wyn, 1979; Paleg *et al.,* 1981 & Nash *et al.,* 1982). Studies suggested that proline act as a protein compatible hydro tope (Srinivas and Bal-asubramaniam, 1995). The production of proline depends upon the degradation or metabolism of proline.

Glycine betaine accumulation has also been observed in the study in response to drought stress, although the rise was less than that of proline. Under abiotic stress accumulation of GB and efficient yield is correlated (Smirnoff & Stewart, 1985; Ibrahim & Aldesuquy, 2003; Giri, 2011).GB accumulates and/or synthesis in plant tissues, have been linked with the development and existence of plants, boostingup of stress responsive genes, membrane equilibrium, osmoregulation of the cytosolic partitions, ROS scavenging, buffering of redox potential, that respond to the metabolism dysfunctions caused by stress. In plant families like Asteraceae, Chenopodiaceae, Poaceae and Solanaceae GB is widespread during stress (Jones &Storey, 1981). Under abiotic stress glycinebetaine (GB) is reported to accumulate in variety of living organisms. The reports of other investigators have undoubtedly shown that plantsknown to accumulate GB naturally grow well under drought and saline environment. The fact is even established by studies of some of the scientist who observed radical improvement in growth and survival rate of plants under drought stress, with the spray of chemicals having GB at the optimum concentrations in soybean fields (Roychoudhury and Banerjee, 2016) by increasing photosynthetic productivity, enlargement of leaf area, nitrogen fixation and seed production (Makela *et al.,* 1996). Another study suggesting the ROS scavenging role of GB in perennial grasses *Alopecurus pratensis* and *Holcus lanatus* (Sui *et al.,* 2012; Gargallo-Garriga *et al.,* 2015) lays the emphasis on its role as osmo protectant (Wang *et al.,* 2010; Giri, 2011) reported an increase in activity of enzymes for GB synthesis, betaine aldehyde dehydrogenase and choline monooxygenase in chloroplast stroma in response to salt and water stress which adds up to the fact of role of glycine betaine in stress tolerance. Wide-ranging work on GB has submitted its diverse roles in plants. Role of GB in defend against enzyme activity, destruction of membrane, photosynthesis and loss of productivity, transcriptome changes and regulation of ROS detoxification have been established in *Oryza sativa* (Alia and Murata, 1998) *Nicotiana tabaccam* (Holmström *et al*., 2000); *Solanum tuberosum* (Ahmad *et al*., 2008).

Advanced indications advise the involvement of differentially expressing endogenous genes in GB facilitated stress tolerance in plants. Further work would found whether the transcriptome changes are uninterrupted aims of GB or are creation of metabolic adjustment in transgenic plants. (Roychoudhury and Banerjee, 2016). To defend the membrane structures the role of GB in declining the level of malondialdehyde (MDA) has been recently described in the leaves of *Kandelia obovata* and *Aegicerascor niculatum* showing drought stress (Guan *et al.,* 2015).

The results of this study provide evidence that drought tolerance in these local traditional trees could be important for improving the variety of other tree species. It is also helps in further research work related to drought tolerant taxa and provide the ideas for sustainable agriculture development.

Summary and Conclusions

To respond several environmental stresses and in many processes of growth proline is involved in plants. Progressive increase of proline to avoid stress is a recognized information, and different roles have been suggested for proline like as an energy source and/or as an osmolyte and/or as an ROS scavenger. But still importance of proline accumulation needs more investigation because none of these functions has been demonstrated in relation to date and adaptation. Proline is associated in flower and embryo development, flower transition and in other developmental processes has been identified. For instance in flower transition proline may act as a signal molecule, on the other hand during flower, embryo and in other developmental processes it maintained the energetic needs of quickly dividing or elongating cells. Therefore, proline is still a query for scientists. A particularly exciting area of future work will be separating the signaling cascades in the developmental processes as well as stress responses proline has been related with, and detecting and explaining any possible cross-talk

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Table 1: Results of proline and glycine betaine for both the post rain and drought samples are shown in following table:

Stress metabolites	*Grewia tenax*			*Grewia asiatica*		
	Post Rain Samples	Drought Samples	Increase in drought sample in comparison to post rain leaf samples (X[a])	Post Rain Samples	Drought Samples	Increase in drought sample in comparison to post rain leaf samples (X[a])
Proline (mg/ml)	1.2	9.6	8.00	1.2	1.6	1.33
Glycine betaine (mg/ml)	5.7	9.8	1.71	0.3	0.4	1.33

[a]Increase in times

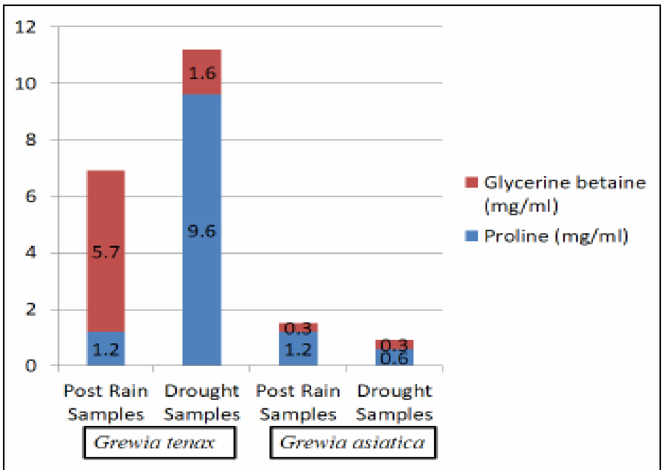

Fig. 1: Relative proline and Glycine betaine content in leaves

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