

Drought Resistance in *Grewia* and *Acacia* Species

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Abstract

Drought remains a major environmental stress, significantly reducing crop productivity and threatening the livelihood of farmers. Water scarcity is a critical challenge, as plants depend on moisture for survival and growth. Consequently, the ability of plants to maintain their internal water balance becomes vital under conditions of water stress. In response to drought, plants employ various physiological and biochemical mechanisms to preserve water and maintain cellular integrity. One such mechanism involves the accumulation of compatible solutes like glycine betaine and proline, which help stabilize cellular structures and protect against oxidative damage. The study on the drought-tolerant species *Grewia tenax*, *Grewia asiatica*, and *Acacia nilotica* revealed significant differences in their biochemical responses to water stress. *Grewia tenax* stood out with a remarkable 41.83% increase in glycine betaine concentration, indicating its strong adaptive response to drought conditions. Similarly, *Grewia asiatica* exhibited a 25% increase in glycine betaine, further supporting its drought resilience. However, *Acacia nilotica*, typically known for its drought tolerance, displayed an unexpected 433.33% decrease in glycine betaine concentration, suggesting that its biochemical response to drought may be more complex or less effective under the specific conditions studied. Proline, another important osmolyte, also showed varying responses across the species. *Grewia tenax* displayed an 87.5% increase in proline concentration, further reinforcing its drought tolerance. In contrast, *Acacia nilotica* showed a decrease of 40.14%, highlighting that its ability to cope with drought might differ depending on the stress level or environmental conditions. These findings emphasize the potential of *Grewia tenax* as a key species for drought-prone areas, where its biochemical responses can be harnessed for agricultural and ecological applications.

Keywords: Drought, stress, glycine betaine, proline and food.

Introduction

Drought is an unavoidable consequence of limited water availability and intense sunlight, making it a significant environmental stress that affects regions worldwide. Turgor pressure plays a crucial role in plant development, particularly in arid environments. Rajasthan, heavily impacted by the Thar Desert, is prone to droughts, with temperatures reaching between 45°C and 50°C and a short rainy season from June to September. The state's abiotic stress factors include high salinity and drought, compounded by less fertile soils. As global warming poses an increasing threat, it is essential to introduce drought-tolerant plant species to mitigate the adverse effects of climate change and combat desertification.

The aim of this study is to identify drought-resistant plant species native to arid and semi-arid regions for afforestation efforts in areas suffering from desertification. The study focuses on species like *Grewia tenax* and *Grewia asiatica*, assessing their physiological traits to confirm their drought resistance. *Acacia nilotica*, known for its drought tolerance, was used as a control group. The introduction of these species in drought-prone areas would not only enhance the state's green cover but also mitigate the effects of climate change and improve soil health. Additionally, this would contribute to the livelihood security of local populations due to the various benefits these plants offer.

Certain adaptive mechanisms, such as the accumulation of osmolytes, increase the osmotic potential of plant cells, helping them retain water. Osmolytes like proline and glycine betaine protect cell membranes, maintain cellular water status, and stabilize proteins during stress (Ashraf and Foolad, 2007). Stress metabolites, although present in small quantities, help in scavenging reactive oxygen species (ROS), stabilizing proteins, and maintaining enzymatic activity under drought conditions (Gill & Tuteja, 2010). These organic compounds remain inactive under favorable environmental conditions but become essential in stress conditions. Plants adopt various survival strategies such as drought avoidance, tolerance, escape, and embolism to cope with the challenges of arid environments (Abobatta, 2019).

The genus *Grewia* is a widely distributed and versatile group of plants used for various purposes across the world. Originally classified under the Tiliaceae family, *Grewia* was later reclassified into the Malvaceae family. The subfamily *Grewioideae* contains approximately 150 species, including shrubs and small trees, which thrive in tropical and subtropical regions globally, including India (Ullah *et al.*, 2012). About 40 species of *Grewia* are found in India, with *G. tenax*, *G. hirsuta*, *G. damine*, *G. lasiodiscus*, *G. optiva*, *G. biloba*, *G. bicolor*, *G. tiliifolia*, and *G. flavescens* being the most commonly found. *Grewia* species are not only important in folk medicine but also provide a source of livelihood for rural families.

Similarly, the genus *Acacia* is renowned for its ability to restore arid ecosystems, particularly through water conservation strategies that involve a balance of physiological and morphological mechanisms (Aref & El-Juhany, 1999; Ramoliya & Pandey, 2002). One notable adaptation of *Acacia nilotica* during drought conditions is the reduction in leaf size or area (Ibrahim, 1995; Ibrahim *et al.*, 1998). Its deep and extensive root system shows morphological plasticity, which helps the plant access water during periods of scarcity (Ramoliya & Pandey, 2002; El-atta *et al.*, 2012). According to Doran *et al.*, 1983, *A. nilotica* is valuable as a source of fuelwood, emergency fodder, and soil conservation. It is well-suited to high temperatures and requires minimal water, making it ideal for drought-prone areas (Bargali & Bargali, 2009).

***Grewia asiatica*:** *Grewia asiatica*, a native shrub of South and East Asia, is also known for its multiple uses. Its fruits are rich in proteins, amino acids, vitamins, and minerals, and they contain valuable compounds such as anthocyanins, tannins, phenolics, and flavonoids. The leaves of *G. asiatica* have antimicrobial, anticancer, antiplatelet, and antiemetic properties, while the fruit exhibits anticancer, antioxidant, radioprotective, and antihyperglycemic effects. The stem bark is known for its analgesic and anti-inflammatory qualities (Zia-Ul-Haq *et al.*, 2013). The fruits of *Grewia asiatica* and its close relatives, *G. subinaequalis* and *G. hainesiana*, are some of the few edible fruits within the Tiliaceae family (Ullah *et al.*, 2012). *Grewia asiatica* is a bushy shrub with horticultural significance, particularly for its small, perishable fruits, which are typically consumed fresh or used to make juice and desserts in local markets in India (Salunkhe & Desai, 1984; Akand, 1960).

***Grewia tenax*:** The local name of *Grewia tenax* is "Gangerun," and it is considered a potentially threatened species in the Thar Desert of India. Its germplasm has been stored and evaluated for future use (Venkatesan *et al.*, 2019). This plant is an important source of fodder, food, timber, fuelwood, fiber, and indigenous medicines, with notable antibiotic properties (Sharma & Patni, 2012; Tahir & Gebauer, 2004). Plant species, particularly those found in semi-arid and arid regions are increasingly affected by unpredictable rainfall patterns and rising temperatures (Walsh & Ryan, 2000; Houghton *et al.*, 2001). The challenges of food insecurity and land degradation can be mitigated by utilizing adaptable local fruit-bearing plants such as *Grewia tenax* (Teketay, 1996; Leistner, 2000; Saied, 2007). This indigenous species is well-suited to drought-prone areas and plays a crucial role in both local nutrition and land reclamation in harsh climatic conditions. It also helps control soil erosion caused by wind (Singh & Kumar, 2003; Orwa *et al.*, 2009). However, overharvesting by rural communities poses a risk to the sustainability of this species (Dalling *et al.*, 2011). Additionally, *Grewia tenax* is valuable for beekeeping, as bees collect nectar from its flowers, and its leaves serve as fodder for animals (Orwa *et al.*, 2009).

Grewia tenax is a small tree or shrub that can grow up to 7 meters in height. The flowers and young shoots are covered with red-brown hairs. The leaves are simple, alternate, and oval with clear venation. The leaf tips are either rounded or pointed with toothed edges. Petioles measure between 0.5 to 1.25 cm in length and the plant has cymose inflorescences. The flowers are actinomorphic, with valvate sepals and imbricate or valvate petals, which may be purple, white, or yellow in color. The plant has numerous stamens, typically arranged in 5 to 10 bundles (Andrews, 1956; Von Maydell, 1986; El Amin, 1990).

***Acacia nilotica*:** *Acacia nilotica*, a member of the Mimosaceae family, is a widely used plant species (Kaur *et al.*, 2005). This tree grows to a height of 5 to 20 meters and has a spreading crown. Trees in the *Acacia* genus are versatile, providing a range of products for various uses (Pandey & Sharma, 2003). *Acacia nilotica* has been shown to improve gas exchange efficiency in crops grown under its shade (Muthuri *et al.*, 2005; Ong *et al.*, 2006). Additionally, this species contributes to soil quality by enhancing the availability of nutrients such as phosphorus (P), nitrogen (N), potassium (K), and organic carbon (Choudhari *et al.*, 2019). *Acacia nilotica* is drought-resistant and has a rapid growth rate, making it an important species for agroforestry, providing timber, fuelwood, and animal fodder (FAO, 2007; Nandal *et al.*, 2007; Solomon-Wisdom, 2010). This tree is known by various names, including *Acacia*, *Acacia arabica*, Babhul (Hindi), Babla (Bengali), Babool (Unani), and Kikar (Punjabi), among others (Steve, 2004). It contains several bioactive compounds such as chlorogenic acids, catechin, flavonoids, and steroids (Pareek & Choudhry, 2013). Native to Egypt, *Acacia nilotica* is widely distributed across arid and semi-arid regions worldwide, including Rajasthan, Uttar Pradesh, Punjab, Haryana, and parts of Himachal Pradesh in India (FAO, 2007; Nandal *et al.*, 2007). It is a moderately sized tree with a spreading canopy and is particularly abundant in Rajasthan.

Effect of drought on *Grewia asiatica*

Phalsa is a hardy plant that thrives in hot climates and can even grow at elevations of up to 1,000 meters (Yadav, 1999). As a deciduous species, it sheds its leaves during the mild winter months. The plant requires warm temperatures for fruit ripening, which is essential for producing high-quality fruit. Protection from cold temperatures is crucial for optimal growth. *Phalsa* can grow on a variety of soils, including fine sand, limestone (when soil fertility is high), and clay. It is often cultivated in marginal lands, which supports the rapid marketing of its fruits. Known for its drought tolerance and resilience,

Phalsa may occasionally need irrigation, particularly during fruiting, as it is cultivated primarily for its fruit (Hays, 1953; Hayes, 1961; Nijjar, 1969).

In Rajasthan, *Phalsa* sheds its leaves in winter, and the fruits typically ripen in June. The plant can tolerate high temperatures, up to 44°C, and thrives in loam soil with a pH range of 7.5 to 8.5. While *Phalsa* can grow in poor soils, occasional irrigation enhances its growth and fruit yield (Singh & Singh, 2017; Gochar *et al.*, 2019).

Effect of drought on *Grewia tenax*

The *Grewia* genus is native to Asia, Africa, and Australia, with around 150 known species, many of which remain underutilized in various countries. *Grewia tenax* is a shrub characterized by small leaves, and it is widely used in horticulture. This plant thrives in sub-humid tropical and arid regions, such as those found in Rajasthan, where it can grow in a variety of soil types, including bushlands, along water bodies, ravine areas, and regions with low moisture content.

The fruits of *Grewia tenax* are highly nutritious and can be eaten fresh or dried for later use. The fruit juice is a popular, refreshing beverage (Sharma & Patni, 2012). *Grewia tenax*, a member of the Tiliaceae family, is commonly found in arid and semi-arid regions, particularly in mountainous and sandy areas. The plant's leaves and branches are relatively small. It is a drought-resistant shrub that performs better in natural, wild environments than when grown in pots (FAO, 1988).

Effect of drought on *Acacia nilotica*

During the dry season, many plants become deciduous, while those growing along water bodies tend to remain evergreen (Fagg and Greaves, 1990). In *Acacia nilotica*, a reduction in growth, osmotic potential, relative water content, and leaf water potential has been observed under water deficit conditions (Kumari & Toky, 2008; Michelsen & Rosendahl, 1990). *Acacia nilotica* is a leguminous tree that can typically grow in temperatures ranging from -1°C to 50°C. According to Dhupper, 2012, this tree is not only drought-tolerant but also beneficial for agro-climatic conditions. It is known for its ability to withstand both drought and salinity. However, during periods of water stress, its root and shoot growth is significantly impaired (El-atta *et al.*, 2012), which can lead to nutritional deficiencies in the plant (Saqib *et al.*, 2013). Water stress has also been shown to negatively impact chlorophyll content, nodulation, and relative water content (Pimratch *et al.*, 2008).

Effect of drought on glycine betaine

Glycine betaine (GB) is an electrically neutral compound, but it becomes dipolar at physiological pH, providing protection to plant cells in several ways. These include stabilizing RuBisCO, regulating osmotic pressure, scavenging reactive oxygen species (ROS), and safeguarding the photosynthetic apparatus (Hussain Wani *et al.*, 2013). Studies by Basu *et al.*, 2010 and Chaum *et al.*, 2010 have shown that abiotic stresses, particularly drought, induce biochemical, physiological, and morphological changes in plants. In response to water deficits, plants accumulate osmoprotectants such as GB and proline, which help them cope with stress. GB, an ammonium compound, is considered an effective osmoprotectant (Burnet *et al.*, 1995). It is found not only in higher plants but also in eukaryotic microorganisms, prokaryotes, cyanobacteria, halophytic plants, and animals (Rhodes & Hanson, 1993). In rice, GB levels were found to be higher in cultivars exposed to polyethylene glycol during water stress but lower in sensitive varieties such as Pusa Basmati and IR-29 (Basu *et al.*, 2010). Cellular

maintenance and osmotic pressure adjustment are crucial mechanisms regulated by plants during unfavorable conditions (Thapa *et al.*, 2011). Research by (Hadiarto & Tran 2011) highlighted that osmoprotectants like glycine betaine, proline, sorbitol, trehalose, and mannitol play a vital role in maintaining plant growth and development under environmental stresses. Factors such as internal water levels, cultivar type, and the crop growth stage affect osmotic adjustment. Furthermore, spraying GB on rice seedlings during drought has been shown to enhance growth activities (Farooq *et al.*, 2010).

Effect of drought on proline

Plants have evolved various mechanisms to cope with the accumulation of osmolytes like glycine betaine and proline. These compounds, including glycine betaine, proline, and sugar alcohols, are found in a wide range of plant species, particularly halophytes, during drought or salinity stress (Hellebust, 1976; Csonka, 1989; McCue & Hanson, 1990; Delauney & Verma, 1993). Proline, in particular, has been identified in protozoa, marine invertebrates, algae, and eubacteria as well (Delauney & Verma, 1993; Roosens *et al.*, 2002). Proline is a water-soluble amino acid with a zwitterionic structure, containing both nitrogen and carboxylic acid groups.

Sasaki *et al.*, 2005, demonstrated that proline plays a multifunctional role in plants, including protecting cellular functions by scavenging reactive oxygen species (ROS), stabilizing membranes, sub-cellular structures, and proteins, all of which help plants combat abiotic stresses. Its synthesis is associated with processes that involve rapid cell division, such as embryo development, floral transition, and the development of shoot apical meristems. Proline is found in high concentrations in seeds and pollen and acts as a dehydration protector for cells, making it crucial during various stages of plant growth.

Additionally, proline accumulates in various plant tissues, cells, vascular bundles, and other organs, with this accumulation being regulated by cellular transport, biosynthesis, and deprivation processes. As an amino acid, proline also plays an essential role in protein synthesis and the formation of other stress-related osmolytes. Research on selective compatible solute transporters and amino acid permeases suggests that these compounds' primary sites can be targeted for genetic manipulation to produce plants resistant to abiotic stress (Armengaud *et al.*, 2004).

Materials and Methods

Before starting work on other parameters, anatomy of leaves and stem of *Grewia tenax* and petiole and stem of *Grewia asiatica* was studied. Fresh material was used in section cutting. Transverse Sections (T.S.) of the stem and leaves of *G. tenax* & stem and petiole of *G. asiatica* were prepared. These sections studied using compound microscope at 4x and 10x magnification (Figure 1.1a to 1.4). Sections were stained with safranin and then mounted with glycerine. The experiments performed on taxa growing in the semi-arid regions such as *Grewia tenax* and *Grewia asiatica*. The results would also be comparative in relation to highly drought tolerant taxa such as *Acacia nilotica*. For each of the experiment, the leaf material was collected at mid noon from semi-arid forests of Jaipur district like Nahargarh & Jhalana sanctuary. The material was collected after rains and in drought condition in the month of October and May respectively, in between 11.00 AM to 1.00 PM.

Analysis of Glycine betaine Concentration

To estimate glycine betaine, finely powdered dry leaf material was used. A 20 ml volume of deionized water was placed in a conical flask, followed by the addition of 0.5 grams of the leaf sample. The flask

was then placed in a vortex shaker at 25°C for 48 hours. Afterward, the leaf samples were strained and stored in a freezer for future analysis. The frozen extract was defrosted and mixed with 2N sulfuric acid in a 1:1 ratio. A 0.5 ml aliquot of the mixture was transferred to a test tube, which was then placed in ice-cold water for one hour.

Subsequently, 0.2 ml of cold potassium iodide-iodine solution was added to the mixture, and it was gently mixed in a vortex shaker. The test tube was kept at 0-4°C for 16 hours. After this incubation period, the sample was transferred into centrifuge tubes and centrifuged at 0°C for 15 minutes at 10,000 rpm. The supernatant was carefully collected using a 1 ml micropipette. Given that the solubility of the periodate complex increases with temperature, it is crucial to maintain a cold environment until the complex is formed. Next, 9 ml of 1,2-dichloroethane was added to dissolve the periodate complex. The sample was placed in a vortex shaker for 2 to 2.5 hours to ensure complete solubility. The absorbance of the resulting solution was measured at 365 nm using a UV-visible spectrophotometer. To create a standard curve, glycine betaine standards (50-200 µg/ml) were prepared in the same manner using 2N sulfuric acid.

Analysis of Proline Concentration

To prepare a homogenizing solution, 0.5 grams of leaf sample was dissolved in 10 ml of 3% aqueous sulphosalicylic acid, and the mixture was filtered. In a separate container, 2 ml of glacial acetic acid and 2 ml of acid ninhydrin were added to 2 ml of the filtrate. The solution was then placed in a boiling water bath for 1 hour. After the reaction, the mixture was cooled in an ice bath to terminate the reaction.

Once cooled, 4 ml of toluene was added to the mixture and stirred for 20-30 seconds. A toluene layer formed which was carefully separated and allowed to stand at room temperature. A red color developed in the toluene layer. The light transmission of this layer was measured at 520 nm using a spectrophotometer. Pure proline was used to create a standard series. A standard curve was plotted, and the amount of proline in the leaf samples was determined based on this curve.

Result and Discussion

The mode of action of *Grewia tenax* and *Grewia asiatica* has not been studied so far. Therefore, the results would be compared in relation to highly drought tolerant taxa *Acacia nilotica*.

Glycine Betaine (GB): Glycine betaine acts as a protector for enzymes and protein complexes under drought stress. It is known that as water stress intensifies, the concentration of glycine betaine typically increases. An increase in GB is considered a marker of stress tolerance. In this study, the concentration of GB significantly increased by 41.83% in *G. tenax* and 25% in *G. asiatica*. In contrast, *A. nilotica*, a known drought-resistant tree, showed a decrease of -433.33%. These findings align with those of Khan *et al.*, 2007; Rezaei *et al.*, 2012 and Khan *et al.*, 2015, who observed increased levels of glycine betaine during arid periods. The studies concluded that higher GB concentrations help in protecting photosynthetic machinery, stabilizing membranes, and enhancing Rubisco activity under drought conditions.

Further studies by Rasheed *et al.*, 2017 also confirmed that increased GB levels stimulate antioxidant enzymes like superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), which help in protecting plants during dry conditions. Additionally, the foliar spray of GB in dry soils has been shown to improve crop productivity in cotton (Gorham *et al.*, 2000) and wheat (Diaz-Zarita *et al.*, 2001). GB has also been reported to enhance the chaperone-mediated protein disaggregation process during stress

(Diamant, 2001). According to Li *et al.*, 2011, GB improves relative water content (RWC) in dry soils and plays a significant role in osmotic adjustment, enhancing plant tolerance under stress.

Park *et al.*, 2004 noted that GB deposits in reproductive parts of plants, promoting better osmotic regulation during drought conditions. Moreover, GB improves the repair system of photosystem II (PSII) and has been identified as a key compound in drought tolerance (Al-Taweel *et al.*, 2007; Allakhverdiev and Murata, 2008; Takahashi & Murata, 2008; Chen & Murata, 2008). It also enhances leaf area and increases yield, as seen in cotton and peanut plants. The protective mechanism of GB during environmental stresses like drought was further explained by Chen and Murata 2008, who concluded that GB protects plants through osmoregulation (Figure 1.5 & 1.6).

However, some studies, such as those by Agboma *et al.*, 1997 and Meek *et al.*, 2003, suggest that the spray of GB has no significant effect on wheat and cotton crops, respectively.

Proline: Proline plays a crucial role in protecting plant cells under moisture deficit stress by providing membrane protection, osmotic adjustment, buffering cellular redox potential, and scavenging free radicals. The concentration of proline in the leaf samples of *Grewia tenax*, *Grewia asiatica*, and *Acacia nilotica* varied, with each species exhibiting different responses to drought stress. *G. tenax* demonstrated a significant increase of 87.5% in proline content, whereas *A. nilotica* showed a reduction of -40.14%. These findings highlight the remarkable ability of *G. tenax* to tolerate drought stress compared to *A. nilotica*.

It is well established that proline accumulation is an early indicator of stress, with its deposition beginning in tissues to prevent cellular injury (Voetberg and Sharp, 1991; Ober and Sharp, 1994). Proline has been found to accumulate in higher amounts in pollen grains, contributing to their desiccation tolerance (Schwacke *et al.*, 1999). Similarly, Verbruggen and Hermans, 2008 observed increased proline concentration in chickpeas during dehydration, helping the plant adjust its osmotic potential for survival. Proline accumulation occurs primarily in the cytosol, where it helps maintain osmotic balance (Anjum *et al.*, 2011). Studies on transgenic plants, such as transgenic *Petunia* expressing the AtP5CS gene from *Arabidopsis thaliana* or the OsP5CS gene from *Oryza sativa*, as well as transgenic *pigeon pea* expressing the P5CSF129A gene from *Vigna aconitifolia*, showed higher proline levels and greater stress tolerance compared to non-transgenic plants, further emphasizing the importance of proline synthesis during water deficit conditions (Surekha *et al.*, 2014). These studies align with our findings that proline content increase significantly under drought stress (Figure 1.5 & 1.7).

However, Boyer, 2010 suggested that proline accumulation does not always have a beneficial effect. He noted that excessive proline may negatively impact carbohydrate synthesis, potentially leading to delays in vegetative and reproductive growth. Moreover, Hellman *et al.*, 2000 and Heuer 2003 observed that the accumulation of proline can be toxic to both plant and animal cells, highlighting a potential downside of its accumulation.

Conclusion

The vegetation of Rajasthan holds substantial ecological and economic importance, home to many endemic and medicinally significant plants. Indigenous plants have long been used for medicinal purposes by local communities, and despite advancements in pharmacognosy, their full potential remains underexplored. With the rising global demand for medicinal plants, coupled with the impacts of climate change and pollution, these natural resources are facing increased pressure, particularly due to

drought. Drought is an escalating issue in the region, severely impacting ecosystems, economies, and communities, resulting in the degradation of agricultural crops, livestock health, and local livelihoods.

The intensification of global warming, driven mainly by greenhouse gas emissions, is increasing both the frequency and severity of droughts. To survive drought, plants have evolved several adaptive strategies, including deep root systems, early senescence, reduced leaf area, and regulation of stomatal movements. However, not all species can withstand prolonged drought, leading to their eventual death. This highlights the need to identify and develop drought-tolerant species by studying their physiological, morphological, and biochemical responses to water scarcity.

This study aims to identify drought-resistant plant species in Rajasthan, focusing on *Grewia tenax*, *Grewia asiatica*, and *Acacia nilotica*. *A. nilotica* was selected as a model due to its well-documented drought tolerance, while the drought tolerance potential of *Grewia* species, particularly *G. tenax*, was investigated due to its promising adaptability in arid regions. Preliminary findings indicate that both *G. tenax* and *G. asiatica* exhibit superior drought tolerance compared to *A. nilotica*. The study, conducted under both post-rain and drought cycles, measured key parameters such as glycine betaine and proline content as indicators of drought resilience. Both *Grewia* species showed increased synthesis of these stress metabolites, with *G. tenax* exhibiting the most significant drought resistance.

G. tenax demonstrated remarkable resilience to high temperatures and flourished in rocky terrains, making it a valuable species for cultivation in arid landscapes. Conserving and cultivating drought-resistant plants like *G. tenax* is essential, not only because of their drought tolerance but also for their ecological benefits, including enhancing regional green cover and contributing to ozone layer protection. Glycine betaine and proline play crucial roles in enhancing drought resistance in plants by stabilizing cellular structures, maintaining osmotic balance, and mitigating oxidative damage. While direct studies on *Grewia* and *Acacia* species are limited, existing research on related species supports the potential benefits of these osmoprotectants. Further research is needed to optimize application strategies and concentrations for these species in arid regions. Further research into drought tolerance mechanisms in these species is critical, and future studies should focus on the following areas:

- Assessing the variation in photosynthetic efficiency using chlorophyll fluorescence measurements.
- Investigating the microanatomy and microecology of the leaves.
- Estimating gas exchange to measure carbon dioxide concentrations in the leaves.

Expanding our understanding of the drought tolerance mechanisms in these plants will help improve conservation strategies and enhance the resilience of Rajasthan's vegetation to the changing environmental conditions.

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Table 1: Relative Glycine betaine Concentration in *G. tenax*, *G. asiatica* and *A. nilotica* in Post Rain and Drought

Samples	Glycine betaine Concentration (mg/ml) Mean $\pm$ S. D.		Percentage Change (%)
	Post Rain	Drought	
<i>Grewia tenax</i>	5.7 $\pm$ 0.14	9.8 $\pm$ 0.26	41.83
<i>Grewia asiatica</i>	0.30 $\pm$ 0.07	0.40 $\pm$ 0.12	25
<i>Acacia nilotica</i>	1.92 $\pm$ 0.40	0.36 $\pm$ 0.32	-433.33

Table 2: Relative Proline Concentration in *G. tenax*, *G. asiatica* and *A. nilotica* in Post Rain and Drought

Samples	Proline Concentration (mg/ml) Mean $\pm$ S. D.		Percentage Change (%)
	Post Rain	Drought	
<i>Grewia tenax</i>	1.2 $\pm$ 0.08	9.6 $\pm$ 0.62	87.5
<i>Grewia asiatica</i>	1.2 $\pm$ 0.04	1.6 $\pm$ 0.17	25
<i>Acacia nilotica</i>	19.9 $\pm$ 0.60	14.2 $\pm$ 0.90	-40.14

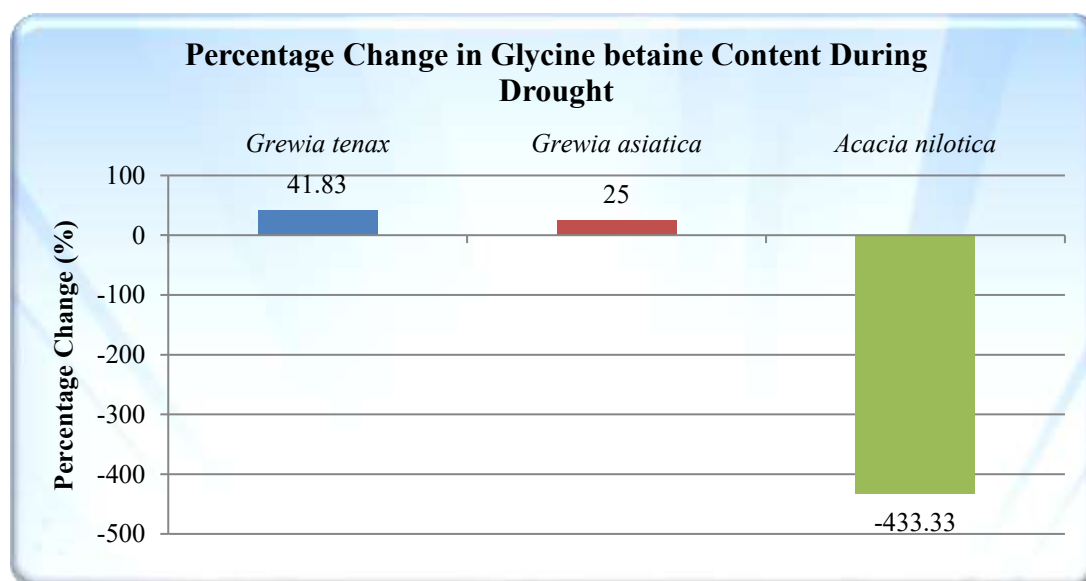


Figure 2: Relative Glycine betaine Concentration in *G. tenax*, *G. asiatica* and *A. nilotica* in Post Rain and Drought

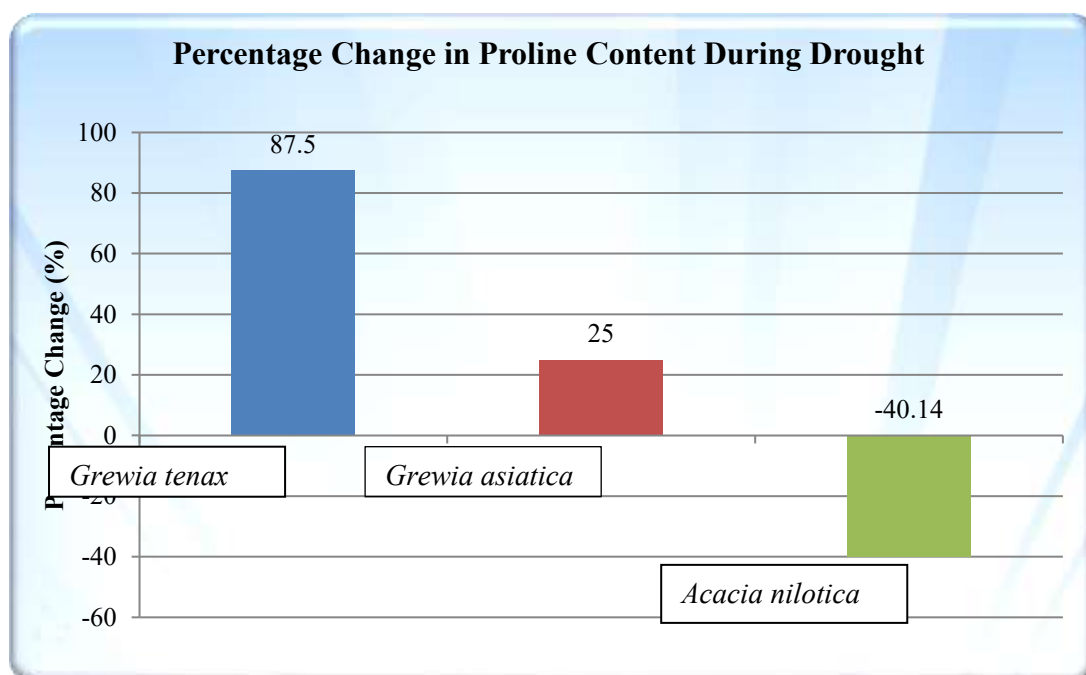


Figure 3: Relative Proline Concentration in *G. tenax*, *G. asiatica* and *A. nilotica* in Post Rain and Drought

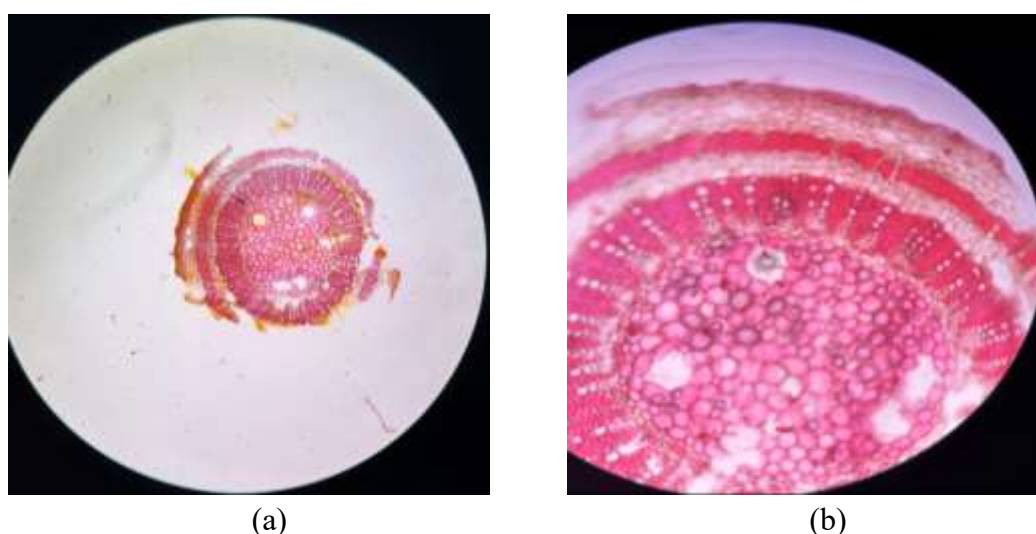


Figure 1.1: a- *Grewia tenax* Stem T.S. (4x magnification), b- *Grewia tenax* Stem T.S. (10x magnification)



(a)

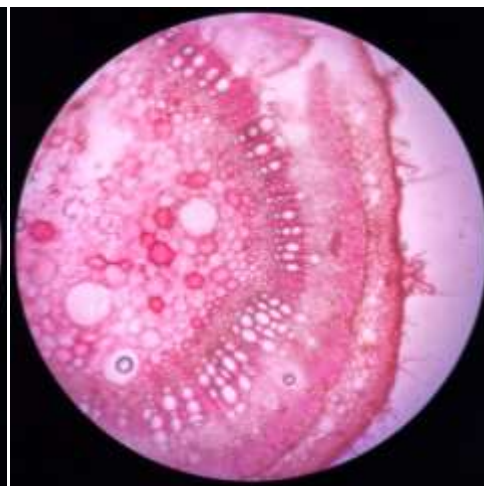


(b)

Figure 1.2: a- *Grewia tenax* Leaf T.S. (4x magnification), b- *Grewia tenax* Leaf T.S. (10x magnification)



(a)



(b)

Figure 1.3: a- *Grewia asiatica*: Stem T.S. (4x magnification), b- *Grewia asiatica*: Stem T.S. (10x magnification)



Figure 1.4: *Grewia asiatica* Petiole T.S.

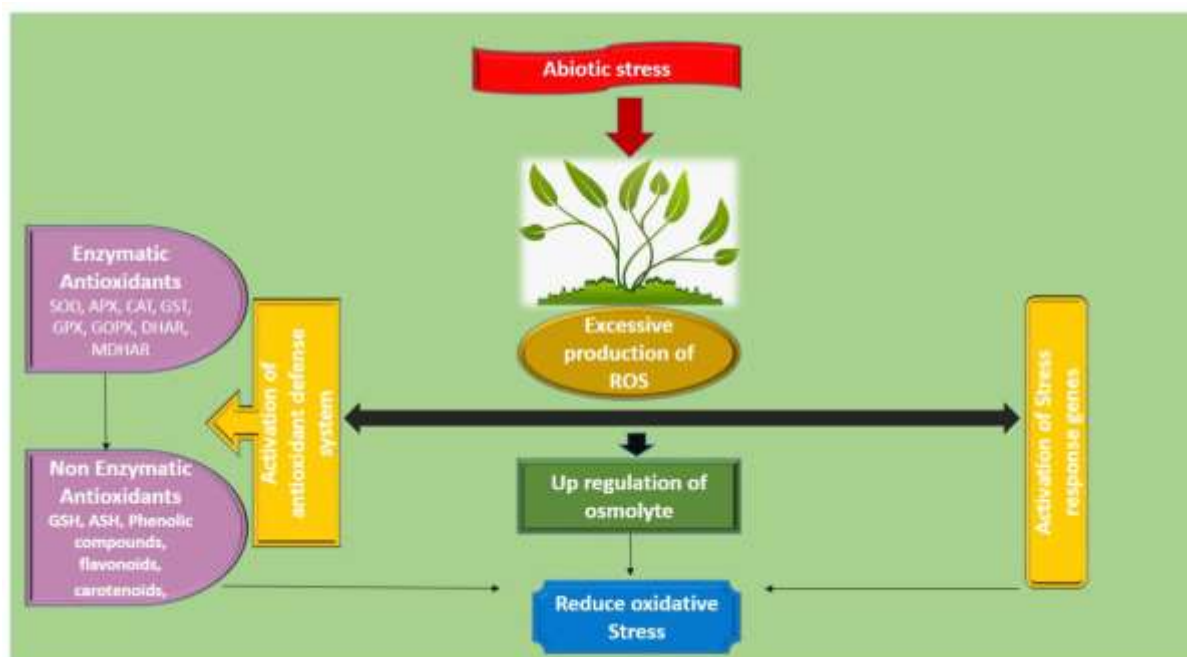


Figure 1.5: The Role of Proline and Glycine betaine in Enhancing Drought Resistance in *Grewia* and *Acacia* Species

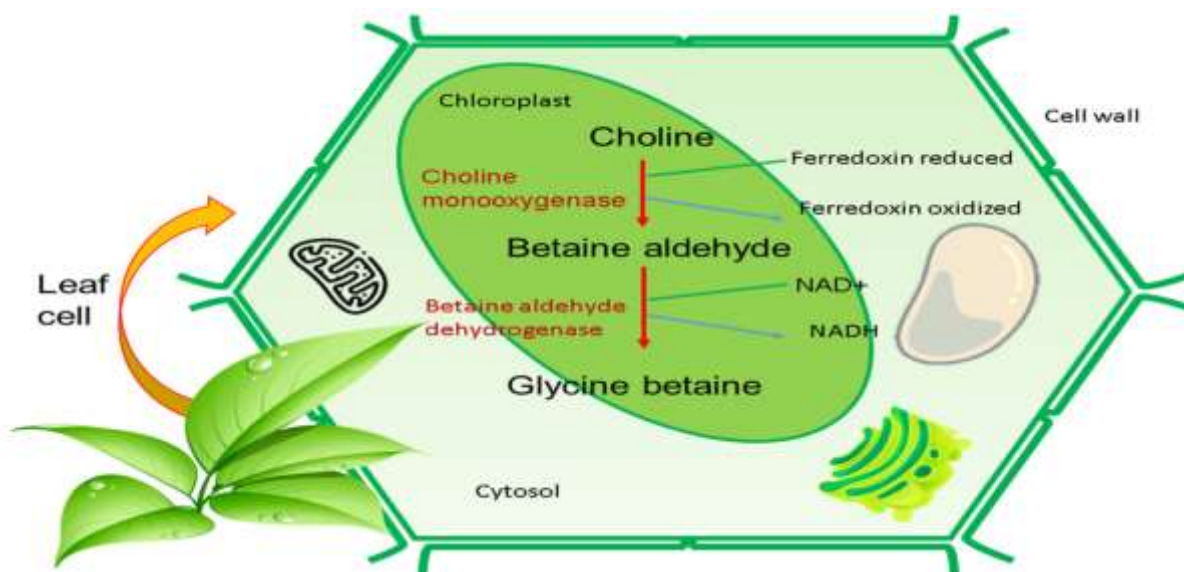


Figure 1.6: Role of Glycine Betaine in the thermo tolerance of Plants

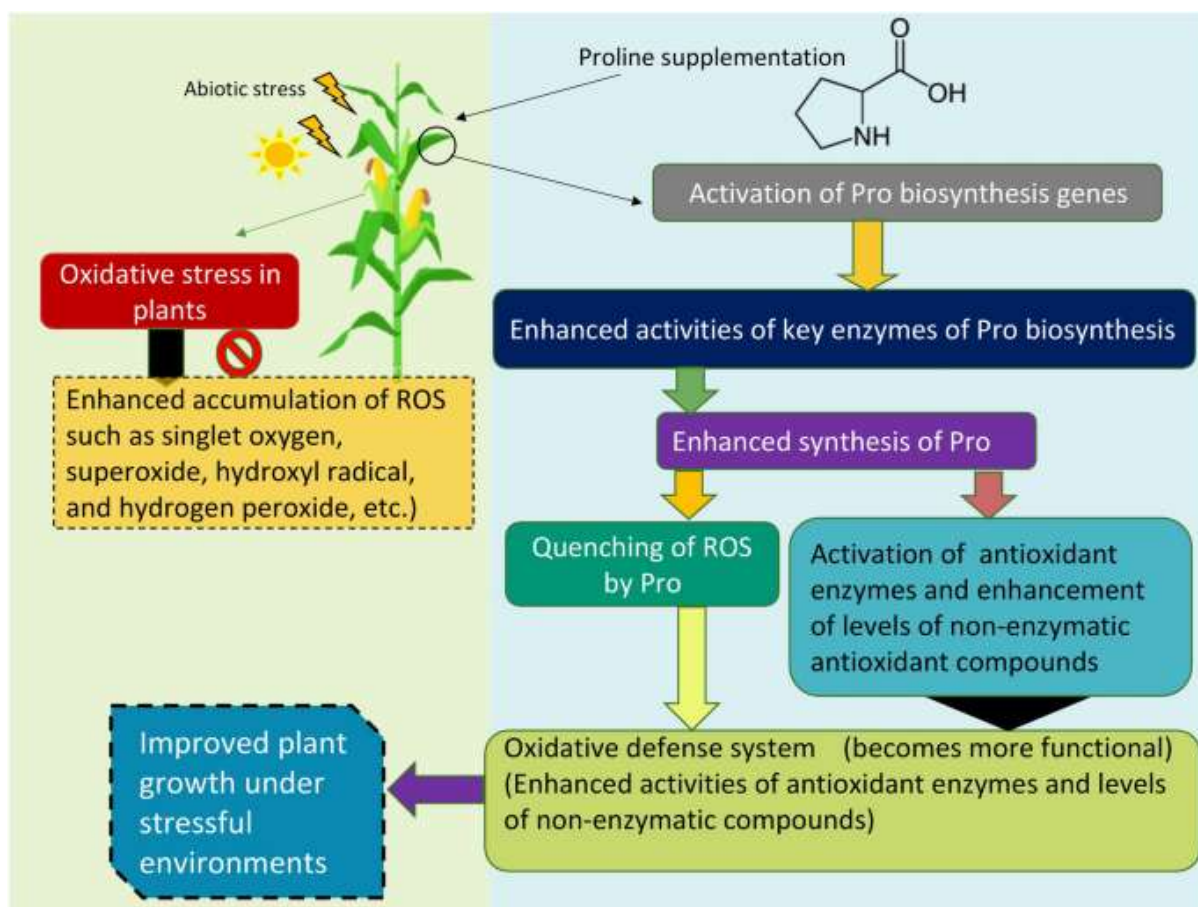


Figure 1.7: Proline Alleviates Abiotic Stress Induced Oxidative Stress in Plants