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Impact of Drought on Growth and Antioxidant Properties of *Grewia* and *Acacia* Species: A Comparative Study

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ABSTRACT: Drought is an important abiotic stress in which availability of water for plants is reduced for a long period of time. Drought is associated with climate change and global warming. It could be more dangerous over worldwide if necessary measures would not be taken. This experimental work was conducted to determine the effects of drought on antioxidants and osmolytes in leaves. For this *Grewia tenax Grewia asiatica* and *Acacia nilotica* were taken as a model plants. The material was collected after rains and in pre rain condition in the Month of October and May respectively, in between 11.00 AM to 1.00 PM. Antioxidant enzymes like peroxidase, superoxide dismutase, catalase and osmolytes like starch; sugar were the parameters of the study. A significant enhancement in peroxidase, catalase, starch and sugar were observed in pre rain samples of *G. tenax*. This was shown a more promising approach of *Grewia tenax* towards drought tolerance as compare to other two species.

KEYWORDS: Drought, global warming, antioxidants, starch, sugar

INTRODUCTION

A period of dry weather condition is one of the phenomena of drought (Nagarajan, 2003). There are so many reasons for drought, like uneven distribution of rainfall, decrease in moisture holding capacity of soils and increasing evaporate demands (Wery *et al*., 1994). The imbalance of water is the reason for prolonged dry weather conditions and this situation is harmful for standing crops. Water is consumed by human life and livestock is also responsible for the shortage of water (Alexander, 1993). This scarcity of water is uninterruptedly going on because of not recharging the resources (Swami, 2001). Drought is the main cause for reduced physiological processes like photosynthesis, reduced plant growth and production capacity (Hu *et al*., 2010), cellular dehydration (Manes *et al*., 2006), degradation of chlorophyll content (Fini *et al*., 2013) and stomatal conductance (Hoshika *et al*., 2013).

Leaf expansion is key parameter for recognizing drought tolerant species (Tardieu and Tuberosa, 2010). It is the first externally visible response in entire plant. Light absorbed is proportional to leaf area and thus it is a major component for biomass production in plants. Reduction in leaf expansion depends on the severity of stress. Cell expansion may be reduced due to cell division and changes in the leaf anatomy (Poorter *et al*., 2009; Tisne *et al*., 2010 and Vile *at al*., 2012). Leaf crown or canopy and leaf expansion were badly affected by the drought and resulted in the alteration of relative water content and entire vegetative growth of the plants (Rahmati *et al*., 2018). Augmentation of antioxidants takes place during drought generated stress and it helps in ROS scavenging, which is a signalling pathway and starts a repair mechanism by controlling lipid peroxidation, integrity of cell membrane & organelles and electrolyte leakage (Gharibi *et al*., 2016). Furthermore, Moller *et al*., 2007 and Cao *et al*., 2017, accepted the relation of ROS and enzyme actions (SOD, CAT and POD enzymes) to control the cell injury by adjusting redox potential of cell. Thus, ROS is considered as a modulator for cell survival at the time of drought stress.

Grewia asiatica is found naturally in South East Asia and the Indian sub-continent. It is native plant of Himalayas. Singh & Singh, 2018 reported about the successful blooms at a height of 3,000 ft. (914 m) in regions of the Himalayas. Among all the species of *Grewia*, *Grewia asiatica* is the only species that is commercially important. Fruit is good source of proteins, amino acids, vitamins and minerals and compounds like anthocyanins, tannins, phenolics and flavonoids are also obtained from it. Leaves possess antimicrobial, anticancer, antiplatelet and antiemetic activities (Zia-Ul-Haq *et al*., 2013). Fruits of *Grewia asiatica* L., *G. subinaequalis* DC. (syn. *G. asiatica Mast*.) and *G. hainesiana* are the only members of Tiliaceae family which have edible fruits (Ullah *et al*., 2012).

Grewia tenax is a small tree or shrub with 7m of height. Red brown hairs are present on flowers and young shoots. Leaves are simple/alternazte, oval, presence or absence of stipules, a network of veins are clear, rounded or pointed tips of leaves with toothed edges (Andrews, 1956). Its local name is 'Gangerun' and a potentially threatened species of Thar Desert in India. For the future perspective, it's germplasm was stored and estimated (Venkatesan *et al*., 2019). It is a good source of fodder, food, timber, fuel wood, fibre, indigenous medicines and possesses antibiotic properties (Sharma & Patni 2012; Tahir & Gebauer, 2004). Plant species which are grown wildly in semi-arid and arid regions all over the world are affected by the unpredictability of rainfall and increased temperatures (Easterling *et al*., 2000; Walsh & Ryan, 2000 and Houghton *et al*., 2001). The threat of food inadequacy and devastation of land are the major problems which can be overcome by local adaptable fruiting plants (Teketay, 1996) like *Grewia tenax* which is appropriate indigenous taxa grown in drought prone areas of the world (Bredenkamp, 2000 and Saied, 2007).

Genus *Acacia* is also known for it's potential for the restoration of arid ecosystems by conserving water through balancing various physiological and morphological mechanism (Aref & El-Juhany, 1999 and Ramoliya & Pandey, 2002). Reduced leaf size or area is one of the adaptations of *A. nilotica* during drought (Ibrahim, 1995 and Ibrahim *et al*., 1998). Roots are very long and deeply penetrating which shows the morphological plasticity during water deficit conditions (Ramoliya and Pandey, 2002 and El-atta *et al*., 2012). Doran *et al*., 1983 stated about the importance of *A. nilotica* as a fuel wood, emergency fooder and soil conservator. It can be grown easily in high temperatures and required minimum water in drought conditions (Bargali and Bargali, 2009).

REVIEW OF LITERATURE

Drought stress not only affects the crop plants but also affects migration of people & livestock, spread of diseases, nutritional ratio in human beings and declines the economic actions (NAAS, 2011). Food security is also badly affected not only at the farm level but also at the economic level in the nation (Samra, 2004; Economic Survey, 2010; NAAS, 2011). Drought is the main cause for reduced physiological processes like photosynthesis resulting in reduced plant growth, reduced production capacity (Hu *et al*., 2010).

Effect of drought on *G. asiatica*

Warm temperature is necessary for ripening of fruits of *G. asiatica* and requires protection from cold temperatures. It can be easily grown on fine sand, limestone (when good fertility) & clay. It is known for its drought resistance and hardy nature. Sometimes irrigation is required during fruiting because it is used as a fruit crop (Hays, 1953; Hayes, 1961; Nijjar, 1969). It can tolerate high temperatures, up to 44° C. 7.5-8.5 ph, loam soil and occasionally irrigation is better for the growth and development of the plant (Singh & Singh, 2017 and Gochar *et al*., 2019).

Effect of drought on *G. tenax*

Grewia tenax is a shrub plant with small leaves. The plant is used for a horticulture purpose and easily grown in sub-humid tropical regions and arid regions of Rajasthan. It can grow in all the soil types like in the bush land, along with water bodies, ravine areas and at low moisture content. Its fruits contain great content of nutrition value and can be consumed either garden-fresh or left to dehydrate for future intake (Sharma & Patni, 2012). *Grewia tenax* belongs to Tileaceae family and is easily found in arid and semi-arid areas like mountains and sand. Leaves and branches of *G. tenax* are small in size. It is a drought tolerant shrub and performed better in wild areas than in pots (FAO, 1988).

A. nilotica is important taxa due to its drought resistant nature and tendency of rapid growth. According to Toky & Bisht, 1992 it's long and strong taproot system helps maintaining soil moisture and nutrients. It is therefore counted as a plant suitable for very harsh environments and used restoration of discarded lands. It has been reported to be significant for agroforestry as it provides timber wood, fuelwood and animal fooder (FAO, 2007; Nandal *et al*., 2007 and Solomon-Wisdom, 2010). It has many names like *Acacia*, *Acacia arabica*, Babhul - Hindi and Napalese, Babla - Bengali, Babool - Unani, Babool Baum - German, Babhoola - Sanskrit, Babul, Babul Tree, Huanlong Kyain - Burmese, Kikar, Mughilan - Arabian Indogom - Japenese and Ummughiion – Persian (Steve, 2004). It contains chlorogenic acids, catechin, catechin-5-galloyl ester, androstene steroid, gallolyated flavan-3, 4-diol, robidandiol, d-pinitol carbohydrate, m-digallic and gallic (Pareek and Choudhry, 2013). In addition, this plant is also used for its phytochemical activities. Leaves have hypolipidemic, hypoglycemic and antioxidant properties (Mathur & Gupta, *2018).*

Acacia nilotica is an indigenous species of Egypt and broadly spread in arid and semi-arid regions all over the world (Raphael *et al*., 2012). In India, it is found in Rajasthan, Uttar Pradesh, Punjab, Haryana and some parts of Himachal Pradesh (FAO, 2007 and Nandal *et al*., 2007). It is a modest sized tree with a scattering canopy. In India, it is generally found in Rajasthan.

Effect of drought on *Acacia nilotica*

During the dry season plants are deciduous whereas plants present on banks of water bodies are evergreen (Fagg and Greaves, 1990). Reduction in growth, osmotic potential, relative water content and leaf water potential was observed in *A. nilotica* during water deficit stress (Kumari & Toky, 2008 and Michelsen & Rosendahl, 1990). *Acacia nilotica* is a leguminous tree and usually grown at -1°C to 50°C temperature. Dhupper, 2012 stated that this tree is not only a drought tolerant plant but also beneficial for agro climate. It is a drought tolerant and salinity tolerant plant. Root and shoot growth was poor during water deficit (El-atta *et al*., 2012) and cause nutritional syndromes in this plant (Saqib *et al*., 2013). It was also reported that water stress caused negative effect on chlorophyll content, nodulation and relative water content (Pimratch *et al*., 2008).

Effect of drought on sugar and starch

In plants carbohydrate is mainly stored in the form of starch. It consists of glucose residues, linked by a-1, 4 linkages, occasional α-1, 6- branches with dense and semi-crystalline particles (Zeeman *et al*., 2010). Starch is commonly found in rhizomes, seeds, stems, tubers and roots. These plant parts are used as a carbohydrate rich diet for human beings and also used for manufacturing purposes (Santelia & Zeeman, 2011). Starch helps to reconcile in growth and development of plants during stress. Under environmental stresses, plants remobilize this starch for their survival. The starch molecules provide energy and act as osmoprotectants to reduce the effect of abiotic stress. Sugar and starch are known as non-structural carbohydrates and prepared by the process of photosynthesis in leaves. These carbohydrates supply energy and carbon through phloem in the plants (Buchanan *et al*., 2015). In plant leaves starch is stored in chloroplast and induced because of high carbon assimilation or low carbon distribution (Beck and Ziegler, 1989). Drought affects the production of carbon and growth is directly associated with carbon stock in leaves (Korner, 2003; Allen *et al*., 2015). In plant source and sink activity is depending on the loss and gain of the carbon molecules in the leaves. During abiotic stress when source activities are limited non-structural carbohydrates act as buffer molecules (Blessing, 2015). Cambial activity promoting growth also depends on the process of respiration and photosynthesis. During moderate drought, it was recorded in many trees that there was no reduction in NSC but in higher drought a great decline was observed (Anderegg *et al*., 2012; Sala *et al*., 2012). Sugar activates the ABA-dependent signaling pathway and stress response cascade by act as a signaling molecule (Rook *et al*., 2006). A reduced amount of starch in leaves was included in many studies during abiotic stress. For instance, decreased amount of starch was reported in *Dunaliella tertiolecta* (green alga) under salt stress (Goyal, 2007). Similarly, in moss *Polytrichum formosum* (Pressel *et al*., 2006); in *Litchi chinensis* and *Hordeum vulgare* (Villadsen *et al*., 2005 and Damour *et al*., 2008) the reduced quantity of starch was observed during abiotic stress. It was reported by Cuellar-Ortiz *et al*., 2008 that drought-sensitive cultivars cut back less quantity of starch as compared to drought tolerant cultivars of *Phaseolus vulgaris* (broad bean); in *Glycine max* (soybean) also starch is higher in drought resistant cultivars. Many investigations are in favor of this concept that the accumulation of starch increases during environmental stresses. It was seen in the *Chlamydomonas reinhardtii* (green alga) (Siaut *et al*., 2011), *Thellungiella halophila* (halophyte) (Wang *et al*., 2013) and Arabidopsis (Kaplan & Guy, 2004; Skirycz *et al*., 2010) as well. Different responses have been noted in different stresses. During cold and salinity stress, improved starch amount was reported in many plants. Likewise, increased starch amount was reported in halophytes (Kanai *et al*., 2007) under salinity stress. It was reported by Cuellar Ortiz *et al*., 2008; Yin *et al*., 2010 that starch was accumulated in the fruits of tomato and broad bean during salt and drought stress respectively. In both leaves and fruits, also soluble sugars were observed in higher quantity during stress. The amount of starch deposited in various vegetative tissues, under stress is different. Soluble sugars are in the petiole of *Lupinus albus* (white lupin) (Pinheiro *et al*., 2001); in *Phragmites australis* (common reed) higher starch content was observed at the base of the shoot than the upper part of the shoot (Kanai *et al*., 2007). Deposition of starch depends on the type of plant species (Kaplan & Guy, 2004; Yano *et al*., 2005 and Thalmann *et al*., 2016). Assembling of Sugar molecules is due to limited demand under stress. In primary carbohydrate metabolism ABA dependent initiation of a multitude gene is involved which was observed in a number of studies of transcriptoms (Zeller *et al*., 2009; Choudhury & Lahiri, 2011; Yoshida *et al*., 2014). In starch metabolism, biosynthesis of ABA is important for stress response (Kempa *et al*., 2008).

In *Triticum durum* Desf variety, concentration of soluble sugars rises much more than that of proline for drought tolerance (Hare and Cress, 1997). According to the investigation, soluble sugars are protective under stress. In the endosperm of cereal seeds metabolization of storage reserves play an important role in dealing with various sugars. Gibberellin and ABA pathways are responsible for drought tolerance (Finkelstein and Gibson, 2002). Sugars are not only the source of energy but act as membrane stabilizers (Hoekstra *et al*., 2001), signal molecules (Sheen *et al*., 1999 and Smeekens, 2000) and as regulators of gene expression (Koch, 1996).

In the present investigation, materials used and the methods followed are given below. The experiments performed on taxa growing in the semi-arid regions such as *Grewia tenax and Grewia asiatica*. For each of the experiment, the leaf material was collected at mid noon from semi-arid forests of Jaipur district like Jhalana & Nahargarh 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.

Effect of drought on Peroxidase (POD), Catalase (CAT) and Superoxide dismutase (SOD)

The present scenario of weather condition is unpredictable and drought is one of the challenges for non-tolerant plants species. Droughts result in decline of CO_2, decreased photosynthesis and morphological functions (Alexieva *et al*., 2001). To overcome the stress conditions plants develop numerous tolerance mechanisms including production of enzymatic & non enzymatic antioxidant compounds like peroxidase (POD), Superoxide dismutase (SOD), catalase (CAT), GSH (Glutathione), ascorbic acid and phenolic compounds (Noctor & Foyer, 1998; Asada, 1999). Antioxidants are known as defence system for plants because they control ROS production (Carocho and Ferreira, 2013). Synthesis and accumulation of secondary metabolites in plants is altered during abiotic stress (Bohnert *et al*., 1995). Flavonoids, phenolic compounds, alkaloids and non-protein amino acids are considered as non-enzymatic antioxidants. These compounds work in coherence with antioxidants like CAT, SOD, POD, POX, GST, PRX, TRX and (APX) that protect plants from extreme ROS destruction (Nath *et al*., 2018; Laxa *et al*., 2019) and regulates redox homeostasis and ROS during stress (Mittler and Poulos, 2005). For example, Superoxide dismutase (SOD; EC1.15.1.1) is a chief scavenger of O_2^- which dismutase superoxide radical anions into H_2O_2 and O_2 (Scandalios *et al*., 1993). For the regulation of the Calvin cycle and stimulation of enzymes in chloroplast, removal of toxic H_2O_2 is necessary (Creissen *et al*., 1994). Up regulation of antioxidant enzymes during drought stress occurs at both transcriptional and post-transcriptional levels. In plants, principal ROS scavenger compounds like GPX, catalase (CAT) and APX are controlled at the transcriptional level (Mittler and Zilinskas, 1994). Dismutation of H_2O_2 into O_2 and H_2O in the peroxisome was catalyzed by CAT which is a tetrameric, heme-containing enzyme. During severe drought conditions, it plays a vigorous role for the survival of plants. Activation of CAT is more than that of APX activity. Protein turnover, gene expression and translation are the few examples of activities controlled by CAT. In fern *Selaginella tamariscinan* activity of POD, CAT and SOD was observed to be better in arid climate. Role of catalase in overcoming drought stress was reinforced by the report of plant death after being sprayed with catalase inhibitor 3-aminotriazole, in water stressed conditions (Gechev, 2013). This was due to decline in CO_2 fixation, increase in photorespiration and increased oxygenation of RUBISCO. These responses were responsible for the release of H_2O_2 in peroxisome. CAT detoxified the H_2O_2 compound and plants are able to survive under drought (Gechev, 2013). Wang *et al*., 2010 observed that level of SOD, APX and CAT was high in the drought tolerant variety of maize whereas levels of APX and CAT were reported low during the hot and dry period in wheat. Peroxidase also produces phenoxyl radical (PhO•) which is known for converting H_2O_2 into H_2O molecule. Peroxidases (POD: EC 1.11.1.7) and catalase (EC 1.11.1.6) is responsible for scavenging H_2O_2 production. Catalase dismutase H_2O_2 into H_2O and O_2; POD decayed H_2O_2 by the process of oxidation of antioxidants and phenolic compounds Mehla *et al*., 2017.

1. Estimation of antioxidants

1.1 Estimation of Superoxide Dismutase (SOD)

Measurement of Superoxide Dismutase (SOD) was done according to the method of (Kakkar *et al*. 1984).

Reagents

1. Sodium pyrophosphate buffer (0.025M, pH 8.3)
2. Phenazine methosulphate (PMS) (186µM)
3. Nitroblue tetrazolium (NBT) (300µM)
4. NADH (780µM)
5. Glacial acetic acid
6. n-butanol
7. Potassium phosphate buffer (50mM, pH 6.4)

Procedure

Preparation of Enzyme Extract

All the collected different leaf samples were ground with 3.0 ml of potassium phosphate buffer and centrifuged for 10 minutes at 2000g. Take out the supernatants for further experiment.

Assay

The assay mix contained 1.2 ml of sodium pyrophosphate buffer, 0.1ml PMS, 0.3ml NBT, 0.2ml of the enzyme preparation and 2.8ml D.W. 0.2ml NADH was added to initiate the reaction. The mixture was incubated at 30°C for 90 seconds. To block the reaction 1.0ml of glacial acetic acid was added. 4.0ml n-butanol was added and allowed to stand. For 10 minutes centrifuge the mixture at 2000g. At 560nm optical density of the chromogen in the butanol layer was measured by spectrophotometer. One unit of enzyme activity is defined as the amount of enzyme that gave 50% inhibition of NBT reduction in one minute.

1.2 Estimation of Peroxidase (POD)

Measurement of Peroxidase activity was done according to the method of (George, 1955).

Preparation of the reagent:

1) Phosphate buffer (0.1 M, 6.4= pH): Phosphate A: 0.2 M monobasic sodium phosphate (27.8g NaH2PO4.2H2O was dissolved upto 1,000ml with DW.

Phosphate B: 0.2 M dibasic sodium phosphate (53.65g Na2HPO4.7H2O was dissolved upto 1,000 with DW.

Phosphate buffer: 73.5ml Phosphate A and 23.5ml Phosphate B were diluted upto 200ml DW.

2) 20mM Guaiacol: 0.22ml Guaiacol was up to 100ml with DW.

The results of enzyme activity were expressed as O.D. difference/min/mg protein.

Procedure: 1ml aliquot of enzyme was taken and mixed with 1ml phosphate buffer (0.1M, pH= 6.4) & 1ml 20ml M guaiacol. On a spectrophotometer optical density was recorded at 420nm. After two minutes 0.5ml H_2O_2 was added. Blank was prepared in the same manner. Calculation was done expressed as O.D difference/minute/gm plant material.

1.3 Estimation of Catalase (CAT)

Measurement of Catalase activity was done according to the method of (Luck, 1974).

Preparation of reagent: - 1) Phosphate buffer: 0.067 M (ph 7.0)

2) Hydrogen peroxide (2mM) in phosphate buffer

Procedure

Preparation of Enzyme Extract: A 20% homogenate mixture of leaf samples was prepared with phosphate buffer. Centrifuged the mixture and take out supernatant for further use.

Assay: H2O2 phosphate buffer (3.0 ml) was taken in a cuvette followed by the rapid addition of 40 µl of enzyme extract and mixed thoroughly the mixture. The time required for a decrease in absorbance by 0.05 units was recorded at 240 nm in a spectrophotometer. The enzyme solution containing H2O2 free phosphate buffer served as control.

One enzyme unit was calculated as the amount of enzyme required to decrease the absorbance at 240nm by 0.05 units.

Estimation of Solute Concentration

3.3.1 Estimation of Sugar

Procedure: Take 100 mg leaf samples and then homogenate using 10 ml 80% ethanol. Mixture was centrifuged at 10 rpm for 10 minutes. Take out supernatant 1 and ethanol (10 ml) was again added to the remaining part. Centrifuge it and take out supernatant 2. Both the supernatants 1 & 2 were mixed together and discarded the residue.

Total sugars: Take 1ml of obtained alcoholic aliquot then add 1ml 1N H_2SO_4. Aliquot was heated in water bath at 49 ℃ for 30 minutes for the process of hydrolysis of the aliquot. Methyl red indicator in quantity of 1-2 drops was added in the mixture. To neutralize the mixture drop wise 1N NaOH was added the color turns pink from yellow. 1ml of Nelson Somogyi's reagent was poured in boiling tubes & set aside in boiling water bath for 20 minutes. 1ml arsenomolybdate was poured after cooling of the tubes. Raise the volume up to 20 ml by D.W. and take the optical density at 540nm. In the same way blank was prepare.

Reducing sugars: 1ml alcoholic aliquot was taken and reagent Nelson Somogyi's was poured in it. The mixture was set for 20 minutes in boiling water bath. 1ml arsenomolybdate was then poured in the mixture after cooling of the tubes. Distilled water was added to raise the volume up to 20ml. O.D. was noted at 540 nm and by the similar method blank was prepared.

Non-reducing sugar = Total sugar – Reducing sugar. The end result was stated as mg/gm leaf samples.

Preparation of reagents:

- 80% Ethanol: Distilled water was added in 80ml Ethanol and makes the volume 100ml.
- 1N Sulphuric acid (H_2SO_4): 2.77ml conc. H_2SO_4 (95-98%) was added in distilled water and makes the volume 100ml.
- 0.1N Sodium hydroxide (NaOH): 4gm NaOH was dissolved in distilled water and makes the volume 100ml.
- Methyl red indicator (1N) - 0.1gm Methyl red powder was dissolved in 5ml 0.02M NaOH and final measurement was raise up to 250ml with distilled water.
- Nelson Somogyi's reagent - Nelson A - 12.5gm Na_2CO_3, 12.5gm Na-K-tartrate, 10gm $NaHCO_3$ and 100gm Na_2SO_4 were dissolved one by one and made the volume rise up to 50ml with distilled water.
- Nelson B - 15gm $CuSO_4 \cdot 7H_2O$ was dissolved in 100ml distilled water.
- Nelson Somogyi's reagent - 50ml Nelson A and 1ml Nelson B were mixed together.
- Arsenomolybdate reagent - 25gm Ammonium molybdate was used to dissolve in 450ml distilled water, 21ml conc. H_2SO_4 was added to it. 3gm Sodium arsenate was used to dissolve in 25ml distilled water. Both the solutions were mixed. After mixing the solution was incubated at 350℃ for overnight in advance for further use.

3.3.2 Estimation of Starch

100 mg leaf samples were weighed and add 10ml 80% ethanol to make a homogenate mixture. Mixture was centrifuged for 10 minutes. Supernatant 1 was discarded. Add 10 ml 80% ethanol was again added in remains. After centrifugation obtained supernatant 2 was discarded. 20ml 0.7% KOH was added to residue and for gelatinization boiled the mixture for 40 minutes. After cooling re-centrifuged the mixture and supernatant was stored. 1ml citrate buffer (0.05 M, pH 5.0), 1ml I_2KI and 0.5ml 20% acetic acid were added to 1ml of supernatant. The mixture was incubated at room temperature (for 10 minutes). At 600 nm optical density was measured on a spectrophotometer. In the same manner blank was prepared. The readings were compared with a standard starch solution. Expression of results was in mg/gm leaf samples.

Preparation of reagents:

1. 80% Ethanol: Ethanol (80ml) was added in distilled water to make the 100ml volume.
2. 0.7% Potassium hydroxide (KOH): KOH (700 mg) was dissolved into 100ml distilled water.
3. 20% Acetic acid: Glacial acetic acid (20ml) was diluted up to 100ml with distilled water.
4. I_2KI Solution: Iodine crystal (200mg) and 2gm KI were dissolved and makes the volume 100ml with distilled water.
   Citrate buffer: (0.05M, pH 5.0).
   0.1M Citric acid (Citrate X).
   Citric acid (2.19gm) was liquefied into 100ml distilled water.
   0.1M Sodium Citrate (Citrate Y).
   Sodium Citrate (2.94gm) was liquefied into 100ml distilled water).
   Citrate buffer: Citrate X (20.5ml) and Citrate Y (29.5ml) were liquefied up to 100ml with distilled water.

CONCLUSION

Results compiled here suggested that *Grewia tenax* is a drought tolerant species & there is a need to investigate the drought tolerant genera. This would help to overcome the negative aspects of global warming and ensure sustainable livelihood security for people living in such areas because of various other benefits associated with these plants.

Peroxidase. Peroxidase enzyme plays an important role in alleviating water stress in plants by rapidly scavenging ROS. The concentration of POD in *G. asiatica* remained unchanged i.e. 2.85 mg/ml in both the samples. Compared with the post rain samples, there was significantly increase in POD activity upon exposure to drought stress in *G. tenax*. This might be an adaptive trait to overcome the damages caused in water limited conditions. The present work is in line with those of Zhang and Kirkham, 1994, who reported an increase in amount of POD which is responsible for oxidising H_2O_2, thus acting as a ROS scavenger. It also normalizes the lignification, cell elongation and oxidation of phenolics. Therefore, increased POD is a mechanism for protection from H_2O_2 (Moradi Talavat *et al*., 2017; Lum *et al*., 2014; Chaparzadeh *et al*., 2004) reported that plants used the peroxidase enzyme for inhibition of free oxygen radicals and are useful in oxidation of phenols. By this way plants create a defence mechanism which protects them during oxidative stress. Further, Kalefetoglu 2006, reported in chickpea that POD level increases and protect the cell membrane when dehydration increases. POD converts H_2O_2 into H_2O to scavenge ROS production in plants during water stress (Hasanuzzaman *et al*., 2020; Savvides *et al*., 2016). Terzi and Kadioglu, 2006 found *Ctenanthe setosa* as a tolerant plant for drought stress due to its increasing activity of POD in leaves and petiole during dehydration. Similar findings are in notice that during arid period POD increases its content (Sharma *et al*., 2012).

However, some results are reverse to our study. Mercado-Blanco *et al*., 2004 in which they observed that activity of POD is related to water holding capacity of leaves. Consequently, when RWC is poor, then it reduced the concentration of POD in the leaves. As an impact of drought, decreased POD and consequently increased ROS were observed in maize seedlings (Hussain *et al*., 2019).

Superoxide Dismutase. SOD constitutes the first line of defence against drought stress by detoxification of H_2O_2. Documented mean change for superoxide dismutase was 20.83% in *G. tenax* which was not a major contribution towards stress in comparison with *A. nilotica* which showed the change by 42.16%. These differences of percentage change might be related to plant's tolerant nature against drought. This is in agreement with the work done by Alscher *et al*., 2002, which showed that plants overcome the abiotic stress by the stimulation of SOD enzyme. These results corroborates with the results of Chutipaijit, 2016; Carrasco-rios; & Pinto, 2014; Tahi *et al*., 2008. SOD prevents the toxic effects of ROS and empowers plants for resistance to drought stress (Kusvuran *et al*., 2016). Similar effect of drought on SOD content was observed in fern *Selaginella tamariscina* (Wang *et al*., 2010) and CE704 genotype of maize (Benesova, 2012). Lum *et al*., 2014 reported that drought tolerant varieties showed enhancement of antioxidant compounds like SOD, CAT and POD to protect plants from toxic effects of H_2O_2. Superoxide free radicals are detoxified by higher activity of SOD during oxidative stress (Hasheminasab *et al*., 2012). Reddy *et al*., 2004 and Chaitanya *et al*., 2002 found that combined effect of SOD and CAT prevent the cellular damage under water deficit by converting H_2O_2 into water and molecular oxygen.

On the contrary, Choudhury *et al*., 2022, reported that reduction of SOD was noted in dehydrated pearl millet. It is due to the high level of H_2O_2 production. This is indicating the week defence mechanism of antioxidants in pearl millet when exposed to water stress events.

Catalase. Catalase is essential for the elimination of the H_2O_2 produced by photorespiration in the peroxisomes. Table 4.18 indicate the significant increase in drought samples in all the generas. Effect of drought in *G. tenax* for catalase concentration was evident at 28.85% changes, which was highly positive value as compared to other plants. These results have shown the similar findings with Sairam and Srivastava, 2001, in which they reported that CAT causes breakdown of hydrogen peroxide into water molecules and oxygen free radicals in the peroxisomes by photorespiration. Similar influence of CAT concentration was reported by Lum *et al*., 2014 in which they concluded the positive relationship between drought tolerant cultivars and CAT activity. In this study CAT concentration increased sharply in upland variety of rice during dehydration whereas reduction was noted in hydrated varieties. Consequently, this led to a better defence antioxidant system for plants to cope with toxic elements activated during stress. H_2O_2 accumulates during photorespiration (low CO_2 and high temperature). Removal of H_2O_2 is a drought induced function of CAT. In wheat leaves marked increase of catalase has been reported during severe drought and vice-versa in normal conditions. Also, its regulation patterns depend on day and night (Luna *et al*., 2005). Hardeland, 2000, investigated that CAT expression patterns were associated with circadian rhythms. These rhythms were often related to regulate photosynthesis, metabolic functions and redox potential. Environmental stress like drought alters circadian controls in plants.

On the other hand some contradictory studies were also presented here in which decreased CAT activity was observed. Severe damage was reported in soyabean line (Vx-08-11614) due to reduction in CAT concentration after dryness. This low concentration of enzyme may allow lipid peroxidation and cellular damages (Rosa *et al*., 2020). Hasanuzzaman and Fujita, 2011 reported diminished activity of CAT in rapeseed seedlings under water limitations. Similar reports were observed by Nguyen *et al*., 2009 under drought, when meiosis division starts for anther development in rice. Moreover, hampered growths were reported in *Malus prunifolia* due to the suppression of CAT under drought (Wang *et al*., 2012).

Total Sugar. Accumulation of sugar prevents oxidation of cell membrane and reduces the rate of photosynthesis. Sugar molecule act as signaling molecules, which cross - talk with the ABA- dependent signaling pathway to activate downstream components in stress response cascade. Enhancement of sugar retains the turgidity of the cells and maintains the volume during scarcity of water. The present data revealed that total sugar concentration in *G*. *tenax* showed a hike i.e. by 50% during drought in comparison to rain fed conditions. (Table 4.8) On the contrary, *A*. *nilotica*, a known drought tolerant taxa and *G*. *asiatica* exhibited a reduction in total sugar content in drought. This re-establishes the fact that *G*. *tenax* has a strong potential towards drought resistance. In bark of loblolly pines, sugars and total carbohydrates were reported increased whereas starch concentration was stable during water deficit phase. It showed the reduction in growth during drought stress (Hodges and Lorio, 1969). Reynolds and Smith, 1995, found that soluble sugars maintain the osmotic balance and regulate gene expression when seeds are in germinating phase respectively. In mature seeds sugar accumulation is higher and it is a characteristic feature of mature seed but during water stress this accumulation was also decreased. Wiedenfeld, 2000, studied that during lower water deficit sucrose concentration is higher and vice versa at the time of higher water deficit. Sugar molecules are not only energy sources but recent studies justified its important role in signalling systems for metabolic reactions (Gibson, 2005). Sugar molecules can cross-talk with the ABA-dependent signalling pathway to trigger downstream mechanisms in the stress response cascade. Boguszewska, 2007, explained that total sugar concentration in potato tubers was reported high due to higher deposition of reducing sugar, non-reducing sugar and starch during water stress. Ramel *et al*., 2009 reported that sugars are accountable for hormone cross-talk and transduction trails during water deficit. Sugar is closely related to ABA signalling cascade. During drought stress one gene of sucrose synthase was associated with ABI3 whereas one gene of neutral invertase, one b-amylase and two sucrose synthases was associated with ABI1 gene. This is a signal of sugar travelling in the xylem cells of plants during drought. Invertase enzymes hydrolyse sucrose molecule to its hexose compound by the regulation of ABA during stress. In addition, the sugar molecules act as an osmoprotectants and signalling molecules to overcome the damaging effect of dry weather conditions (Krasensky & Jonak, 2012).

Reducing Sugar. Reducing sugar transporter genes stimulated during low availability of water to regulate sugar transport in the roots. Result analysis of the reducing sugar content under drought showed significant increase in *G*. *tenax*. Among all the plants, *A*. *nilotica* showed the maximum reduction in mean change. Again observations exhibited the concept of xerophytic nature of *G*. *tenax* which is in good agreement with our other findings. Other researchers has been made similar observations of reducing sugar like Rathinasabapathi, 2000 informed about the progressive studies on metabolic reactions in plants. A significant enhancement of reducing sugar during dehydration in drought stressed plants was reported by Krasensk and Jonak, 2012. Reducing sugars play a positive role by increasing their accumulation in the plant to modulate various physiological activities during water deficit. Cell turgor, water potential and absorption of water can rise up the amount of reducing sugars (Williams *et al*. 2012). Reducing sugars provide intermediate compound for the growth of stressed plants during abiotic and biotic stress (Richardson *et al*., 2015). Holland *et al*. 2016, reported that increased amount of sugar retains turgidity of cells. The experiment showed that increased reducing sugars, improved the occurrence of big vessels in the xylem. These vessels are then important for direct interchange of ions, water and maintain the osmoregulation of the water stressed cells of woody plants. Consistent results were obtained by Tomasella *et al*., 2019 that non-structural carbohydrate decrease in the stem due to embolism and this reduction is further responsible for the higher sugar concentration to recover the water stress. Therefore, plants tolerate the condition of drought by keeping their reducing sugar level elevated and available till the end of stress. In contrast, Amjad *et al*., 2019 emphasized the reduction of reducing sugar (glucose) in water stressed varieties. However, these changes are affected by meteorological and agro-technical factors. On the basis of above studies, Grudzinska and Zarzynska, 2022, noted the decline in reducing sugar content in water stressed potato tubers but with higher respiration rate. Therefore, further exploration is required to authorize these associations.

Non-reducing sugar. Non-reducing sugar prevent protein degradation during severe arid period. Non-reducing sugar showed increase only in *G. tenax* accounting to 58.82% increase. The concentration of post rain samples was 0.07 mg/ml whereas it was 0.17 mg/ml in drought samples. On the other hand, *G. asiatica* and *A. nilotica* showed reduction in drought samples. Thus, positive response of *G. tenax* during dehydration was seen. This outcome is in agreement with previous reports. Fu *et al*., 2010 reported low level of osmotic potential and higher amount of non-reducing sugar in leaves of *Festuca arundinacea* when availability of water was low. This phenomenon was reported to occur due to enzymes like acid invertase, sucrose phosphate synthase and sucrose synthase.

In the study of Du *et al*., 2020, non-reducing sugar (sucrose) increased in leaves of Glycine max during water stress. In addition, activity of carbohydrate metabolism and sugar metabolism enzymes were reported as stimulator of sucrose enhancement. Also, sucrose transporter genes such as GmSUC2*,* GmSWEET6 and GmSWEET15 up-regulate their mechanism during arid period. Environmental stress like drought affects the physiological and biochemical processes in plants. Sucrose is responsible for maintaining sugar homeostasis under water deficit in roots, leaves and phloem sap of rice cultivars. Activation of *SWEET* genes and ABA-responsive transcription factor increases the transportation of sucrose in plant tissues. SUTs and SWEETs genes are involved in modifying mechanism towards drought stress (Aluko *et al*., 2021). On the contrary, in sugar beet, dehydration reduces the sucrose accumulation (Hoffmann, 2010).

Starch. Starch provides resistance to the seedling and maintaining leaf growth during water deficit. Under unusual dryness photosynthesis may be limited resulting in remobilization of starch to provide energy and carbon. Figure 4.15 illustrates that in all the three generas, starch content was reduced which showed that moisture stress induced a significant reduction in starch content. *G. tenax* exhibited minimum reduction i.e. -1.66%. Therefore, in the view of the above obtained results *G. tenax* gives a promising approach under adverse condition and proved to be drought tolerant species. Starch is important for increasing of soluble sugars in stressed conditions. Starch biosynthesis was affected by starch synthase enzyme which decreases its metabolic levels in drought affected species (Ahmadi and Baker, 2001). Increased amylase activity is also responsible for the downstream and is accountable for the deprivation of carbohydrates and starch content (Li & Li, 2005). Degradation of starch into sugar due to drought stress has also been reported by Zanella *et al*., 2016. Degradation in starch provides carbon and energy to plant when photosynthesis was limited. Starch is known as a key molecule in between plant response and abiotic stresses like drought, salinity etc. On the other hand, in Bonica var: Rose and Galine var: Brinjal, starch content in leaf was noticeably higher during water stress with less lipid peroxidation activity (Hannachi *et al*., 2022).

Present aimed at identifying the drought tolerant taxa for Rajasthan. For this *Grewia tenax*, *Grewia asiatica* and *A. nilotica* were taken as model plants. *A. nilotica* was opted being an established plant with drought tolerant nature. The observed growth potential of *Grewia* under arid conditions generated the thought of investigating the resistant properties of these taxa in perspective of drought. The expected results emphasized higher drought tolerant potential of *G. tenax* & *G. asiatica* as compared to *A. nilotica*. The experiments in all the three taxa were conducted under post rain and drought cycles. For drought tolerance various parameters were studied like peroxidase, superoxide dismutase, catalase. *Grewia tenax* can tolerate drought stress by endorsing stress metabolites (glycine betaine & proline content); antioxidants (peroxidase, superoxide dismutase & catalase concentration). It is the need in present scenario to conserve and cultivate these types of domestic plants not only for their ability to endure drought but also in view of other benefits associated with them. These efforts would also enhance the green cover of the state and helps to protect the ozone layer as well. However, following are the parameters which can be explored more in future in terms of drought for this plant:

- Determination of variation in photosynthetic efficiency by measurement of chlorophyll fluorescence.
- Investigation of microanatomy and micro ecology in leaves.
- Estimation of gas exchange to measure carbon-di-oxide concentration in leaves
- Determination of phaeophytin content in relation to a change in Specific Absorbance Coefficient (SAC).
- Analysis of endophytic (fungal) growth in leaves.
- Analysis of seed viability

Table 1: Relative Superoxide Dismutase Concentration in *G. tenax*, *G. asiatica* and *A. nilotica* in PR* and DR**

Samples	**Superoxide Dismutase Concentration (mg/ml) Mean ± S. D.**		**Percentage Change (%)**
	Post Rain	Drought	
Grewia tenax	0.038 ± 0.00	0.048 ± 0.00	20.83
Grewia asiatica	0.078 ± 0.00	0.111 ± 0.02	29.72
Acacia nilotica	0.096 ± 0.00	0.166 ± 0.00	42.16

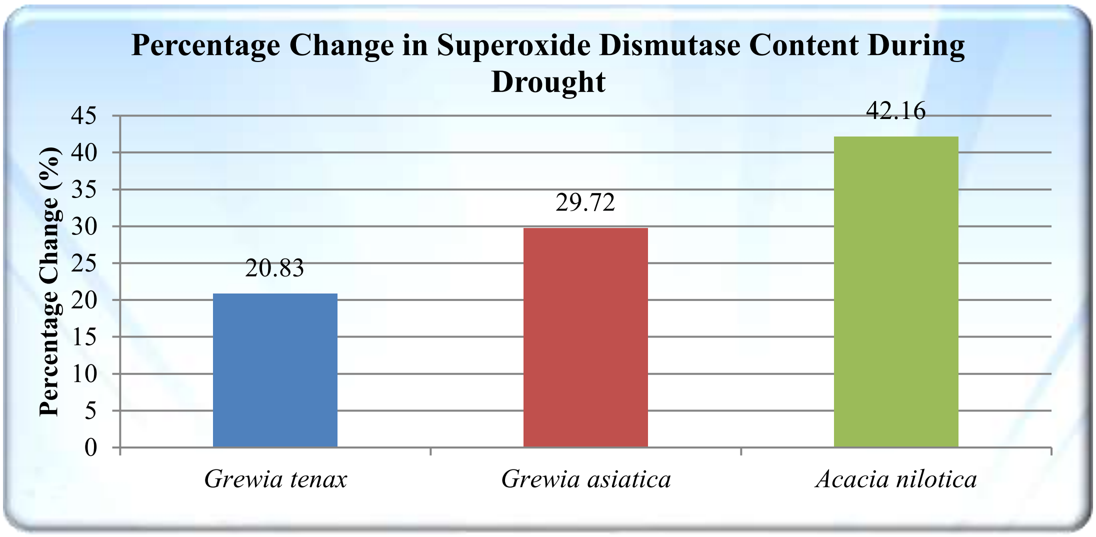

Figure 1: Percentage Change in Relative Superoxide Dismutase Concentration in *G. tenax*, *G. asiatica* and *A. nilotica* in PR* and DR**

Table 2: Relative Peroxidase Content in *G. tenax*, *G. asiatica* and *A. nilotica* in PR* and DR**

Samples	**Peroxidase Concentration (mg/ml) Mean ± S. D.**		**Percentage Change (%)**
	Post Rain	Drought	
Grewia tenax	0.24 ± 0.00	0.31 ± 0.00	22.58
Grewia asiatica	2.85 ± 0	2.85 ± 0	0.00
Acacia nilotica	2.85 ± 0.00	2.09 ± 0.00	-36.36

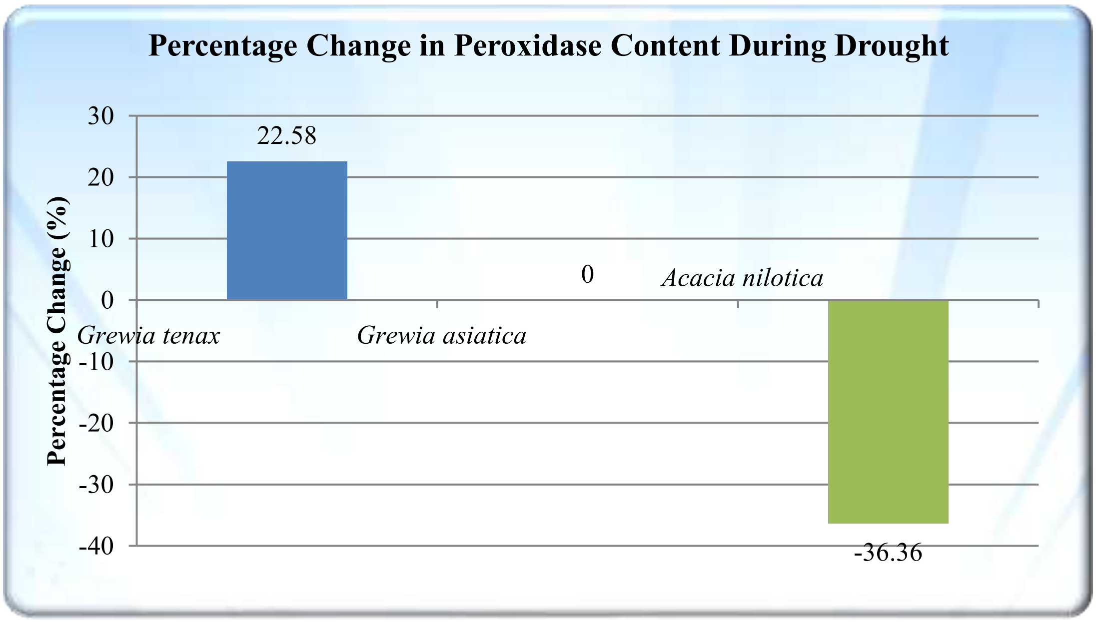

Figure 2: Percentage Change in Relative Peroxidase Concentration in *G. tenax*, *G. asiatica* and *A. nilotica* in PR* and DR**

Table 3: Relative Catalase Concentration in *G. tenax*, *G. asiatica* and *A. nilotica* in PR* and DR**

Samples	**Catalase Concentration (mg/ml)** **Mean $\pm$ S. D.**		**Percentage Change (%)**
	Post Rain	Drought	
Grewia tenax	4.980 $\pm$ 0.43	7.000 $\pm$ 0.33	28.85
Grewia asiatica	10.550 $\pm$ 0.38	13.070 $\pm$ 0.52	19.28
Acacia nilotica	10.810 $\pm$ 0.68	13.760 $\pm$ 0.87	21.43

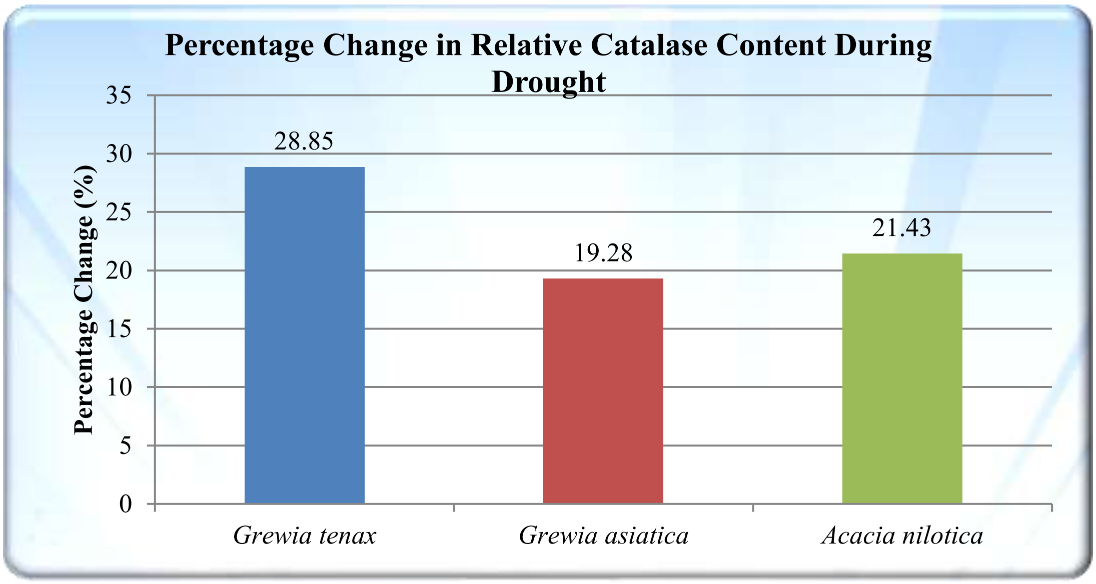

Figure 3: Relative Catalase Concentration in G. tenax, G. asiatica and A. nilotica in PR* and DR**

Table 4: Relative Total Sugar Concentration in *G. tenax*, *G. asiatica* and *A. nilotica* in PR* and DR**

Samples	**Total Sugar Concentration (mg/ml)** **Mean $\pm$ S. D.**		**Percentage Change (%)**
	Post Rain	Drought	
Grewia tenax	0.13 $\pm$ 0.00	0.26 $\pm$ 0.00	50
Grewia asiatica	0.15 $\pm$ 0.00	0.11 $\pm$ 0.00	-36.36
Acacia nilotica	0.45 $\pm$ 0.00	0.32 $\pm$ 0.00	-40.62

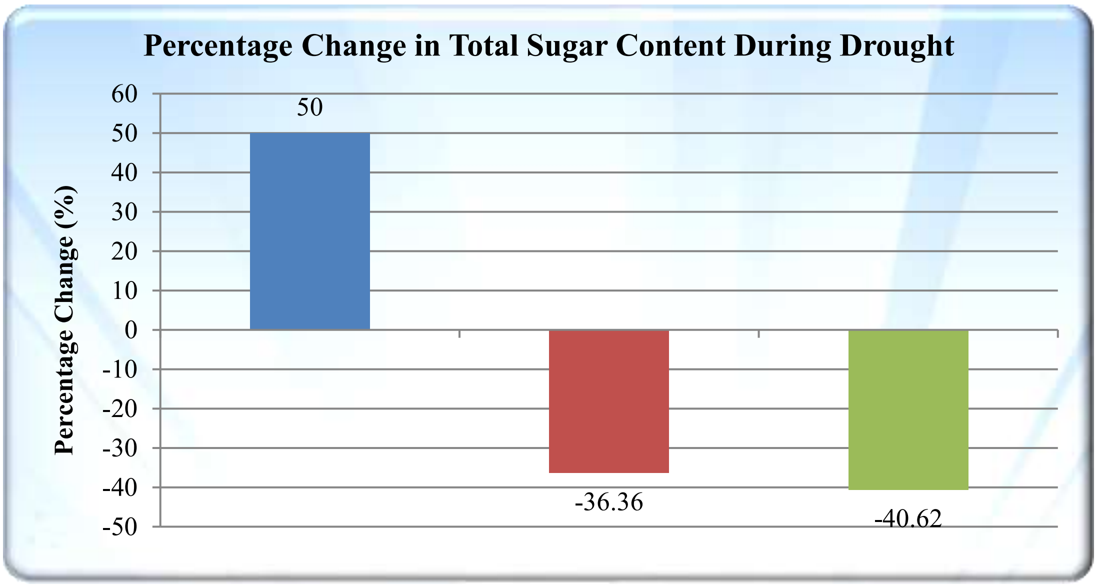

Figure 4: Percentage Change in Relative Total Sugar Concentration in *G. tenax*, *G. asiatica* and *A. nilotica* in PR* and DR**

Table 5: Relative Reducing Sugar Concentration in *G. tenax*, *G. asiatica* and *A. nilotica* in PR* and DR**

Samples	**Reducing Sugar Concentration (mg/ml) Mean ± S. D.**		**Percentage Change (%)**
	Post Rain	Drought	
Grewia tenax	0.05 ± 0.00	0.08 ± 0.00	37.5
Grewia asiatica	0.09 ± 0.00	0.07 ± 0.00	-28.57
Acacia nilotica	0.09 ± 0.00	0.05 ± 0.00	-80

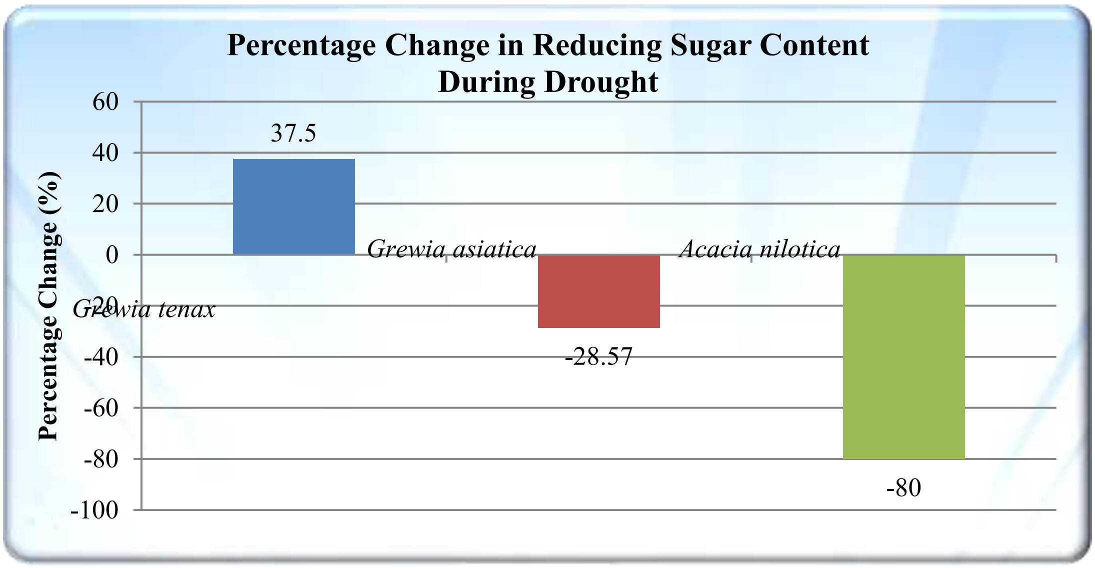

Figure 5: Percentage Change in Relative Reducing Sugar Concentration in *G. tenax*, *G. asiatica* and *A. nilotica* in PR* and DR**

Table 6: Relative Non Reducing Sugar Concentration in *G. tenax*, *G. asiatica* and *A. nilotica* in PR* and DR**

Samples	**Non Reducing Sugar Concentration (mg/ml) Mean ± S. D.**		**Percentage Change (%)**
	Post Rain	Drought	
Grewia tenax	0.07 ± 0.00	0.17 ± 0.00	58.82
Grewia asiatica	0.06 ± 0.00	0.04 ± 0.01	-50
Acacia nilotica	0.36 ± 0.00	0.26 ± 0.01	-38.46

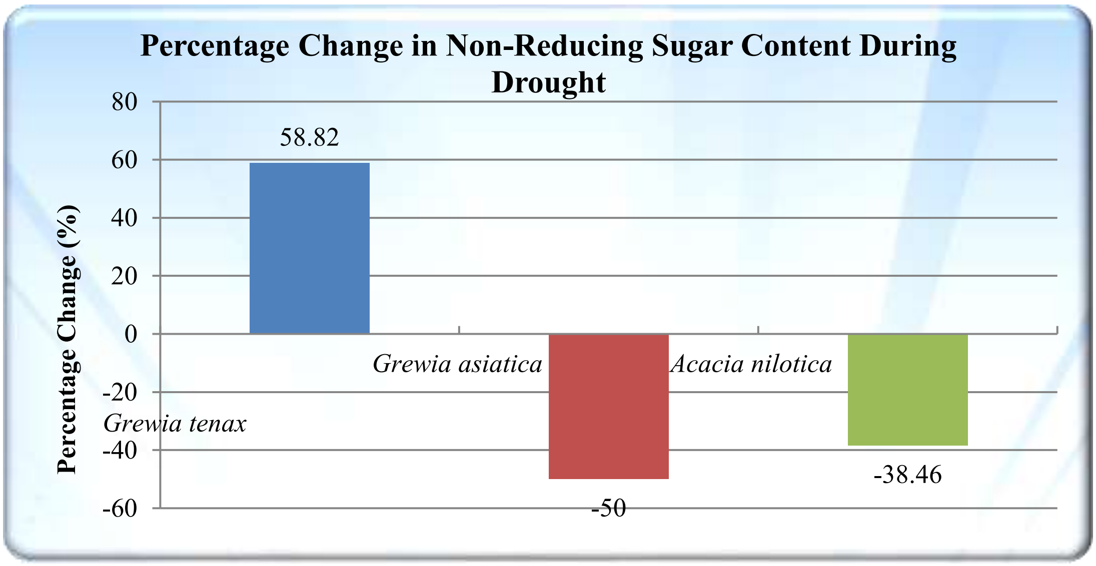

Figure 6: Percentage Change in Relative Non-Reducing Sugar Concentration in *G. tenax*, *G. asiatica* and *A. nilotica* in PR* and DR**

Table 7: Relative Starch Concentration in *G. tenax*, *G. asiatica* and *A. nilotica* in PR* and DR**

Samples	**Starch Concentration (mg/ml) Mean ± S. D.**		**Percentage Reduction (%)**
	Post Rain	Drought	
Grewia tenax	6.1 ± 0.13	6.0 ± 0.58	-1.66
Grewia asiatica	12.64 ± 0.38	5.92 ± 0.85	-113.51
Acacia nilotica	6.22 ± 1.00	3.44 ± 0.37	-80.81

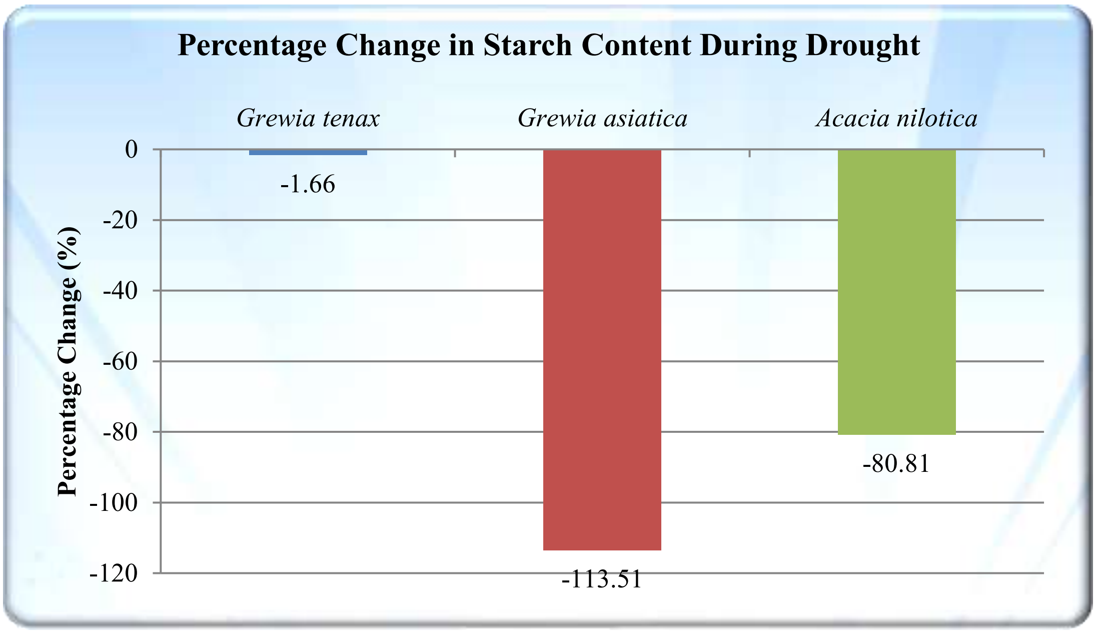

Figure 7: Percentage Change in Relative Starch Concentration in *G. tenax*, *G. asiatica* and *A. nilotica* in PR* and DR**

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