Study on synthesis, characterization and effect of silica nanoparticles on drought tolerance in *Eleusine coracana*

Thesis

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Pantnagar
June, 2018

(Sikha Snehal)
Author

CERTIFICATE

This is to certify that the thesis entitled **"Study on synthesis, characterization and effect of silica nanoparticles on drought tolerance in *Eleusine coracana*"** submitted in partial fulfillment of the requirements for the degree of **Master of Science in Agriculture** with major in **Molecular Biology and Biotechnology** of the College of Post-Graduate Studies, G.B. Pant University of Agriculture and Technology, Pantnagar, is a record of *bonafide* research carried out by **Mr. Sikha Snehal, Id. No. 51105**, under my supervision and no part of the thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation have been duly acknowledged.

(Pushpa Lohani)
Chairperson
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June, 2018

CERTIFICATE

We, the undersigned, members of the Advisory Committee of **Mr. Sikha Snehal, Id. No. 51105**, a candidate for the degree of **Master of Science in Agriculture** with major in **Molecular Biology and Biotechnology** and minor in **GENETICS AND PLANT BREEDING**, agree that the thesis entitled **"Study on synthesis, characterization and effect of silica nanoparticles on drought tolerance in *Eleusine coracana*"** may be submitted in partial fulfillment of the requirements for the degree.

(Pushpa Lohani)
Chairperson
Advisory Committee

(Sandeep Arora)
Member

(Sundip Kumar)
Member

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LIST OF ABBREVIATIONS

Abbreviation		Full form
MDA	:	Malondialdehyde
SNPs	:	Silica nanoparticles
PCR	:	Polymerase chain reaction
µg	:	Microgram
mg	:	Miligram
ml	:	Mililiter
µg /mg	:	Microgram per miligram of extract
SE(m)±	:	Standard error of mean
S.E	:	Standard error
Chl(a)	:	Chlorophyll A
Chl(b)	:	Chlorophyll B
RWC	:	Relative Water Content
ABA	:	Abscissic acid
RWC	:	Relative water content

Introduction

Chapter 1 INTRODUCTION

Due to the "population explosion" in today's world, the load on increasing the crop production to satisfy the hunger of the ever increasing population has also incremented drastically over the past few decades. To prevent the humankind from the agony of low food production, intensive agriculture is mainly practiced all round world. Large scale application of fertilizer, herbicides and pesticides is done in order to get more production per unit area. A heavy use of chemical fertilizer culminates in severe environmental issue in agriculture. Out of the total synthetic fertilizers applied, only half is utilized by the plants and the major portion of these chemical fertilizers are leached down the soil. This contributes to pollution of the ground water and the nearby water bodies. The effect of synthetic fertilizers on one hand increases the crop production and on the other hand it causes soil mineral imbalance and reduces the soil fertility. The recent widespread application of the synthetic chemicals has led to the irreversible changes in the soil structure, soil microbial flora, minerals cycles and even on the food chains leading to drastic effects on the future consumers. In the recent scenario biotic stress, abiotic stress and land degradation are three major concerns in agriculture.

Among the different abiotic stresses drought is a major abiotic stress that is a cause of severe agony to humankind. It is generally defined as an extended period of (a season, a year, or several years) of deficient precipitation compared to the statistical multi-year average for a region that results in water shortage for some activity, group, or environmental. It is major abiotic stress that culminates in severe loss of yield to crops and has proven to be more scourging than any other physical hazard. Since 1900 more than 11 million people have died as a consequence of drought and more than 2 billion have been affected by drought **(FAO, 2013)**. In order to endure the problems related to drought an important approach is to generate crops with enhanced drought tolerance, by utilizing biotechnology and molecular biology techniques. This strategy requires the identification of traits relating to drought tolerance and then of the genes that determine these traits. Plants often carry genes that enable them to maintain their performance despite of severe moisture stress conditions. Such plants are referred to as

drought tolerant and a number of genes that impart this trait have been identified and characterized. The genetic and molecular basis of drought tolerance has been investigated extensively and the genes encoding drought-related transcription factors and functional proteins have been identified **(Grover *et al.,* 2011)**. These stress-inducible genes can be classified into two groups. The first group includes proteins that most probably function in abiotic stress tolerance. These include molecules such as chaperones, late embryogenesis abundant (LEA) proteins, osmotin, antifreeze proteins, mRNA-binding proteins, key enzymes for osmolyte biosynthesis, water channel proteins, sugar and proline transporters, detoxification enzymes, and various proteases **(Hayano-Kanashiro *et al*., 2009)**. The second group is comprised of regulatory proteins. These include various transcription factors, protein kinases, protein phosphatases, enzymes involved in phospholipid metabolism, and other signaling molecules such as calmodulin-binding proteins.

Drought tolerance is a quantitative trait involving complex genetic control as it is multigenic in nature. Till date researchers have not been completely successful in developing drought tolerant cultivars by targeting a single gene. With increasing knowledge to decipher comprehensively the complicated mechanisms of drought tolerance in model plants, it is a formidable challenge to modify genetically such complex, interacting metabolic systems in crops to achieve greater production under drought conditions. It may probably require considerable time. The use of the genetically modified crops having drought tolerance properties is both challenging as well as expensive. Prior to market approval, genetically modified plant varieties, including drought-tolerant plant varieties, require in most countries an extensive food and environment safety assessment, contrary to similar conventionally bred crops. These limitations provide set back to the research on genetically modified crops, as a result recombinant drought tolerant crop have no contribution in ensuring food security of the nation. Another constraint accompanying the genetically modified crops is the surety, whether the transgenes may impart increased fitness under actual field conditions or not. There is also an apprehension of change in population ecology of wild relatives with the introduction of genetically modified crops in the field. As a result both direct as well as indirect environmental assessment of the newly introduced genetically modified drought-tolerant crops becomes difficult to estimate. Even with

advances in basic research and genetic technology, there remains a considerable margin of uncertainty with predicting introgression from crops to wild relatives as well the desired expression of the introduced gene for drought tolerance.

In order to overcome this severe problem, some alternative ways must be discovered to increase the crop production with limited land and water resources. This can be possible only by increasing the resource use efficiency along with minimum damage to the soil and environment. One way in which this can be done is by use of nanotechnology. The new and emerging branch of science i.e., Nanotechnology has the potential to act as an alternative for the conventional agriculture system and thus can be a cure for the major problems faced by agriculture in present times. Nanotechnology has emerged as one of the leading fields of the science having tremendous application in diverse disciplines including biotechnology and agriculture; the use Nano-fertilizers which have many advantages over the conventional fertilizers can be seen as the new idea for improving the quality of the agricultural system.

Nanoparticles (NPs) have high surface area; this is very important property for the systemic and targeted release of the chemicals for plants in improving the mineral deficiencies and other growth and development parameters. This is mainly due to the physiochemical properties of nanoparticles such as the large surface area to volume ratio which provide an enormous reactive interface between the particle and its local environment. For sustainable development, application of biosynthesized nanoparticles can be done in the agriculture field. They can be used for site directed delivery of different nutrients needed for increase in growth, development as well as productivity of plants. The nanoparticles are added at low levels as compared to the chemical fertilizers. The refined use of nanoparticle also reduces the harmful effect of synthetic fertilizers on the environment. Nanoparticles play an importance role in the protection of plant from diseases, pests and pathogens. The nanoparticles can also affect the modification of the plant gene expression, which in turn results in the changes in the biological and metabolic pathways controlled by the associated genes, and thus affects the plants growth and development.

Among the other nanoparticles silica nanoparticles has much importance in the life cycle of plants. Fertilizers having silica nanoparticles are gaining importance in agriculture due to its beneficial effects on the plant growth and development. Silica is

the second most abundant element present in the soil. It is one of the four beneficial elements after sodium, cobalt and selenium. It is mainly absorbed by the plants roots in the form of silicic acid through the aquaporin type channel (nod26-like intrinsic proteins and NPs **(Ma *et al*., 2006).** Based on the amount of biogenic silica present in their tissues the plants are classified into 3 groups: accumulators, excluders and intermediate type. Rice is the example of accumulators, tomato under excluders and *Urtica dioica* are the intermediate types **(Mitani and Ma, 2005; Trembath-Reichert *et al.*, 2015).**

Silica plays an important role in protecting the plant from biotic and abiotic stress tolerance. It has been observed in some crops (rice and equisetum) the absence of silicon increases the susceptibility to fungal pathogens **(Datnoff and Rodrigues, 2005).** Application of silica also increases plant vigor and resistance to various diseases **(Azeem *et al.,* 2015)**. Under salinity stress conditions, the application of silica nanoparticles has shown to increase the leaves fresh and dry weight, chlorophyll content and proline accumulation in plants. The tolerance of plants to endure abiotic stress has increased with the increase in the accumulation of free proline, free amino acids content, antioxidant enzymes activity as a result of application of silica nanoparticles **(Kalteh *et al*., 2014; Haghighi *et al*., 2012; Li *et al*., 2012; Siddiqui *et al*., 2014).** The application of silica nanoparticles have shown to increase the plant growth and development by enhancing the gas exchange via stomata and chlorophyll fluorescence parameters, which are as follows net photosynthetic rate, stomatal conductance for gas exchange, transpiration rate, potential activity of PSII, actual photochemical efficiency, electron transport rate, effective photochemical efficiency and photochemical quench during water stress **(Siddiqui *et al*., 2014; Xie *et al*., 2012).**

Chemical synthesis of silica nanoparticles is heath hazardous process. Therefore the use of green synthesis of silica nanoparticles can be done with the use of bamboo, sugarbeet and rice husk. Rice husk is a major agricultural waste and its disposition is of great concern for the environment.

Synthesis of silica nanoparticle from rice husk can cater to this concern in two ways:

a) Rice husk can be used to produce silica nanoparticles and easily disposed off.

b) These silica nanoparticles when applied to the plants will not only help in good growth and production of plants but also helps the crops to tolerate drought stress as well as attack of pest and pathogens. This will not only increase earning of the farmers as well as save the soil from degradation which is otherwise caused by the indiscriminate use of fertilizers and pesticides.

Objectives :

In view of above, present investigation was undertaken with the following objectives:-

1. To synthesize silica nanoparticles from rice husk that is a major agricultural waste.
2. Characterization of silica nanoparticles.
3. Study of morpho-physiological characters in different genotypes of *Eleusine coracana* treated with silica nanoparticles and subjected to experimental drought stress.
4. Study of biochemical parameters (Chlorophyll, Malondialdehyde, proline content) in contrasting genotypes (one drought tolerant and another drought sensitive) of *Eleusine coracana* treated with silica nanoparticles and subjected to experimental drought stress.

Review of Literature

REVIEW OF LITERATURE

Agriculture has always been the backbone of most of the developing countries. It does not only fill the people abdomen but also fuel the economy. One of the major challenges faced by agriculture today is the global water shortage caused by worldwide climate change and increasing world population. Greenhouse emission rise has led to erratic precipitation, increase in arid land area, desertification and ultimately results in reduced crop productivity. Water shortage is threatening sustainable crop production as agricultural activities uses 75% of global water and in many developing countries irrigation requires over 90% of it **(UNEP, 2009; Yang *et al.*, 2010)**. Researchers anticipate that developing countries will be severely affected due to climate change by 2030, because: (a) Sub tropics and tropics will be facing the greatest impact due to climate change (b) Developing countries are to witness most of the predicted population growth till 2030 (c) Most of the workforce in developing countries, is involved in agriculture and related activities **(Reynolds and Ortiz, 2010)**. The convergence of variable climate and population growth is anticipated to jeopardize food security at a worldwide scale.

Drought is the single largest abiotic stress factor leading to reduced crop yields. Approximately one-third of the earth's land area is arid or semi-arid. The situation is aggravated by the shortage of water resources because of widespread water pollution and unpredictable climatic change. Water availability is particularly critical for agricultural crops to maintain high yields in variable growing seasons. Thus, agricultural drought, which is also known as water deficiency, adversely affects plant and crop production by reducing leaf size, stem extension and root proliferation. It also disturbs plant water and nutrient relations and inhibits its water-use efficiency, resulting in reduced rate of photosynthesis, less number of leaves and tillers etc. as shown in **(figure 2.1)**. During periods of severe drought, the losses can be much higher and can potentially result in complete crop failure. Drought is currently the leading threat to the world's food security. Maintaining food security therefore in today's scenario will demand systemic approaches involving the use of drought tolerant germplasm as well as other agricultural strategies that could provide drought tolerance to crop plants **(Reynolds and Ortiz, 2010)**.

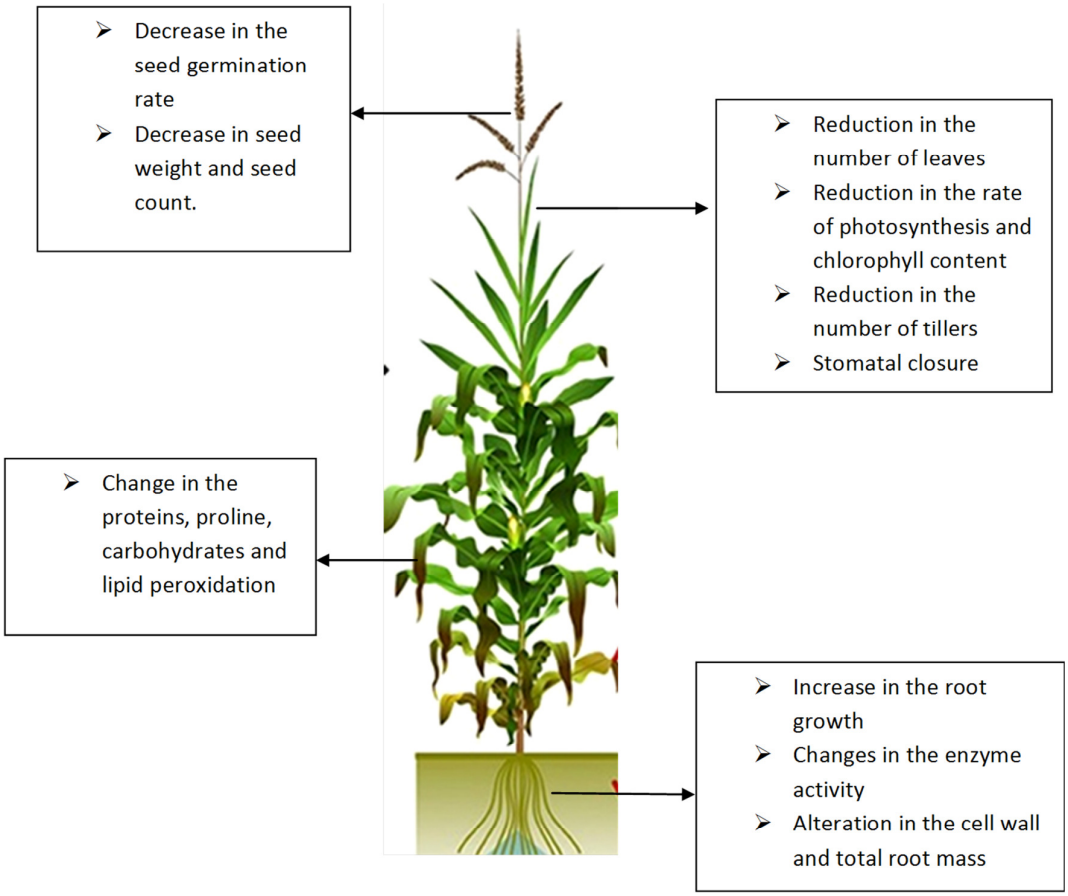


Fig. 2.1: Schematic representation of effect of water stress on finger millet plant

One of the important germplasm used in drought tolerant studies is finger millet which is a potential climate-resilient and nutracuetical crop, maintaining high socio-economic importance in the context of sustainable agriculture of Indian and African semi-arid tropic regions **(Gull *et al.*, 2014)**. The grains are a rich source of calcium, iron, dietary fiber, essential amino acids such as methionine, isoleucine, leucine, phenylalanine and are free from gluten **(Chandrashekar, 2010)**.Seed coat of finger millet is highly enriched with variety of phytochemicals such as dietary fiber and polyphenols. It also contain very high amount of minerals especially calcium **(Devi *et al.*, 2014)**. Rich protein, vitamins, minerals, fiber content and energy containing grains of finger millet are crucial for the diets of pregnant and lactating mothers, and children as well for the economy of marginal farmers **(Vadivoo *et al.*, 1998)**.

Beside rich nutritional value, it also has the C4 carbon assimilation mechanism of photosynthesis which helps in utilizing water efficiently under arid and hot

conditions without having any severe effect on the yield **(Wang *et al.*, 2009)**. Thus finger millet can flourish under a variety of harsh environmental and organic conditions like low fertility soils **(Gull *et al.*, 2014)**. Finger millet have different genotypes with genes for early and vigorous growth, large panicle size, increased finger number and branching as well as high-density grains. Some genotypes are water-proficient with high CO_2 fixation rates and negligible leaf area performing extraordinary well in semi-arid climates **(Gupta *et al.*, 2014)**. However such hardiness and ability to withstand environmental stresses varies amongst the different genotypes. Therefore development of suitable approaches for the utilization of genomic resources of this crop is important for moving towards climate resilient agriculture.

2.1 Metabolites and Osmoprotectants In Drought Response:

2.1.1 ABA Metabolism

One of the quick responses of plants to drought stress is the accumulation of ABA in plant cells **(Hsiao, 1973)**, which triggers ABA-inducible gene expression **(Yamaguchi-Shinozaki and Shinozaki, 2006)** and stomatal closure to reduce transpirational water loss **(Schroeder *et al.*, 2001)**. ABA *de novo* biosynthesis occurs in leaves, stems, and roots of almost all plant species. The initial four catalytic steps of ABA biosynthesis, which involves the conversion of β-carotene to xanthoxin, primarily take place in plastids (e.g. chloroplasts in the leaf) via ABA1/low expression of osmotically responsive gene 6 (LOS6), ABA4, and 9-*cis*-epoxycarotenoid dioxygenase (NCED) enzymatic activities, while the last two steps occur in the cytoplasm to produce bioactive ABA via ABA2 and ABA3/LOS5 **(Wasilewska *et al.*, 2008; Wan *et al.*, 2009)**. In the cytoplasm, ABA is readily perceived by the recently identified ABA receptors (PYR1/ABI1, **Park *et al.*, 2009**), which then relay the signal to the AREB TFs to regulate the ABA-dependent expression of numerous downstream target genes for drought adaptation. ABA can also be directly converted from its inactive glucose ester conjugate storage form in vacuoles to bioactive ABA via β-glucosidase, AtBG1, **(Lee *et al.*, 2006)** and *vice versa*. After rehydration or drought release, ABA is catabolized to phaseic acid or dehydrophaseic acid by Cyp707A-mediated hydroxylation to maintain its homeostasis **(Kushiro *et al.*, 2004)**.

LOS5/ABA3 is a key enzyme working in the last step of ABA biosynthesis, and its loss-of-function mutant *los5* had reduced tolerance to drought, salt, and cold stresses

(Xiong *et al.*, 2001). The constitutive or drought-inducible over-expression of *LOS5* in rice significantly increased the spikelet fertility and yield of transgenic plants under field conditions **(Xiao *et al.*, 2009)**. NCED is drought-inducible and a rate-limiting enzyme for ABA biosynthesis.

2.1.2 Phospholipid Signaling

Membrane-component phospholipids and their metabolites are important second messengers in plant development and in response to environmental stimuli **(Testerink and Munnik, 2005; Xue *et al.*, 2009)**. Phosphatidylinositol-specific phospholipase C2 (PtdIns-PLC2) hydrolyzes PtdIns-4, 5-biphosphate (PtdIns(4, 5)P2) to produce Inositol 1, 4, 5, -triphosphate (Ins(1, 4, 5)P3), which acts as a second messenger for Ca^{2+} release from internal storages. The Ca^{2+} oscillation mediated by PtdIns(4, 5)P2 and Ins(1, 4, 5)P3 is crucial for guard cell movement as well as for other plant growth processes. In addition, phospholipase D (PLD) hydrolyses phospholipids at the terminal phosphodiester bond to generate phosphatidic acid (PA), which interacts with and represses the activity of ABI1, resulting in enhanced ABA response and stomatal closure **(Zhang *et al.*, 2004b)**. PA is likely the functional link between the phospholipid and ABA signaling pathways with respect to stomatal movement and drought response.

Arabidopsis PLC **(Tasma *et al.*, 2008)** and *PLD* **(Katagiri *et al.*, 2001)** genes are both induced by dehydration and involved in drought tolerance **(Sang *et al.*, 2001; Hong *et al.*, 2008)**. Maize over-expressing *ZmPLC1* **(Wang *et al.*, 2008)** exhibited a higher cellular solute content and increased rate of photosynthesis, whereas canola over-expressing *BnPtdIns-PL2* **(Georges *et al.*, 2009)** exhibited a lower rate of stomatal transpiration and a smaller stomatal aperture.

2.1.3 Heat or Cold shock protein (HSP or CSP) chaperons:

Drought, high temperature and salinity cause the misfolding and denaturation of several proteins and RNAs. Some genes induced by such stresses code for proteins that secure the conformation of other RNAs, proteins or cell structures. These are the numerous CSPs and HSPs required for quick adaptation to temperature changes and also for quick recovery following heat or cold release. Small HSPs (sHSP) are the major family of HSPs which are induced by stress in plants. For example, Endoplasmic reticulum (ER) luminal binding protein (BiP), which is a member of HSP 70 protein

chaperone family, when over expressed resulted in delayed drought induced leaf senescence **(Valente *et al.*, 2009)**.

2.1.4 Late Embryogenesis Abundant (LEA) protein:

These accumulate in the embryos at the time of seed desiccation and are also generated due to dehydration, salinity, cold and ABA treatment in the vegetative tissues. These highly hydrophobic proteins are involved in providing adaptive responses to hyperosmotic conditions by maintaining the protein or membrane structure, binding to water, sequestration of ions and acting as molecular chaperons **(Bray, 1997)**.

2.1.5 Osmolytes:

Accumulation of metabolites functioning as adaptive osmolytes or antioxidants can improve tolerance to drought. The compatible osmolytes includes certain amino acids (notably proline), quaternary ammonium compounds (e.g. glycine betaine, proline betaine, B-alanine betaine, and choline-*O*-sulfate), and the tertiary sulfonium compound 3-dimethylsulfoniopropionate (DMSP). These compounds share the property of being uncharged at neutral pH, and are of high solubility in water. Moreover, at high concentrations they have little or no perturbing effect on macromolecule-solvent interactions. Unlike perturbing solutes (such as inorganic ions) which readily enter the hydration sphere of proteins, favoring unfolding, compatible osmolytes tend to be excluded from the hydration sphere of proteins and stabilize folded protein structures. These compounds are thought to play a pivotal role in plant cytoplasmic osmotic adjustment in response to osmotic stresses, protection of membranes and proteins from dehydration and act as reactive oxygen scavengers. Hence these solutes are called as osmoprotectants. Osmolytes like glycine betaine are accumulated by many plants under drought, salinity and temperature (high and low) stresses. Its precursor is choline and two enzymes viz., choline monooxygenase and betaine aldehyde dehydrogenase play crucial role in its synthesis in bacteria and plants. It serves as an osmolyte by lowering the osmotic potential of the cell and thus prevents movement of water from the cell, as well as a compatible solute by preventing denaturation of macro-molecules like enzymes/proteins **(Grieve and Grattan, 1983)**.

2.2 Morpho-physiological adaptations in response to drought stress:

2.2.1 Root traits:

Root system has a critical role to play in response to moisture deficit stress. In some plants there is the robust capability to enhance their root growth in the early phase of the drought to absorb water from the deeper soils. Researchers have found a positive correlation between degree of drought resistance and the penetration ability of the roots **(Mohamed *et al.*, 2002)**. It is the depth and range of soil moisture that mainly influences rooting depth, volume and distribution. Plants very dynamically adapt to moisture deficit conditions by modifying their root system architecture. This is done by altering their root growth in diverse ways, depending upon the species **(Herder *et al.*, 2010; Smith *et al.*, 2012; Malamy, 2005)**. Severe water deficit reduces root elongation, branching and cambium layer formation. Root tips get suberified in arid soils **(Kramer, 1969)**. It has been observed that root to shoot ratios often increase in drought conditions **(Wu *et al.*, 2000; Fulda *et al.*, 2011)**. Thus in order to describe the ability to tolerate drought, the root to shoot ratio is used as an important criteria in many studies now **(Pallardy, 2010; Tavakol *et al.*, 2007; Champoux *et al.*, 1995; Ali *et al.*, 2009).**

2.2.2 Leaf traits

On facing severe water shortage, plants respond by drooping or rolling their leaves due to the loss of cell turgor pressure, a phenomenon called as wilting **(Poorter, 2008)**. Wilting is a passive movement of leaves for preventing excessive consumption of water under moisture stress conditions. Apart from this, some plants have the ability to actively adjust leaf blade orientation in parallel to the incident solar radiation. Thus the interception of the incident solar radiation can be regulated by the phototropic movement of the plant leaves. Upright leaves during moisture stress lose less water and have a better overall water status as they receive less radiation. This indicates that erect leaves form an effective mechanism of drought avoidance **(Stevenson and Shaw, 1971; Meyer and Walker, 1981; Oosterhuis *et al.*, 1985)**.

Leaf rolling, a drought adaptive trait that is induced due to turgor pressure can be delayed by osmotic adjustments **(Hsiao *et al.*, 1984)**. Leaf rolling is a common response of plants to drought. Both active and passive movements of the leaf perform a role in reducing leaf surface temperature by reducing the incident solar radiation and

thus protecting the plant from excessive water loss. Plants having increased resistance to drought have been found to have various xeromorphic structures like smaller and thicker leaves, denser stomata, more epidermal trichomes, thick palisade tissue and more developed vascular bundle sheath **(Esau, 1960)**.

Under the conditions of moisture stress, the role of stomata is particularly important in adjusting the rate of transpiration. Stomata of the plants tolerant to drought stress are sensitive to moisture stress, and they close when the leaf water status is about to approach wilting levels, thus exerting a drought avoidance function. The osmotic potential changes in the guard cells bring about the movement of stomata, and K+ is one of the major ions that alters the osmotic potential in the guard cells **(Hosy *et al.*, 2003)**. There are several proteins such as the substrate binding proteins (ABA binding protein, GTP binding proteins, acetyl choline receptors and light receptors), channels and pumps on the guard cell's plasma membrane involved in the control of movement of stomata **(Cousson and Vavasseur, 1998)**. These proteins play a crucial role in the perception and transduction of stress signals in the guard cells and form the fundamental basis for the stomatal movement under moisture stress conditions. Studies suggest that ABA signaling triggered by the roots in drying soil profile also controls the movement of stomata **(Schroeder *et al.*, 2001; Zhang *et al.*, 2006)**. Stomatal movement constitutes an important strategy by the plants to conserve water at the time of drought stress. Therefore a proper insight into the mechanisms associated with stomatal movement is crucial for understanding the factors related to drought tolerance.

Other than these, other leaf associated traits like epidermal hairs, relative water content, water loss rate, cuticular wax and canopy temperature are used as important parameters for the appraisal of drought avoidance.

2.2.3 Photosynthesis

Light reaction as well as the assimilatory efficiency of dark reaction is affected by water deficiency, thus causing reduction in contents of photosynthetic output **(Herppich and Peckmann, 2000; Pagter *et al.*, 2005; Basu *et al.*, 1999)**. In order to assimilate atmospheric carbon dioxide plants undertake three types of photosynthetic mechanism namely C3, C4 and Crassulacean acid mechanism (CAM). In general, plants using the C4 and CAM pathways can adapt better to drought stress conditions **(Ashraf and Harris, 2013)**. C4 plants can concentrate their carbon dioxide within the

bundle sheath cells using a metabolic pump and they can separately bring about the fixation of carbon dioxide in the bundle sheet and in the mesophyll cells **(Chaves *et al.*, 2003)**. This mechanism particularly enhances the water use efficiency in C4 plants and therefore provides better chances for survival in arid regions. In the CAM pathway the water use efficiency is dramatically increased, useful for adaptations in extremely arid environments. This is achieved by opening the stomata and performing carbon dioxide absorption and fixation in the night and keeping the stomata closed during the day to reduce transpirational water loss **(Chaves *et al.*, 2003)**. Researchers reported that phosphoenol pyruvate carboxylase, the key enzyme of the CAM pathway is transcriptionally regulated by water stress **(Sanchez *et al.*, 2006)**.

2.2.4 Chlorophyll content

In the chloroplast, thylakoid membrane forms the structural foundation for the absorption, transmission and transformation of light. This is performed by the various pigment protein complexes located on the membrane of thylakoid. Moisture deficit conditions results in the destruction of membrane system in the plant cells including the thylakoid membrane leading to disruption of physiological processors. It has been found that a positive correlation exists between decreasing soil moisture and declining chlorophyll content, and the total chlorophyll content decline is mainly due to the destruction of chlorophyll A molecules **(Sayed, 2003; Li *et al.*, 2006; Kocheva *et al.*, 2004; Guo *et al.*, 2008)**.Thus, the plants which hold the ability to maintain higher chlorophyll content despite moisture deficit conditions are more efficient in utilizing light energy and hence believed to have increased drought tolerance.

2.2.5 Antioxidant defense mechanism

Oxidative stress generally accompanies drought stress. Show plants possess antioxidant defense mechanism as one of the drought tolerance mechanisms. The intracellular formation of reactive oxygen species (ROS), under normal conditions is under dynamic equilibrium. This dynamic equilibrium is disturbed when plants are exposed to drought stress and results in over accumulation of reactive oxygen species in the cells, which injuries the cells and ultimately leads to cell death **(Cruz, 2008)**. Damage to membrane phospholipids and fatty acids occurs due to excessive ROS production resulting in peroxidation of the membrane lipids **(Moller, 2001)**. Also it leads to disturbance of the spatial configuration of several enzymes and proteins,

causing membrane permeability with iron leakage, perturbations in the metabolism and destruction of chlorophyll and other pigments, eventually leading to the death of the plant **(Gill and Tuteja, 2010)**. Excessive reactive oxygen species production results in induction of lipid peroxidation, carboxylation of protein, and DNA damage. This results in impairment of function, leading to catastrophic cascade of events **(Moller *et al.*, 2007)**. To safeguard the cells from harmful effect of over accumulation of reactive oxygen species, plants have evolved several non-enzymatic and enzymatic antioxidant defense mechanisms for maintaining the homeostasis of the intracellular redox state. The enzymatic components include catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPX), ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR), monohydroascorbate reductase (MDAR), thioredoxin peroxidase (TPX), glutathione S-transferase etc. **(Apel and Hirt, 2004; Mittler *et al.*, 2011)**. From these the SOD, H2O-H2O, cycle, the AsA-GSH cycle, GPX and CAT cooperate to constitute the main ROS scavenging pathways **(Dixon *et al.*, 1998; Mittler, 2002; Miyake, 2010)**. Balance between the activities of SOD, CAT and APX is crucial for maintaining the homeostasis of H_2O_2 in plants. The non-enzymatic components of the antioxidant defense mechanism comprises of various reducing substances glutathione (GSH), ascorbic acid (AsA), carotenoids, cytochrome f (cyt f), alfatocopherol (vitamin E), anthocyanins, flavanones etc. **(Blokhina *et al.*, 2003; Apel and Hirt, 2004; Gill and Tuteja, 2010).**

Thus it could be concluded that water is vital resource for the survival of the plant and is also for the proper transport of nutrients, so in case of drought and water stress the vitality of the plant is affected **(Martinez-Vilata and Pinol 2002),** there is marked reduction in the growth **(Bigler *et al.,* 2006),** and increased mortality of the plants **(Rebetez and Dobbertin, 2004).** Plants often carry genes that enable them to maintain their performance despite of severe moisture stress conditions. Such plants are referred to as drought tolerant.

With the concern of providing food to a big population there has to be a new technology giving more yield even in the stress condition. In that manner, nature is complex, which will have imbalances which directly affects plants and crops, and indirectly animals and human. With the growing limitation in arable land and water resources, the development of agriculture sector is only possible by increasing resources use efficiency with the minimum damage to agro ecology through effective

use of modern technologies. The new and emerging branch of science ie., Nanotechnology has the potential to act as an alternative for the conventional agriculture system and thus can be a cure for the major problems faced by agriculture in today's world. Nano-technology has the potential to revolutionize agricultural systems. In order to overcome this severe problem some alternative ways must be discovered to increase the crop production with limited water resources. This can be possible only by increasing the resource use efficiency along with minimum damage to the soil and environment.

2.3 Nanotechnology:

Nanotechnology, a new emerging and fascinating field of science, permits advanced research in many areas, and nanotechnological discoveries could open up novel applications in the field of biotechnology and agriculture. Nanotechnology is working with the smallest possible particles which raise hopes for improving agricultural productivity through encountering problems unsolved conventionally. The word "Nanotechnology" is derived from the Greek word "Nano" which means "dwarf" meaning one billionth part of a meter. "Nano" means one-billionth, thus nanotechnology refers to materials that are measured in a billionth of a meter. A nanometer is 1/80, 000 the diameter of a human hair or approximately ten hydrogen atoms wide. For a particle to be considered as nanoparticle the dimension must be less than 100nm **(Thakkar *et al.,* 2010).** The surface area to volume ratio of nanoparticles is very high. The unique properties of nanoparticles make it highly applicable to agriculture research, which in turn can help in solving numerous agricultural problems. Nanoparticles have high surface area, sorption capacity, and controlled-release kinetics to targeted sites making them very proficient in smart and targeted delivery. Nanostructured materials having a particular element (as silica in our experimental setup) can increase the nutrient use efficiency through mechanisms such as targeted delivery, slow or controlled release. They could precisely release their active ingredients in responding to environmental triggers and biological demands.

The biological role of Silica in plants has not been deeply studied by plant physiologists because it has not been classified as essential plant element **(Ma and Yamaji, 2006)**. Nevertheless, many researchers believe that Silica is an important element for plants **(Siddiqui and Al-Whaibi, 2014; Epstein, 2009; Gong and Chen, 2012; Currie and Perry, 2007).** Silica, acts as a physic-mechanical barrier, as it is a

part of the epidermal cell walls and vascular tissues in stems, pods, leaves and bark **(Siddiqui and Al-Whaibi, 2013)**. Various studies indicate that Silica might decrease the negative effects of oxidative stress and offer slight resistance to some abiotic and biotic plant stressors **(Liang *et al.,* 2007, Ma 2004** and **Pei *et al.,* 2010)**. Thus, using Silica instead of herbicides and pesticides could reduce harmful environment effects **(Vasanthi *et al.,* 2012; Balakhnina and Borkowska, 2013; Karmollachaab *et al.,* 2013)**. The positive effects of the Silica (in bulk size) have been demonstrated in plants by investigators; however, compared with Si bulk size, absorption of Si in plants is greater when nanoparticles of silicon are used **(Suriyaprabha *et al.,* 2012b)**. Nanomaterials consist of particles smaller than 100 nm. The small size of Silica nanoparticles implicates unique physical, chemical and biological properties **(Monica and Cremonini, 2009).** At the global level, the use of nanotechnology in agriculture is at a nascent stage, yet it is increasing. Nanosciences led to the development of a wide range of applications for enhancing plant growth **(Nair *et al.,* 2010)**.

2.4 Method for the synthesis of silica nanoparticles

There are various methods that have been used to obtain silica particles can be categorized into two main approaches: top-down and bottom-up **(Reverchon and Adami, 2006**; **Klabunde, 2001)**. Top-down is characterized by reducing the dimension of the original size by utilizing special size reduction techniques (physical approach). Bottom-up or chemical approach involves a common route used to produce silica nanoparticles from atomic or molecular scale. Some of the widely used methods to synthesize silica nanoparticles are sol-gel process, reverse microemulsion, and flame synthesis. The sol-gel process is widely used to produce pure silica particles due to its ability to control the particle size, size distribution and morphology through systematic monitoring of reaction parameters. Some of the methods used to synthesize silica nanoparticles are reverse microemulsion and flame synthesis and widely utilized sol-gel **(figure 2.2)**. In reverse microemulsion, the surfactants molecules dissolved in organic solvents forms spherical micelles **(Tan *et al.,* 2011)**. In the presence of water, the polar head groups organize themselves to form microcavities containing water, which is often called as reverse micelles. The major drawbacks of the reverse microemulsion approach are high cost and difficulties in removal of surfactants in the final products.

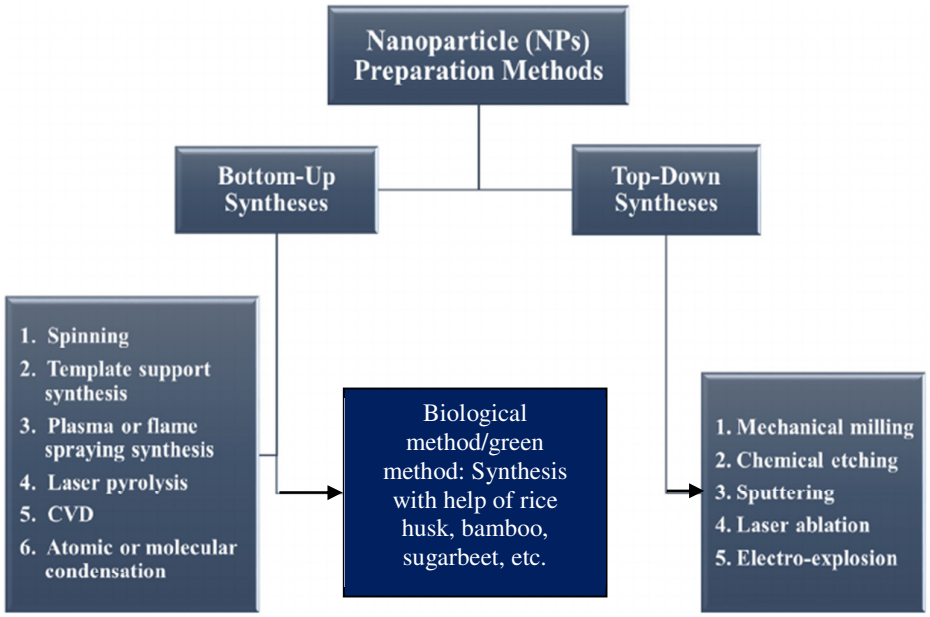


Fig. 2.2: Different methods for the synthesis of nanoparticles.

But the objective was not to synthesize silica nanoparticle by the chemical methods, so the synthesis of silica nanoparticles was done in a green way.

2.5 Green synthesis of silica nanoparticles:

Considering the immense importance of silica nanoparticles in agriculture, its green synthesis and frequent use in agricultural field is the need of the house. Along with drought, waste management is also one of the most complex and cost-intensive public services. Nanotechnology is an effective tool in the field of productive science, which helps in the utilization of agricultural food waste into bio-energy for human welfare. Energy recovery from waste can play a role in minimizing the impact of agricultural waste on the environment with the additional benefit of providing source for the synthesis of nanoparticles. Preparation of silica nanoparticles from rice husk is a good alternative to the conventional production materials from tetraethylorthosilicate (TEOS), tetramethyl ortho silicate (TMOS), sodium silicate solution (water glass) which are more expensive and carcinogenic **(Kumar *et al.*, 2013).**

Rice husk, also known as rice hull, is the outer covering on a seed or grain of rice. It is formed from hard materials; including silica and lignin, the presence of these compounds protect the seed during the growing season. Approximately 600 million tons of rice crops are

produced all around the world every year. After milling each kg of rice results in roughly 0.28 kg of rice husk as a by-product of rice production during milling. 20% of the rice plant accounts for the husk, therefore the production of rice husk turn out to be approximately 120 million tons silica comprises about 60% of rice husk **(Pham *et al.*, 2016)**. Rice husk as such is considered as an agricultural waste material from the rice milling process and it is usually eliminated by dumping and/or burning. Burning of rice husk is the most economical method for the disposal but this practice generates smoke, as well as breathable dust that contains crystalline silica and other health hazard substances, causing worldwide environmental and health problems. Rice husk can easily be collected from rice mills and can be utilized in synthesis of silica nanoparticles. Silica extracted from rice husk.

Various steps are involved in extraction of silica from rice husk. The silica obtained from the rice husk is further processed to form silica nanoparticles.

2.5.1 Acid washing step:

An acid washing step was used to remove the small quantities of minerals prior to silica extraction from rice husk ash in the following manner. Rice husk ash samples were dispersed in distilled water, and the pH was adjusted to 5 or 7 using HCl. These dispersions were stirred for 2 h, filtered through Whatman No. 41 (ashless filter paper) and then the rice husk ash residues were washed with water. The residues were used for silica extraction. **2.5.2 Silica extraction step:**

Silica was extracted from Rice husk ash adapting the method **of (Kamath and Proctor, 1998).** Sodium hydroxide was added to the washed rice husk ash. The solutions were filtered through Whatman No. 41 and the carbon residues were washed with boiling water. The filtrates were titrated with HCl with constant stirring to pH 7. Silica gels started to precipitate when the pH decreased to <10. The silica gels formed were aged for 18 h. The gel was then centrifuged for 20 min at 1200 rpm, the clear supernatants were discarded and the washing step was repeated. The gels were transferred into a beaker and dried at 65˚C.

2.5.3 Obtaining silica nanoparticles from silica:

Silica obtained from the rice husk was dispersed in NaOH aqueous solution and heated at 100 °C for 2 h under vigorous stirring so that silica is dissolve and produce sodium silicate. The solution obtained was filtered to remove the nonreactive

impurities. The transparent filtrate of sodium silicate solution was allowed to cool to room temperature and titrated with sulphuric acid to pH 7 under vigorous stirring. Sodium silicate has shown to be neutralized with diluted sulfuric acid to precipitate silica **(Lu and Hsieh, 2012)**. Hereafter, the solution was first stirred for 24 h and then aged for 48 h to allow the silica gel to slowly precipitate. The formed gel was fragmented, filtered, and washed with water to remove the sulfate salt. The clean silica gel was freeze-dried overnight to remove water to obtain powder silica nanoparticles.

2.6 Characterization of silica nanoparticles:

The characterization of the synthesized nanoparticles is important for understanding their properties and applications. Nanoparticle size and morphology are the most important among the different parameters of characterization of nanoparticles. Morphology and size are measured by electron microscopy. The characterization methods used are discussed below. The techniques adopted for characterization of the prepared nanoparticles are: Scanning Electron Microscopy (SEM), Scanning Tunneling Microscopy (STM), Fourier Transform Infrared Spectroscopy (FT-IR), X – ray diffraction (XRD), UV - Visible Spectroscopy and Energy Dispersive X-ray spectroscopy (EDX). Particle size distribution and morphology are the most important parameters of characterization of nanoparticles. Morphology and size are measured by electron microscopy. The major application of nanoparticles is in element release and targeting. It has been found that particle size affects the element release. Smaller particles offer larger surface area. Consequently smaller particles tend to aggregate during storage and transportation of nanoparticle dispersion. Therefore there is a mutual compromise between maximum stability and small size of nanoparticles.

2.6.1 Scanning Electron Microscopy (SEM):

The scanning electron microscope (SEM) as shown in **(figure 2.3)** is one of the important imaging techniques that utilize a focused beam of high-energy electrons to generate a variety of signals at the surface of solid sample **(Reed, S.J.B. 1993)**. The signals that are derived from the interactions between electrons and sample, gives the information about the sample including its external morphology (texture), chemical composition, crystalline structure and orientation of materials of which the sample is made up of. In most applications, data are collected over a selected area of the surface

of the sample, and a 2-dimensional image is generated that displays the variations in these properties **(Reimer, L., 1985)**. The range of areas observed under microscope is approximately 1 cm to 5 microns in width which can be imaged in a scanning mode using SEM techniques (the range of magnification is from 20X to approximately 50, 000X, spatial resolution of 50 to 100 nm) **(Goldstein *et al.*, 2003)**.

For SEM characterization, nanoparticles powder was mounted on a sample holder then it was coated with a conductive metal, usually gold is used for this purpose, using a sputter coater. The nanoparticle sample is then scanned with the help of a focused fine beam of electrons **(Jores *et al.*, 2004).** The surface characteristics of the sample are obtained from the secondary electrons emitted from the mounted nanoparticle sample surface. The nanoparticles should be able to withstand vacuum as the entire process of scanning is performed under vacuum condition, and the electron beam can damage the polymer (**Molpeceres *et al.*, 2000).**

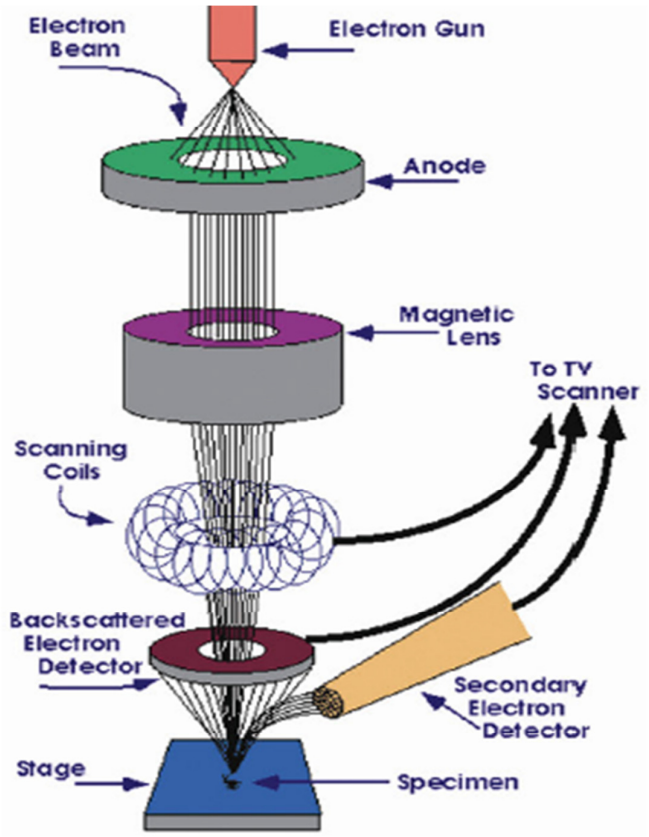


Fig. 2.3: Schematic diagram of SEM

(Source: Joshi *et al.*, 2008)

2.6.2 Scanning Tunneling Microscopy (STM):

Scanning tunneling microscopy is a technique which is used for producing surface image with atomic scale and lateral resolution. A fine probe tip is used to scan over the nanoparticle sample which must be conducting in nature, if the sample is not conducting then it is coated with a conducting material. The probe movement is controlled with the help of a piezoelectric crystal, the distance ranging between 0.5 to 1nm **(figure 2.4)**. The tunneling current thus produced is measured. STM is a very powerful tool in the field of nanotechnology used in the characterization of different types of nanoparticles.

The concept of STM is based on quantum tunneling. When the conducting tip is brought very close to the metallic surface, a bias between the two allows the electrons to tunnel through vacuum between them. For low voltage this tunneling current is the function of the local density of the electrons on the surface. Variation in current passes as the probe passes over the surface. For STM good resolution is considered to be 0.1nm lateral resolution and 0.01nm depth resolution.

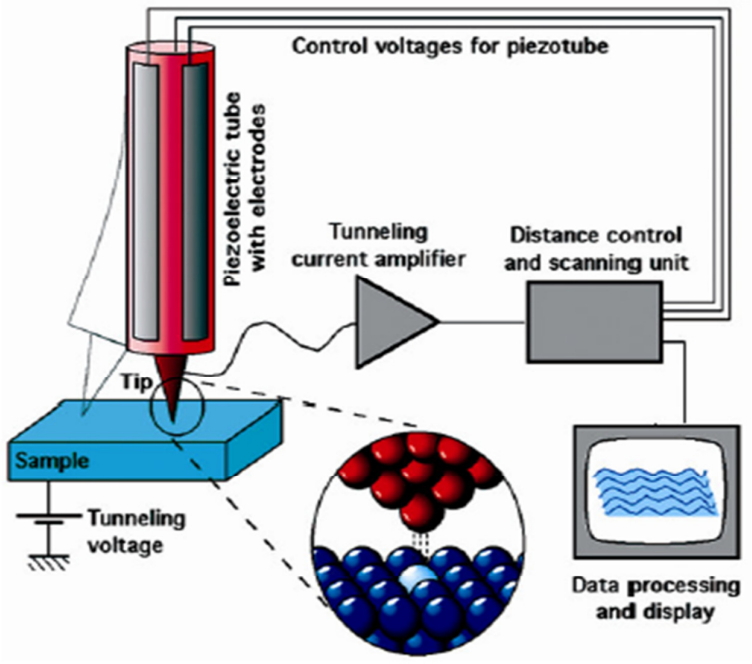


Fig. 2.4: Schematic view of Scanning Tunelling Microscopy

(Source: Joshi *et al.,* 2008)

2.6.3 Fourier Transform Infrared (FTIR) Spectroscopy:

Fourier transform spectrometers have recently replaced dispersive instruments due to its superior speed and sensitivity. They have greatly used the capabilities of infrared spectroscopy and have been applied to many areas that are very difficult or nearly impossible to analyze by different instruments. The infrared spectroscopy (IR) is one of the powerful technique for the characterization of nanoparticles by matching spectrum of unknown compound with reference spectrum "finger printing", it also help in the identification of functional groups in the nanoparticles (**Rafiee *et al.*, 2012**). The IR region of the electromagnetic spectrum covers the range from 50 to 12, 500 cm^{-1} approximately.

The infrared light is passed through a sample of the nanoparticle, and then some frequencies are absorbed, while other frequencies are transmitted without being absorbed by the nanoparticles. The transitions that are involved in the infrared absorption are mainly associated with the vibrational changes in the molecule of which the nanoparticles are made up **(Figure 2.5)** Different bonds / functional groups present in the nanoparticles have different vibrational frequencies and hence the presence of these bonds in a molecule can be detected by identifying this characteristic frequency as an absorption band in the infrared spectrum. The graph plotted between transmittance against frequency is called infrared spectrum, this graph is used to deduce the result of the nanoparticles.

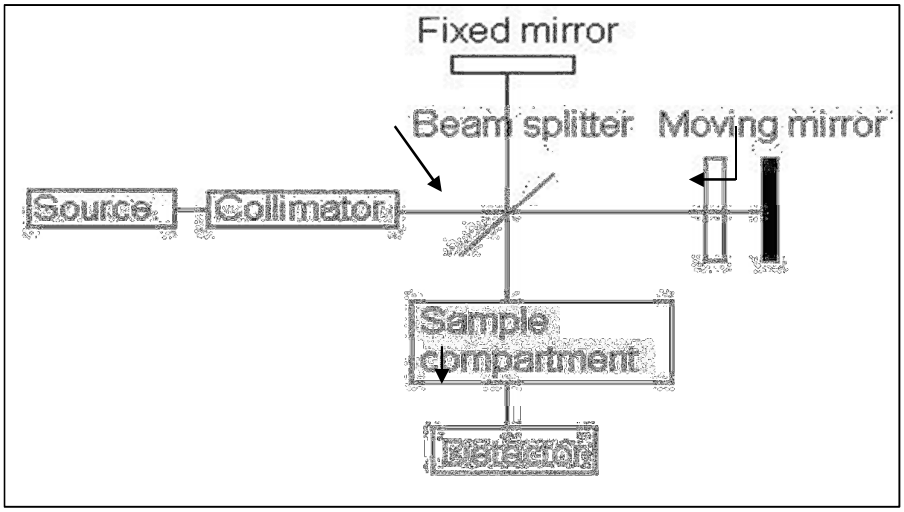


Fig. 2.5: Schematic diagram showing the working of FT-IR

2.6.4 Energy Dispersive X-Ray (EDX) spectroscopy:

Energy Dispersive X-Ray Spectroscopy technique make use of the X-ray spectrum emitted by a solid nanoparticle sample which is bombarded with a focused beam of electrons in order to obtain a localized chemical analysis of the elemental composition of sample. All elements which is in the range of the atomic number 4 (Be) to 92 (U) can be detected with the help of this technique; it is very efficient as not all instruments are equipped for the analysis of 'light' elements (Z < 10) **(Russ, 1984)**. 'Characteristic' X-rays result from electron transitions between inner orbits, which are normally full. An electron must first be removed in order to create a vacancy into which another can 'fall' from an orbit further out. X-ray lines are identified by a capital Roman letter indicating the shell containing the inner vacancy (K, L or M), a Greek letter specifying the group to which the line belongs in order of decreasing importance α, β**,** etc., and a number denoting the intensity of the line within the group in descending order (1, 2, etc.). Thus the most intense K line is Kα**1** (The less intense Kα**2** line is usually not resolved, and the combined line is designated Kα**1, 2** or just Kα). The most intense L line is Lα**1.** Because of the splitting of the L shell into three subshells, the L spectrum is more complicated than the K spectrum and contains at least 12 lines, though many of these are weak as shown in **(figure 2.6)**.

An electron transition associated with X-ray emission can be considered as the transfer of a vacancy from one shell to another, the energy of the X-ray photon being equal to the energy difference between the levels concerned. For example, the Kα line results from a K-L3 transition (Figure 3). Energies are measured in electron volts (eV), 1 eV being the energy corresponding to a change of 1 V in the potential of an electron (= 1.602 x10**-**19 J). This unit is applicable to both X-rays and electrons. X-ray energies of interest in electron probe analysis are mostly in the range 1- 10 keV.

The identification of the lines in the spectrum is used for the qualitative analysis and is very simple owing to the simplicity of X-ray spectra. Quantitative analysis is important for the determination of the concentrations and composition of the elements present in the sample nanoparticles. Measuring line intensities for each element in the sample is used for the quantitative analysis, the intensities of the X-rays is measured against the calibration standards of known composition. After scanning process is complete the beam is processed in a television-like raster and displays the intensity of a selected X-ray line, element distribution images or 'maps' are thus produced. The images produced by electrons collected from the sample reveal surface topography or mean atomic number differences according to the mode which is selected for further analysis.

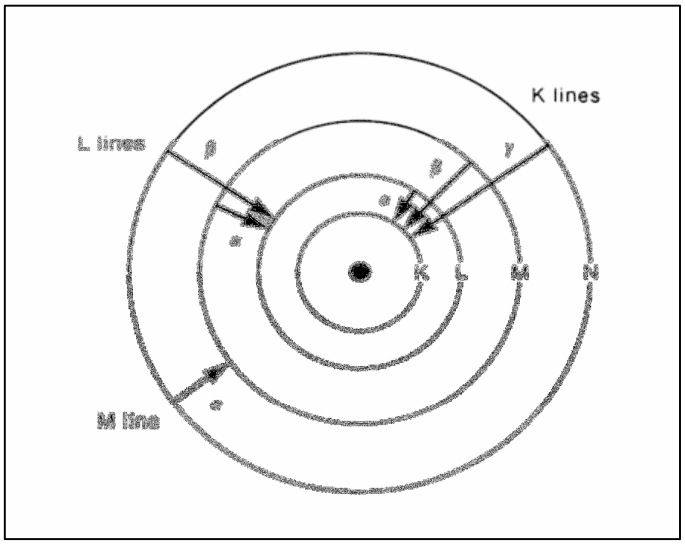


Fig. 2.6:) X-Ray Nomenclature

2.6.5 X - ray diffraction

This technique is most common and very efficient for the determination of structure and material identification of the nanoparticles. XRD is a method which is used to examine whether the nanoparticles has amorphous or crystalline nature. When an X-ray beam hits an atom, the electrons around the atom start to oscillate with the same frequency as the incoming beam. This will result in destructive interference in almost all directions. The atoms in a crystal are arranged in a regular pattern, and in a very few directions will have constructive interference. The waves will be in phase and there will be well defined X-ray beams leaving the sample at various directions. Hence, a diffracted beam may be described as a beam composed of a large number of scattered rays mutually reinforcing one another) as shown in **(figure 2.7).**

X - ray diffraction is technique that is based on constructive interference of monochromatic X - rays from the crystalline nanoparticle. The X - rays, generated by a cathode ray tube are filtered so that a monochromatic radiation is produced, which collimated and is directed towards the nanoparticle sample. X - ray primarily interact with electrons in the nanoparticles atoms, collide and some photons from the incident beam are deflected away from original. The X - rays obtain after the interaction with the sample forms constructive and destructive diffraction pattern on the detector. The incident X - ray produces a Bragg peak if they are reflected from the various planes interfered constructively **(Amutha *et al*., 2010)**

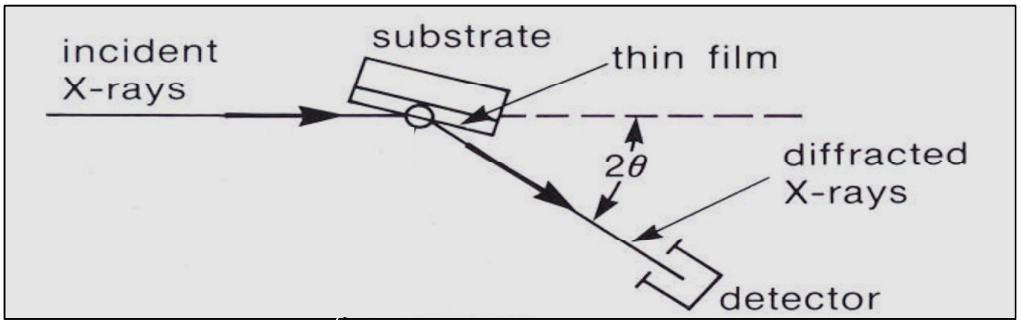


Fig. 2.7: Schematic diagram showing X-Ray Diffraction.

2.6.6 UV - VISIBLE SPECTROSCOPY (UV - Vis)

UV- vis spectroscopy refers to absorption spectroscopy that lies in the ultra - violet and visible spectra region. In this region of the electromagnetic spectrum, molecules of the nanoparticles undergo electronic transition. When sample molecules are exposed to light having an energy, which matches a possible electronic transition within the molecule of the nanoparticles, some part of the light energy is absorbed as the electron is excited to a higher energy orbital. An optical spectrometer is used to record the wavelengths at which absorption occur, together with the degree of absorption at each wavelength. This is presented as a graph of absorbance (A) versus wavelength. The optical properties of materials can be studied with the help of UV-Vis spectra are used to measure the optical properties of the nanoparticle.

2.7 Importance of Silica

Silica is very ubiquitous element and it is difficult to verify its essentiality in higher plants (**Epstein 1994)** based on the criteria of essentiality of elements established by **(Arnon and Stout, 1939).** According to the recent definition of the essentiality of elements which is proposed by **(Epstein and Bloom, 2005),** silica should be considered an essential element for higher plants because silica deprived plants tend to grow abnormally. Silica when present in excess is not detrimental to plants **(Epstein 1994; Ma *et al.* 2001)**. Silica has been reported to be very important in alleviating both biotic stresses (e.g., plant diseases and pest damage) and abiotic stresses such as drought, salinity, nutrient imbalance, aluminum toxicity, heavy metal toxicity, lodging, radiation, high temperature, wounding, and freezing **(Richmond and Sussman, 2003; Ma and Yamaji, 2006; Liang *et al.,* 2007; Kim *et al.,* 2011; Van Bockhaven *et al.,***

2013) Studies reports show that silicon application may increase tolerance to drought in plants **(Liang *et al.,* 2007; Bauer *et al.,* 2011)**. Silica can improve drought tolerance of plants, its application may reduce the need for irrigation, and this in turn results in the reduction of salinity of crop land **(figure 2.8)**. Silica is pollution-free and non-corrosive, and thus, silica nano-fertilizer is a high-quality fertilizer for developing ecologically green agriculture.

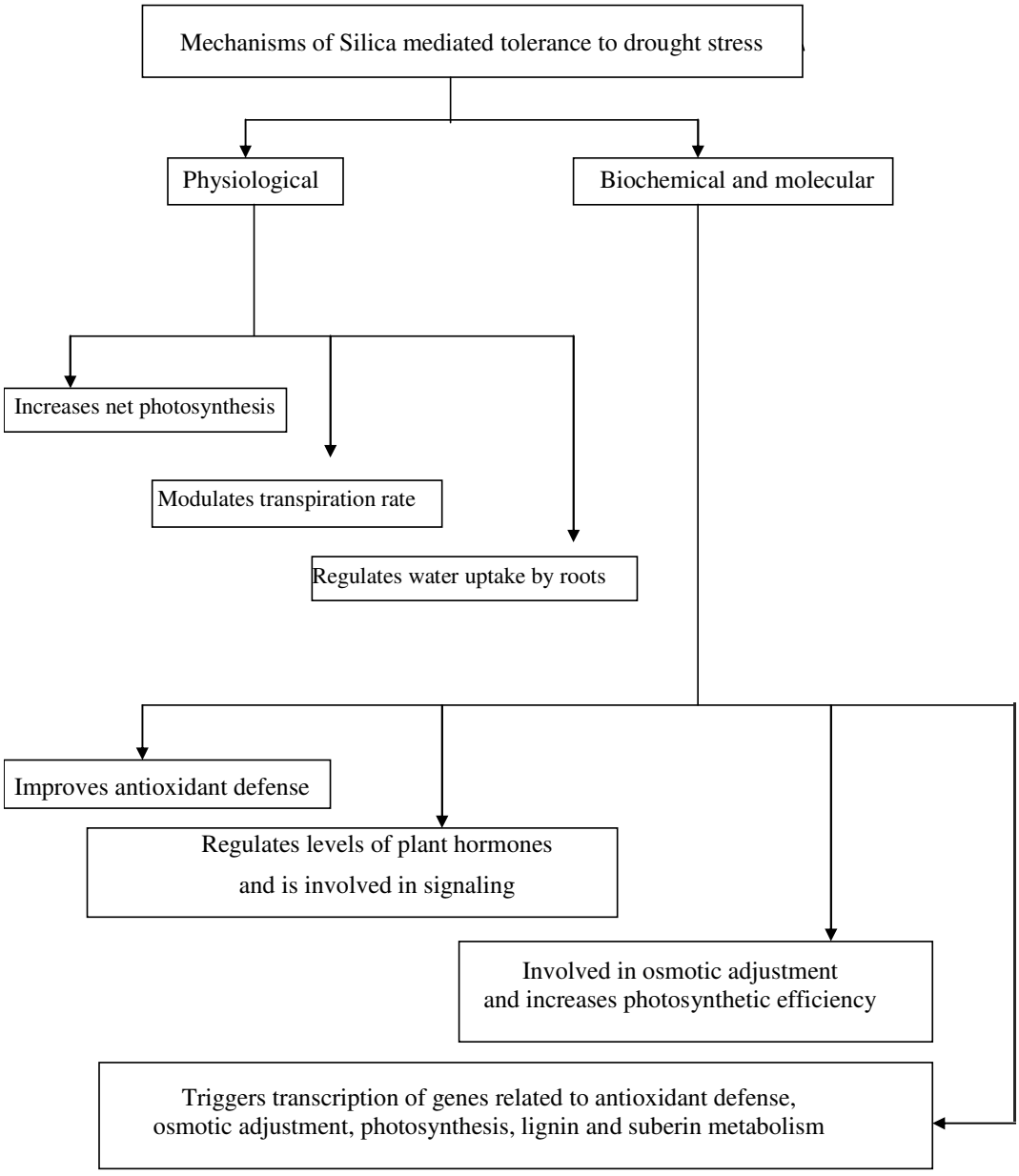


Fig. 2.8: Possible mechanisms for silica-mediated tolerance in plants during water stress.

2.7.1 Silica uptake, transport, and accumulation in plants

The silica content of soils is in the range of <1 to 45 % dry weight **(Sommer *et al.,* 2006).** Silicon is abundant in soil, but generally it cannot be absorbed directly by plants. The uptake of silica by plant root is generally in the form of soluble silicic acid [Si(OH)4] **(Mitani *et al.,* 2005),** which is an un-dissociated molecule that is normally present at a concentration of 0.1–0.6 mM in the soil solution at most naturally occurring pH (pH 1–9) **(Takahashi and Hino, 1978; Ma *et al.,* 2006).** Many factors are responsible for the formation of soluble silicic acid such as soil pH, temperature, water conditions, presence of cations, and organic compounds in soil and thus affect silicon accumulation in plants **(Liu *et al.,* 2003, Savant *et al.,* 1997).** It is very important to apply silica in silica deficient areas to reap both economic and ecological benefits. All plants contain silica in their tissues **(Epstein, 1994; Ma and Yamaji, 2008),** and silicon content in plants varies greatly among species and genotypes **(Ma and Yamaji, 2008).** The silica uptake and transport in plants can be classified as passive and active. Therefore, plants can be classified as high-, intermediate-, or non-silica accumulators **(Henriet *et al.,* 2006).** Some species such as rice (*Oryzae sativa*) and barley (*Hordeum vulgare L.*) can take up silica actively, whereas tomato (*Lycopersicon esculentum* Mill.) limits silica transport from the root to the shoot **(Nikolic *et al.,* 2007).** In cucumber (*Cucumis sativus L.*), **Mitani and Ma, 2005 and Liang *et al.,* 2005** suggested that different xylem loading of silica in cucumber is mediated by passive diffusion. **Fu *et al.* (2002)** reported that, silica nanoparticles can be absorbed directly and selectively from soil, in the cortex of a species of fern (*Matteuccia*); both passive and active silica uptake may be simultaneously present in plants **(Liang *et al.,* 2006a; Henriet *et al.,* 2006).** Many studies suggested that transpiration rate of an organ reflects the silica concentration in that particular plant organ **(Guntzer *et al.,* 2012).** It was observed that the major transpiration sites in plants were having highest concentrations of silica **(Raven, 2001).**

2.7.2 Silica alleviates effect of drought stress in plants

Drought is one of the major environmental stresses in agriculture, having various deleterious effects on plant growth and metabolic processes, including water relations, photosynthetic assimilation, and nutrient uptake **(Cattivelli *et al.,* 2008;**

Xiong *et al.,* 2012). It has been reported that silica increases drought tolerance in plants such as rice **(Agarie *et al.,* 1998),** sorghum **(Hattori *et al.,* 2005, 2008a; Ahmed *et al.,* 2011a, b; Sonobe *et al.,* 2011),** cucumber **(Hattori *et al.,* 2008),** maize **(Gao *et al.,* 2004, 2006)**, wheat **(Gong *et al.,* 2005, 2008; Gong and Chen, 2012),** pepper **(*Capsicum annuum* L., Lobato *et al.,* 2009)**, and sunflower **(Gunes *et al.* 2008).** Moreover, silica also enhances tolerance to heat stress by the process of maintaining membrane stability **(Agarie *et al.,* 1998)**. Drought is usually accompanied by high temperature **(Halford ,2011)**, application of silica nanoparticles may be an alternative to reduce the deleterious damage caused by both drought and heat stresses.

2.7.3 Silica influences water relations and improves Photosynthesis under drought stress

Leaf water potential and water content decreases to a large extent when plants are exposed to drought **(Siddique *et al.*, 2000; Farooq *et al.,* 2009).** Water status in non-irrigated crops can be significantly improved by the application of silica. Reports shows that the water potential of drought-stressed wheat leaves applied with silica is maintained to a large extent compared with stressed plants without silica treatment, showing that silica can improve the water status of wheat plants during the drought condition **(Gong and Chen, 2012)**. A similar observation was recorded by **Pei *et al.* (2010)** in wheat which was exposed to polyethylene glycol induced water stress condition.

Stomatal conductance and transpiration rate are two most important characteristics of the plants that influence plant water relations **(Farooq *et al.,* 2009).** The advantageous effects of silica on plant growth have been observed to be related with a variation in transpiration. Researchers have postulated that the formation of a double layer of silica cuticle on leaf epidermal tissue may be responsible for the observed lowering in leaf transpiration in the plants treated with silica **(Yoshida 1965; Wong *et al.,* 1972; Matoh *et al.,* 1991). Gong *et al.* (2003)** observed that leaves of wheat in drought stress become thicker upon application of silica and speculated that silica might improve drought tolerance by lowering water loss by transpiration. Therefore, minimizing transpiration—by cuticle or stomata—is one of the mechanisms for the observed silica-mediated increase in drought tolerance.

Sonobe *et al.* (2011) suggested that if silica is included in culture solution then it can enhance root water uptake by root hairs under water-deficit stress by the process of active accumulation of soluble sugars and amino acids. Proline is one of the most important and compatible solutes that accumulates during stress conditions and has been considered to play an importnat role in osmotic adjustment **(Nayyar andWalia 2003). Pei *et al.* (2010)** reported that proline concentration increased in wheat leaves under water scarcity and that application of silica reduced the proline accumulation in the leaves. This result supports the view that proline accumulation is a symptom of stress-related injury.

The first response of plants exposed to serious water deficit is the closure of stomata and is usually been accepted to be the major limiting factor of photosynthesis (**Reddy *et al.,* 2004; Farooq *et al.,* 2009).** Due to the closure of stomata CO2 influx decreases and leaves many electrons for the formation of active oxygen species **(Farooq *et al.,* 2009).** In drought conditions, **(Meyer and Genty, 1998)** observed that the reduction of photosynthetic rate in *Rosa rubiginosa* was mainly due to CO_2 deficiency. The application of silica affects stomatal movement, along with the photosynthetic pigments. Studies showed that addition of silica maintains the content of photosynthetic pigments (chlorophylls a, chlorophylls b, and carotenoids) in Capsicum annuum L. under drought condition (**Lobato *et al.,* 2009**). This may be as a result of silica-mediated improvement of chloroplast ultrastructure and elevated activities of Antioxidant enzymes such as superoxide dismutase and catalase **(Liang 1998; Gong *et al.*, 2005**). **Chen *et al.* (2011)** observed that added silica not only increases the content of photosynthetic pigments but also plays a role in the increase of the basal quantum yield (Fv/F0) and maximum quantum efficiency of photosystem II photochemistry (Fv/Fm) of rice plants subjected to drought.

The activities of certain photosynthetic enzymes are regulated by silica**. Adatia and Besford (1986)** reported that there was an increase in the activity of ribulose-bisphosphate carboxylation in cucumber grown hydroponically when silica was added to the medium. There is an increase in the activity of phosphoenol pyruvate carboxylase and the concentration of inorganic phosphorus in wheat leaves under drought conditions, when silica was applied **(Gong and Chen, 2012**). The above mentioned studies suggest that silica plays an important role in both stomatal dynamics and

photochemical reactions and as a result regulates photosynthesis. The beneficial effects of silica in water-stressed plants can be partially attributed to its positive effect on plant water status and photosynthesis.

2.8 Applications of silica nanoparticles in agriculture:

Mesoporous Silica Nano particles (MSNPs) usually 20nm in size are generally used. The MSNPs is taken up by the root system through the apoplastic and symplastic pathways and is translocated to the aerial parts of the plants through the xylem system. MSNPs are mainly deposited in the cell walls, so they have very high with the other cell wall components. For the efficient uptake of the nanoparticles there must be a fine tuning between the pH and the surfactant concentration, these two decides the size and nature of the MSNPs, the entry of the MSNPs takes place through the pores present in the cell wall **(Sun *et al.,* 2014).**

With the use of MSNPs the total protein content increased, the growth was boosted; the photosynthesis of lupin and wheat seedlings increased but there were no changes in the antioxidant enzyme activity**.** The shift of 14/cm and 10/cm in the Raman peak of chlorophyll was observed in wheat and lupin, this suggested that there was a change in the chlorophyll structure **(Sun *et al.*, 2016).**. In tomato which comes under the category of silica excluders, the application of silica Nanoparticles resulted in increased germination of the tomato seeds. The SNPs was applied at the concentration of 8g/L along with the increase in the germination rate of tomato seedlings there was also an increase in the fresh weight and the dry weight by 1116.6% and 117.5% respectively **(Siddiqui and Al-Whaibi, 2014).**

Silica Nanoparticles were observed to stimulate the antioxidant defense system in order to protect the wheat seedling against the UV-B abiotic stress **(Tripathi *et al.,* 2016).** The SNPs decreased the damage caused by the UV-B stress such as tissue damage, low fresh weight and decreased chlorophyll content. The protective roles through the modification of the NO levels were observed as the nitric oxide attained a peak after SNPs+UV-B treatments. When the Nanostructured SiO2 (TMS) was applied to the roots of 1 year old larch seedlings by soaking for 6 hours, the results showed promotion in the chlorophyll content, lateral root growth, and main root length **(Bao-shan *et al.,* 2004).**

The application of low concentrations of silica nanoparticles have shown to improve seed germination rate of tomato **(Siddiqui and Al-Whaibi, 2014).** Silica nanoparticles application has increased seed germination in maize seeds as it helps in providing better nutrients uptake, the pH and conductivity of the growing medium was also maintained by its use **(Suriyaprabha *et al.*, 2012)**. The exogenous application of silica nanoparticles on Changbai larch (*Larix olgensis*) seedlings showed that silica nanoparticles is observed in improving seedling growth and quality, including the mean height, the diameter of the root collar, the number of lateral root and the main root length of seedlings and also geared up the process of synthesis of chlorophyll **(Bao-shan *et al.*, 2004)**. In tomato silica nanoparticles application augments seed germination under abiotic stress **(Haghighi *et al.*, 2012).** In squash application of silica nanoparticles enhanced rate of seed germination and also stimulated the effect of antioxidant system under salinity stress **(Siddiqui *et al.*, 2014).** Exogenous application of silica nanoparticles resulted in the improvement of the seed germination rate of soybean by increasing the concentration of nitrate reductase **(Lu *et al.*, 2002)** and also by enhancing ability of the seeds to absorb and utilize nutrients and water **(Zheng *et al.*, 2005).** Under salinity stress conditions, the application of silica nanoparticles has shown to increase the leaves fresh and dry weight, chlorophyll content and proline accumulation in plants. The tolerance of plants to endure abiotic stress has increased with the increase in the accumulation of free proline, free amino acids content, antioxidant enzymes activity as a result of application of silica nanoparticles **(Kalteh *et al.* 2014; Haghighi *et al.* 2012; Li *et al.* 2012; Siddiqui *et al.* 2014).** The application of silica nanoparticles have shown to increase the plant growth and development by enhancing the gas exchange via stomata and chlorophyll fluorescence parameters, which are as follows net photosynthetic rate, stomatal conductance for gas exchange, transpiration rate, potential activity of PSII, actual photochemical efficiency, electron transport rate, effective photochemical efficiency and photochemical quench under water stress **(Siddiqui *et al.* 2014; Xie *et al.* 2012).**

Materials and Methods

Chapter 3 MATERIALS AND METHODS

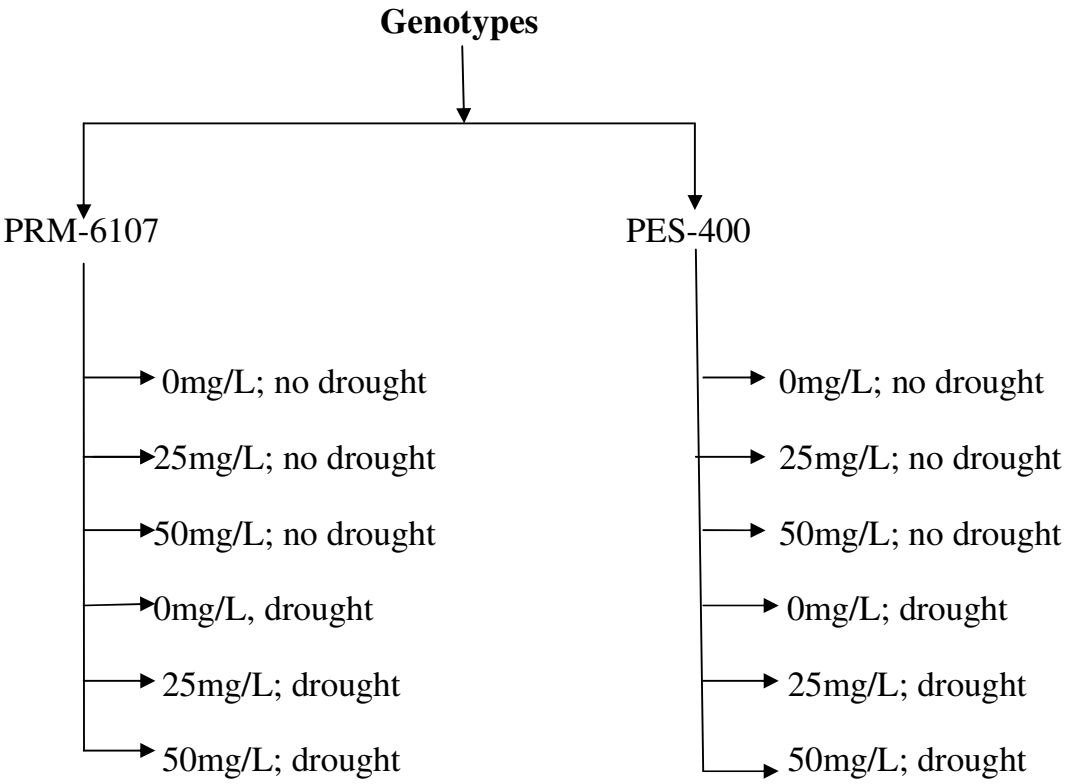
The present study was conducted in the Department of Molecular Biology and Genetic Engineering, College of Basic Sciences and Humanities, G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand (29.0210^{o} N, 79.4897^{o} E). Details pertaining to the materials used, methods followed and the individual experiments performed are given below.

3.1 Seed sowing, germination and imposition of water stress

Seeds of two contrasting genotypes of finger millets were used in the study. The drought resistant genotpe (PRM-6107) and the drought sensitive genotype (PES-400) were used. The seeds of these genotypes were obtained from the Department of Molecular Biology and Genetic Engineering, College of Basic Sciences and Humanities, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar. Seeds of the two genotypes were washed with tween 20 and were then sterilized with 0.5% sodium hypochlorite. The Seeds of the two genotypes of finger millet were sown in trays filled with soil and vermicompost mixture in the ratio 2:1. After a period of 14 days of sowing, when the plants were in its 2-3 leaf stage it was transferred to the small pots and was kept in a polyhouse. All the pots used for the experiment was of the same dimension, the pots were also filled with soil and vermicompost mixture in a ratio of 2:1. They were allowed to grow in controlled polyhouse conditions at optimum temperature and moisture for 30 days. There were 6 pots for genotype PRM-6107 and 6 pots for PES-400. To each pot 4 plants of *Eleusine coracana* was planted. 300ml of suspension of silica nanoparticles was prepared in water and was applied at three concentrations 0 mg/L, 25mg/L and 50mg/L on alternate days for a period of 45days. After the application of silica nanoparticles for 45 days, one pot of each concentration was exposed to drought stress by withholding the water for 5 days. The other set of pots with the 3 different concentrations of nanoparticles was watered continuously these control pots were regularly irrigated to keep the soil moisture content at 60% to 70% of the total water retaining capacity. Similar set up was done for both genotypes. The 3

pots of each genotype that were watered acted as the control for that particular silica nanoparticle concentration. The whole experiment was carried out in triplicate.

The plants in the pots that were treated with silica nanoparticles of different concentrations were compared with the control for the following morpho-physiological and biochemical parameters following the 5 day drought treatment.



3.4 Glasswares and plasticwares

Glasswares used for the experiments were procured from Borosil company. Plastic wares which included centrifuge tubes, test tubes, beakers, conical flask, centrifuge tubes, glass rod, funnel, quartz cuvette, magnetic beads, measuring cylinders, micropipette tips were brought from Tarson. Micropipettes : T-10, T-20, T-100, T-200 and T-1000 were purchased from Nichipet.

3.5 Source of Chemicals

Sl No.	Name of Chemical	Make
1.	2-Thiobarbituric acid	HiMedia Laboratories
2.	5-Sulfosalicylic acid	HiMedia Laboratories
3.	Acetic acid-glacial	HiMedia Laboratories
4.	Sodium hydroxide	HiMedia Laboratories
5.	Cetyltrimethylammonium bromide	HiMedia Laboratories
6.	Chloroform	HiMedia Laboratories
7.	Methanol	HiMedia Laboratories
8.	Dimethyl sulphoxide	Sisco Research Laboratories
9.	EDTA disodium salt dihydrate	HiMedia Laboratories
10.	Ethanol	Sisco Research Laboratories
11.	Sulfuric acid	HiMedia Laboratories
12.	Whatman filter paper	HiMedia Laboratories
13.	L-proline	HiMedia Laboratories
14.	Hydrochloric acid	Sisco Research Laboratories
15.	Isoamyl alcohol	HiMedia Laboratories
16.	Isopropanol	HiMedia Laboratories
17.	L-proline	HiMedia Laboratories
18.	Ninhydrin	HiMedia Laboratories
19.	Phenol	HiMedia Laboratories
20.	Phosphoric acid	Sisco Research Laboratories
21.	Potassium bromide	HiMedia Laboratories
22.	Sodium acetate	HiMedia Laboratories
23.	Sodium chloride	HiMedia Laboratories
24.	Sodium hydroxide	HiMedia Laboratories
25.	Toluene	Sisco Research Laboratories
26.	Trichloro acetic acid	Sisco Research Laboratories
27.	Tris-free base	Sisco Research Laboratories
28.	β-Mercaptoethanol	Sisco Research Laboratories

3.6 Equipments Used

Sl No.	Equipment	Make
1.	Autoclave	Narang Scientific Works
2.	Centrifuge	Eppendorf, Rainin
3.	Magnetic stirrer	Biogen scientific
4.	Electronic Balance	Citizen
5.	Electrophoresis Assembly	Bangalore GeNei
6.	Gel Documentation System	Alpha Innotech
7.	Hot air oven	Lab Companion
8.	Magnetic stirrer with heater	Biogen scientific
9.	Micropipette	Nichipet, Astra
10.	Microwave Oven	LG
11.	pH meter	Bangalore GeNei
12.	Refrigerator	Whirlpool
13.	Spectrophotometer	Thermo Electron Corporation
14.	pH meter	Biogen scientific
15.	Vortex	JSGW
16.	Water bath	Lab Companion

3.7 Synthesis of silica nanoparticles from rice husk:

The rice husk, also known as rice hull, is the outer covering on a seed or grain of rice. It is formed from hard materials; including silica and lignin, the presence of these compounds protect the seed during the growing season. Approximately 600 million tons of rice crops are produced all around the world every year. After milling each kg of rice results in roughly 0.28 kg of rice husk as a by-product of rice production during milling. 20% of the rice plant accounts for the husk, therefore the production of rice husk turn out to be approximately 120 million tons silica comprises about 60% of rice husk **(Pham *et al.*, 2016).**

The process of synthesis of silica nanoparticles from rice husk is as follows:

1. The rice husk was burned in order to obtain rice husk ash.
2. 10g of this rice husk ash is taken and dispersed in 60ml water.
3. The pH was adjusted to 5.
4. This solution was stirred continuously at medium speed for 2 hours.
5. This solution was filtered with the help of whatman filter paper.
6. The residue obtained after filtering was dispersed in 60ml 0.1N NaOH solution.
7. This solution was filtered with whatman filter paper and to the residue 100ml boiling water was added.
8. This solution was again filtered and the filterate was collected. The filterate was allowed to cool.
9. The filterate was titerrated with 0.1N HCl so that the pH of the solution comes to pH 7
10. The gel thus formed was allowed to set for 2 hours.
11. This gel was centrifuged at 12000 rpm for 20 minutes.
12. The supernatant was discarded and the residue was washed with distilled water to remove any impurities.
13. The gel was allowed to dry at 65˚C until it become dry to form silica crystals.
14. 5g of the silica thus obtained was dispersed in 250ml of 0.05N NaOH solution.
15. This solution was heated for 2 hours at 80˚C along with vigourous stirring; the stirring was required to silica.
16. The solution was allowed to cool and then filtered to remove the nonreactive impurities.
17. The transparent solution thus obtained after filtration was titrated against 5% sulfuric acid under vigorous stirring to reach the pH 7.
18. When the pH of the solution becomes 7, then the solution was stirred for 24hrs
19. This solution along with gel which is formed as a result of titration was allowed set for 48 hours to allow the silica gel to slowly settle down.

20. The upper aqueous phase is discarded and the gel is freeze dried overnight to remove the water.
21. After drying the gel changed to powder form, this powder obtained was silica nanoparticles.
22. The silica nanoparticles were stored at 4°C for further use and characterization.

3.8 Characterization of the silica nanoparticles synthesized from rice husk:

The following characterization was done at Department of Institute Instrumentation Centre, IIT, Roorkee.

3.8.1 Scanning Electron Microscopy:

The size, shape and morphology of the silica nanoparticles were measured with the help of field emission scanning electron microscope (Carl Teiss Ultra 5S FE-SEM). The SEM is an instrument that produces a very highly magnified image by the use of electrons in place of light to form an image. For performing FE-SEM a drop of 250 ppm well sonicated sample was placed on a carbon tape which was then pasted on a holder. After this the solvent was evaporated and the particle was coated with ≈10Å thin gold film and then it was examined in FE-SEM. An electron gun present at the top of the microscope was used to produce a beam of electrons. The electron beam thus produced followed a vertical path through the microscope, which is held within a vacuum environment. This beam of electrons travels through electromagnetic fields and lenses, which as a result focused the beam down toward the silica nanoparticle sample. , electrons and X - rays are ejected from the sample surface, when the beam hits the sample. Detectors collect these X - rays, backscattered electrons and secondary electrons and convert them into a signal that was sent to a screen similar to a television screen. This produced the final image**.** The size of the nanoparticles was estimated with the help of analysis image processing software.

3.8.2 Scanning Tunneling Microscopy (STM):

Scanning tunneling microscopy is used to obtain single nanometer resolution of the nanoparticles. In this type of microscopy a very small and fine metal tip was placed at a very minute distance from silica nanoparticle sample which acted as the conducting surface. When these two surfaces are in close proximity but not actually touching each

other, then this can allow electron to tunnel through the vacuum in between them. This phenomenon gives rise to a tunneling current, which was measured and the tunneling current was the function of the electron density on the surface. Electron density is more around atoms or bonds in a molecule. The movement of the metal tip is controlled by a piezometer. The histogram of different sizes of silica nanoparticles were obtained by means of the STM results.

3.8.3 Fourier Tranform Infrared Spectroscopy (FT-IR):

In order to authenticate the purity of the synthesized silica nanoparticles and its chemical structure in accordance to the related functional groups, FT-IR analysis was carried out. Standard KBr pellet technique was followed for the Fourier Transform Infrared measurements (FTIR), using Perkin Elmer FTIR spectrophotometer. Instead of viewing single component frequency sequentially, all the frequencies are examined simultaneously in Fourier transform infrared (FTIR) spectroscopy. There are three major spectrometer components in the FT system are as follows: radiation source, interferometer and detector. The Infra-Red radiation from a broadband source was first directed into an interferometer, where the radiation was divided and then recombined. After this the split beams travelled different optical paths to form constructive and destructive interference. This resulting beam now passes through the sample compartment where the silica nanoparticle was placed and then reaches to the detector.

Sample preparation was very easy, the silica nanoparticles (minimum of 10 μg), to be analyzed was grounded in the presence of KBr matrix or dissolved in a suitable solvent (CCl4 and CS2). For solid samples (silica nanoparticles) it is mixed with solid KBr (transparent in the mid - IR region), then ground and pressed. If there are chances of moisture to be present in the sample then the water should be removed

3.8.4 Energy Dispersive X-ray spectroscopy (EDX):

For the compositional analysis of the silica nanoparticles XRD was performed by Horiba Energy Dispersive X-ray micro analyzer which was attached to the SEM machine. Since the electron probe analyses only to a shallow depth, silica nanoparticles were well polished so that surface roughness has no negative affect the results. Sample preparation was essentially same as that for the FE-SEM, and the samples that are vacuum compatible are applicable for this technique. Opaque nanoparticle sample was

embedded in epoxy resin blocks. For viewing the transmitted light polished thin film of nanoparticles sample was coated on the glass slides. Holders are commonly provided for 25mm (1") diameter round specimens in a rectangular glass slides on which the sample is placed. The silica nanopaticle samples are electrically non-conducting and a conducting surface coat was applied to provide a path for the incident electrons to flow to ground. Since the sample prepared was same as that for FE-SEM so it was coated with gold (~10nm thick)

3.8.5 X-ray diffraction pattern (XRD):

For the analysis of the silica nanoparticle crystal structure, the silica nanoparticles was analyzed by Bruker D8 Advance X-ray Diffractrometer with θ to 2θ geometry by using Cu-Kα radiation (Cu-Kα = 1.540 Å) in the range 2θ = 5˚ to 90˚.

The X - ray diffractometer which was used consisted of a source for radiation, a monochromator to adjust/select the wavelength, slits for the adjustment of the shape of the beam, the silica nanoparticle sample and the detector. For further fine adjustments of the sample and the detector positions goniometer is used. The goniometer allowed the precise movement of the sample and the detector. The source which emits X - rays contains several components; the most important being Kα and Kβ. Monochromators and filters are one of the most eminent parts and are used to absorb the unwanted emission with Kβ wavelength, while allowing the desired wavelength, Kα to pass through it . The X - ray radiation was emitted by copper, whose characteristic wavelength for the Kα radiation was equal to 1.540 Å. The filtered X - rays were collimated and directed towards the sample. The sample was ground to a more fine powder before it was loaded it in the glass sample holder. The powdered silica nanopaticles should be placed in a way that it completely occupies the square glass well. When the incident beam strikes the powder silica nanoparticle sample, then the diffraction occurred in every possible orientation of 2θ. The diffracted beam was detected with the help of a moveable detector (Geiger counter), which is connected to a chart recorder.

The counter was set to scan over a range of 2θ (i.e. 5 to 90 degrees) values at a constant angular velocity. The scanning speed of the counter was 2θ of 2˚ min¯1. The detector recorded and processed this X - ray signal and converted the signal into a

count rate which was then fed to a device such as a printer or computer monitor in order to obtain the results.

3.8.6 UV-vis absorption spectroscopy:

The optical behavior of the silica nanoparticle was analyzed using UV-vis spectroscopy. The major components of the UV-Vis spectrometer are a monochromator, a light source, double beams (reference and sample beam), a detector and a recording device. The source in the spectrophotometer is generally a tungsten filament lamp for visible and deuterium discharge lamp for the measurements of UV wavelength of light. The light coming out of the source is split into two beams - the reference and the sample beam. The silica nanoparticles sample is prepared according to the concentration to be used for the application in plants. The solution was well sonicated and was filled 2/3 in the rectangular quartz cuvette. The blank solution is used to set the baseline which acts as reference in accordance to which the readings are measured. The recorder plots a graph of the absorbance (A) against the wavelength (nm).

3.9 Screening for Drought Tolerance Traits:

3.9.1 Morpho-Physiological parameters

3.9.1.1 Shoot Length

A meter scale was used to record the plant height from soil base to the tip of flag leaf of each genotype. Measurements were performed before and after stress treatment for both control and drought treated plants. Shoot length was expressed in centimeters.

3.9.1.2 Root length

The plants from both control and treatment pots were uprooted and length of the longest root was measured using a meter scale. Root length was expressed in centimeters.

3.9.1.3 Number of Tillers

The number of tillers of each plant, both control and drought treated was counted and recorded.

3.9.1.4 Number of leaves

Number of leaves present in each plant of all genotypes was counted and recorded.

3.9.1.5 Leaf Rolling and Drying

Leaf rolling and drying in all genotypes was observed and recorded.

3.9.1.6 Leaf area

Leaf area of leaves from both control and treatment plants of all genotypes was measured by the method described by **Rajappa *et al.* (1972)**. A factor of 0.75 multiplied by the product of the leaf length and width of the flag leaf was used to determine the leaf area of both control and drought treated plants. Leaf area was expressed in cm^2.

Leaf area = 0.75 x Leaf length x Leaf width

This method can provide non- destructive leaf area estimates to within 0.05 accuracy.

3.9.1.7 Soil Moisture Content

Gravimetric method of soil moisture content determination is most commonly used in which a known weight of wet soil is oven dried at 100-110ºC and the moisture removed is calculated. Soil moisture content is expressed as wet weight percentage or volume percentage.

1. Soil samples were collected from the experimental pots from a depth of 10 cm.
2. The soil samples were placed immediately in an aluminum soil moisture box and they were closed with the lid of the box.
3. Weight of the soil moisture box containing moist soil sample (W1) was measured.
4. The soil moisture boxes containing soil samples were placed in an oven at 100-110ºC for 24 hours.
5. The dry weight of the soil along soil with moisture box (W2) was recorded.
6. The dry soil was discarded and the empty soil moisture box (C1) was weighed.

Calculation:

Moist soil weight = W1-C1

Dry soil weight = W2-C1

Water content = (W1- W2)-C1

Gravimetric Soil moisture content (*g*) % = [(W1-C1)-(W2-C1)]/(W2-C1)}*100

3.9.1.8 Relative water content

Relative water content (RWC), considered the appropriate measure of plant water status in terms of the physiological consequence of cellular water deficit was estimated by the method of **Barrs and Weatherley (1962)**. Leaves of the three varieties under stress condition as well as control condition were taken.

1. Fully expanded topmost leaves were collected at 11AM – 12 in noon.
2. Immediately after sampling the samples were placed in a polythene bag and sealed properly to minimize water loss from the leaves.
3. The bags were placed in an ice box (around 10°C-15°C) and brought to the lab as soon as possible.
4. 5-10 cm length mid-leaf sections were cut from the leaves while avoiding large veins and mid-rib.
5. The samples were weighed quickly to record the fresh weight.
6. The samples were hydrated to full turgidity by floating on de-ionized water in a closed petri-dish for 24 h in a lab refrigerator.
7. After 24 hours the samples were taken out and any surface moisture was removed lightly with filter paper and immediately weighed to obtain fully turgid weight.
8. Then the samples were dried in a hot air oven at 80°C for 24h
9. The dry weight of the samples was weighed after proper drying (till the weight become constant).

The relative water content was then calculated using the following equation:

RWC (%) = [(Fresh weight – Dry weight) / (Turgid Weight – Dry Weight)] x 100

3.9.1.9 Chlorophyll and total carotenoid content -

The assay for chlorophyll content was performed based on the various equations for the determination of pigments based on their absorption in organic solvents at various wavelengths as described by **Lichtenthaler and Wellburn (1983)**.

50 mg leaf material was placed in 10 ml of DMSO in test tubes, and thereafter the test tubes were placed in a constant temperature incubator at 65^{o}C for 4 h.

1. After the completion of period the tubes were cooled to room temperature. The absorbance of the solvent was recorded at 470, 645 and 663 nm against DMSO blank.

2. The chlorophyll-a, chlorophyll-b and total carotenoid content was calculated as per the equations given below. The values obtained for chlorophyll-a, chlorophyll-b and total carotenoids are in µg ml $^{-1}$ of leaf extract.

   Chl-a = 12.21 A_{663} - 2.81 A_{645}

   Chl-b = 20.13 A_{645} - 5.03 A_{663}

   Total carotenoids = [1000 A470 - (3.27 Chl-a + 104 Chl-b)]/229

3.9.2 Biochemical parameters

3.9.2.1 Proline content

Proline content was estimated using the method devised by **Bates *et al*. (1973).**

1. 2.48 g of Ninhydrin was dissolved in 60 mL glacial acetic acid and 40 mL of 6N phosphoric acid.

2. 0.5 g leaf sample was grounded in 10 mL of 3% aqueous sulfosalicylic acid. It was then filtered through Whatman #2 paper.

3. To 2 mL of this filtrate, 2 mL of acid-ninhydrin and 2 mL of glacial acetic acid were added in a test tube.

4. The test tube was then heated at 100°C in a water bath for 1 h. The reaction was stopped by removing the tubes from hot water bath and placing them in an ice bath.

5. 4 mL of toluene was added to the mixture and vortexed for 15 to 20 s. The chromophore was aspirated from the aqueous phase.

6. The absorbance of toluene phase was measured at 520 nm. A blank absorbance was recorded at 520nm, which was subtracted from the sample absorbance. A standard graph by using L-Proline as a standard was prepared.

3.9.2.2 Malondialdehyde content

Lipid peroxidation is oxidative degradation of lipid-fatty acids by reactive oxygen species. The level of lipid peroxidation was measured in terms of thiobarbituric acid reactive substances (TBARS) content, which includes products like malondialdehyde, fatty acid-hydro-peroxides, by the method given by **Heath and Packer (1968)**.

1. 0.1% Trichloro-acetic acid solution was prepared by dissolving 0.1 g TCA in water and then the volume was made to 100 ml.

2. 0.5% Thiobarbituric acid (TBA) in 20% TCA was prepared by first preparing 20 % TCA (20 g TCA in 100 ml). 0.5g TBA was dissolved in 20 % TCA and then volume was made to 100 ml by 20 % TCA.

3. Leaf sample (0.5 g) was homogenized in 10 ml, 0.1 % trichloro-acetic acid (TCA). The homogenate was centrifuged at 15000 g for 15 min.

4. Supernatant was used for the estimation of TBARS contents. Assay was done by adding **t**o the 1.0 ml aliquot of the supernatant 4.0 ml of 0.5% thiobarbituric acid (TBA) in 20% TCA.

5. The mixture was heated at 95℃ for 30 min in the laboratory electric oven and then cooled in an ice bath. After cooling the aliquot was centrifuged at 10 000 g for 10 min the absorbance of the clear supernatant was recorded at 532 nm.

6. Values of non-specific absorption recorded at 600 nm were subtracted from the values recorded at 532 nm. The TBARS content was calculated according to its extinction coefficient,ϵ = 155 $mM^{-1}cm^{-1}$.

Results and Discussion

Chapter 4 RESULTS AND DISCUSSION

Drought stress has been reported to affect many processes needed for plant growth and development. For instance, drought stress reduces cell division, inhibits cell expansion, decreases photosynthetic rate and modifies the root morphology. Drought stress affects both cell expansion and elongation. Cell expansion and cell growth are greatly suppressed as a result of water stress. The effect of drought can be pacified by the use of silica nanoparticles.

The effect of silica nanoparticles on the drought tolerance of the two genotypes of finger millet at vegetative stage was studied. The nanoparticles were synthesized in the lab using rice husk. The particles were applied at three different concentration (0, 25 and 50 mg/L) to drought responsive (PRM-6107) and drought sensitive (PES-400) genotypes of *Elusine coracana* and their effect on drought tolerance was studied. Results are based on mean values of 3 replicates of the experiments. For all the parameters the significant difference amongst the genotypes was found to be P<=0.05.

4.1 The synthesis of silica nanoparticles from rice husk:

The rice husk was obtained from CRC, GBPUAT, Pantnagar, Uttrakhand **(figure 4.1a).** The burning of the rice husk resulted in the formation of rice husk ash **(figure 4.1b).** After dissolving the rice husk ash in NaOH, the solution was filtered and the filtrate was titrated with 1N HCl to produce the gel **(figure 4.1c).** After further process when the gel was dried at 65˚C then it formed dried silica nanoparticles **(figure 4.1d).**

4.2 Characterization of silica nanoparticles was done by various methods to evaluate the dimension of the synthesized silica nanoparticles:

The characterization of silica nanoparticles synthesized from rice husk was carried out by means of the following techniques: Scanning Electron Microscopy (SEM) which was done to determine the external morphology and size of the nanoparticle. Energy dispersive X-ray (EDX) was performed to find out the composition of the nanoparticle. Scanning tunneling microscopy (STM), was done to measure the average size of the nanoparticle. The average size was obtained by the

mean value from the analysis of 100 observed nanoparticles. Fourier Transform Infrared Spectroscopy (FT-IR), was conducted to study the various functional groups present in the nanoparticle. X-ray diffraction pattern (XRD), was carried out to determine whether the nanoparticle is of crystalline or amorphous nature. UV-vis spectroscopy revealed the presence of defects in the silica nanoparticle formation. These techniques enabled not only a detailed examination of silica nanoparticle powder morphology, but also its chemical composition. The results that were obtained showed that the particles had narrow size distribution which is well described by normal distribution.

4.2.1 Scanning Electron Microscopy (SEM):

Surface morphological attributes of silica nanoparticles were measured using Scanning Electron Microscope at an accelerating voltage of 15-20 kV. The samples were analysed under Variable Pressure (VP) mode with gold coating so as to improve the surface conductivity. The micrograph illustrates that amorphous silica particles were obtained **(figure 4.2.1).** . The majority of primary SiO2 particles had a uniform size varying from ~50 to ~ 70 nm in size. The scanning electron microscopy showed that at 50000X magnification the size of the nanoparticle was 100 ± 20nm **(Qasim *et al.,* 2014)**. Since the silica nanoparticles are nonconductive, they tend to be present in close proximity with each other **(Lu and Hsieh, 2012).** As there in no repulsion among the silica nanoparticles due to its uncharged surface, the nanoparticles shows the tendency to grow in size by particle aggregation and decrease in number of the nanoparticles. The result reveals that almost spherical silica nanoparticles (SiO2) were synthesized using rice husk. The above information on microstructural characterization of the silica nanoparticles have influence on the properties of the nanoparticles as it mainly affect the physical stability and the distribution of the nanoparticles, when solution of different concentrations of nanoparticles was made.

4.2.2 Scanning tunneling microscopy (STM):

In scanning tunneling microscope, image of the conducting surfaces of the sample was obtained with single nanometer resolution. The sample prepared was stable during the study and lacked coagulation processes. Observation of immobile single nanoparticles enabled the analysis of their size distributions. The obtained histogram

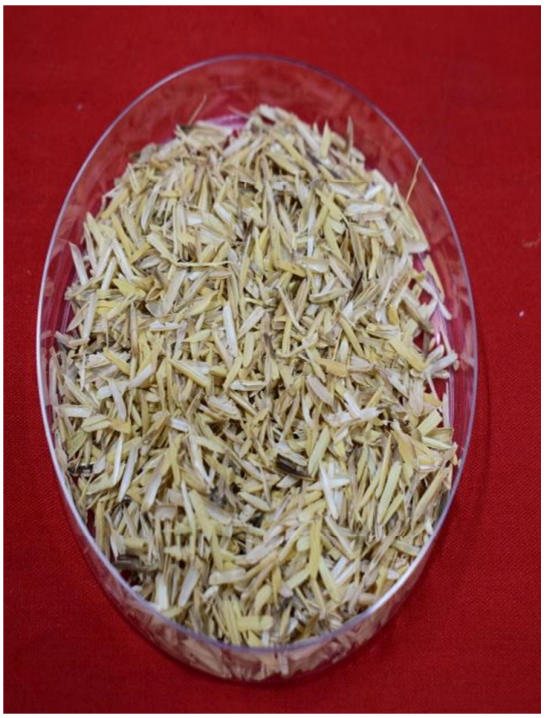


Fig.4.1a Rice husk

Fig.4.1b Rice husk ash

Fig. 4.1c Formation of gel

Fig.4.1d Powder silica nanoparticles

Fig. 4.2.1: Scanning Electron Micrograph at 50000x magnification of silica nanoparticles synthesized from rice husk.

confirms the fact that for 100 observed nanoparticles. The distribution of their sizes was having maximum number of nanoparticles ranging between 50-100nm as shown in **(figure 4.2.2).** The average value of silica nanoparticles synthesized from rice husk was observed to be 80nm and a 1.4 nm standard deviation. The theoretical curve of standard distribution was calculated by means of MS Excel program. The nanoparticle sizes calculated by this method are comparable with the results of similar studies conducted with other measurement techniques.

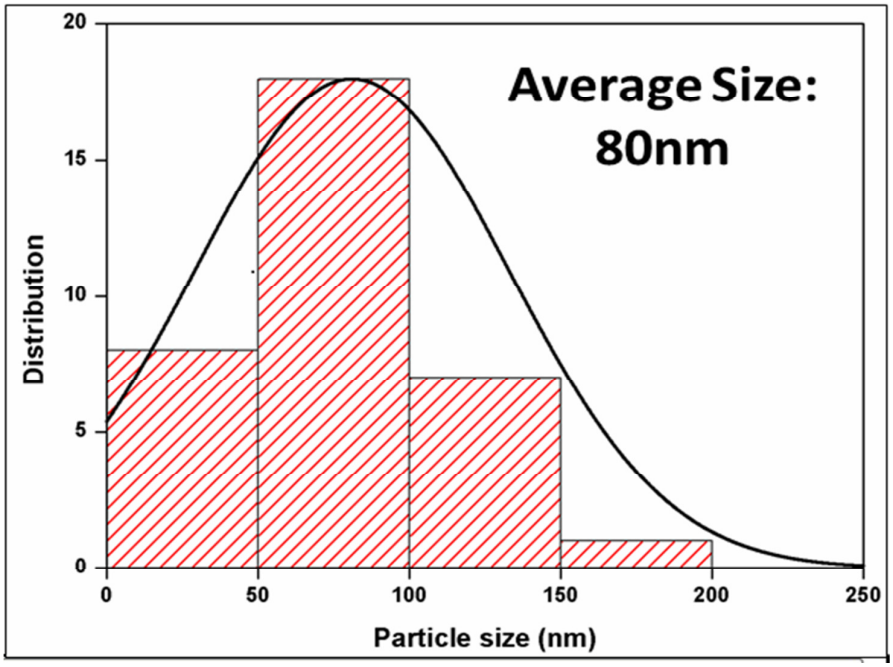


Fig. 4.2.2: The histogram of silica nanoparticles sizes obtained by means of the Single Nanometer Resolution (STM) results

4.2.3 Fourier Transform Infrared Spectroscopy (FT-IR):

The FT-IR spectra of silica nanoparticles synthesized from rice husk is shown in **(figure 4.2.3)**. There is an intense and wide band appearing at ~1079 to 1167 cm^{-1} which represents the asymmetric stretching vibrations of SiO_2 bond. The band appearing at ~1079 cm^{-1} is also known as absorption band. The band that appears at 960 cm^{-1} is due to the asymmetric vibrations of SiOH bond and at 801 cm^{-1} is due to asymmetric vibrations of Si– O bond. The peak at ~468 cm^{-1} represents the bending mode of SiO2 bond **(Qasim *et al.,* 2014)**. An intense and sharp characteristic absorption band is observed at ~3300-3500 cm^{-1} which is as a result of OH bond

stretching vibrations (**Kalapathy *et al.*, 2000**). This states the bonding of the solid state network structure of silica or may be due to the absorbed water that is unbounded. The sharp band at ~1631 cm^{-1} is caused because of the scissor bending vibration of molecular water (**An Dongmin *et al*., 2010).**

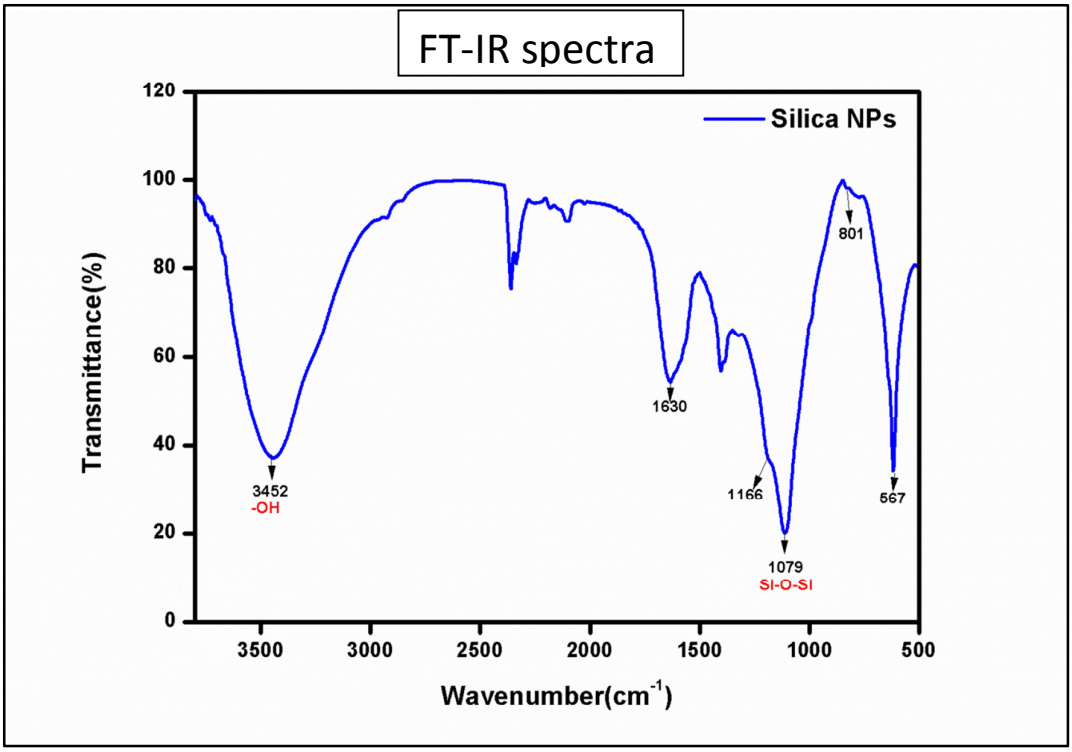


Fig. 4.2.3: FT-IR spectra of silica nanoparticles synthesized fron rice husk

4.2.4. Energy Dispersive X-ray spectroscopy (EDX):

The results in **(figure 4.2.4)** shows the area of EDX map, which was produced by the incidence of electron beam in order to estimate the elements present in the silica nanoparticles. The results provided in the table represent the semi quantitative estimation both in atomic percent and weight percent of the different elemental composition of the silica nanoparticles. The map shows that silica is the main element present in the sample (**Qasim *et al.,* 2014**). Since alkali treatment was carried out in order to precipitate the silica, so small amount of sodium still remained in the sample and represented as a separate peak. The silica peak in the EDX map is represented at around 1.7 keV and oxygen peak is present at around 0.5 keV **(Kalapathy *et al*., 2000).**

Since gold was used as the coating material during the preparation of the silica nanoparticles sample to be characterized by EDX, so small peaks of gold is also observed. Since the sample prepared was same as that for FE-SEM so it was coated with gold (~10nm thick), because of which there was an additional unwanted peaks to the X-ray spectrum. The coating material such as vacuum-evaporated carbon (~10nm thick), can be used which has a minimal influence on X-ray intensities as the atomic number of carbon is low, and does not add unwanted peaks to the X-ray spectrum.

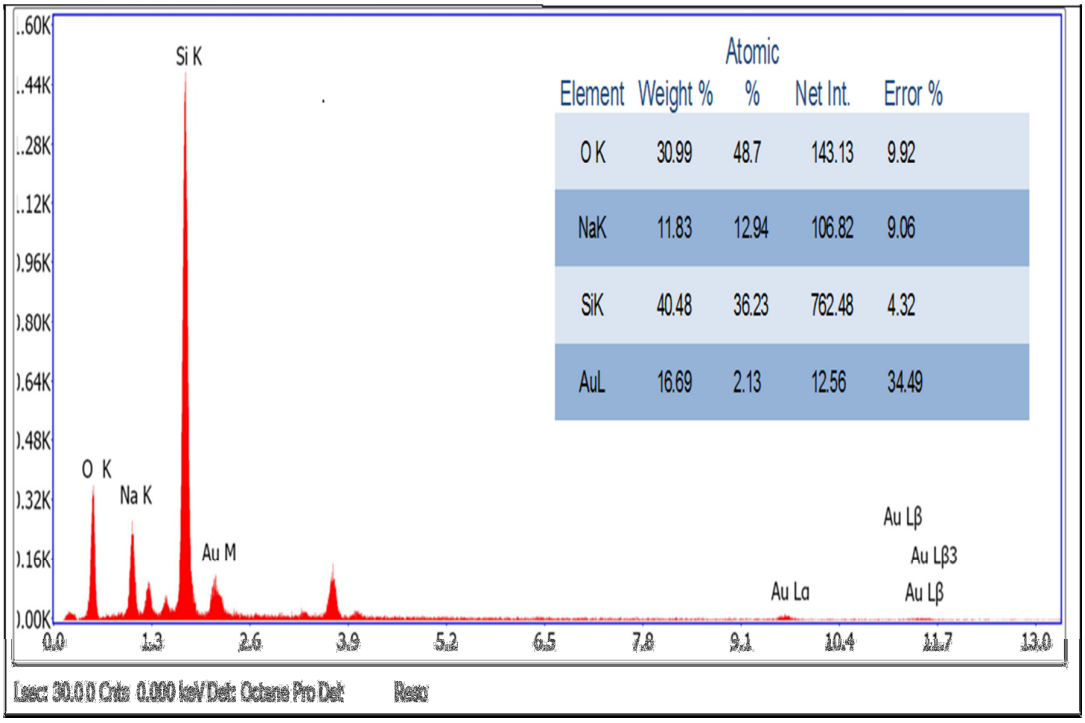


Fig. 4.2.4: Energy Dispersive X-ray spectroscopy (EDX) spectrometric data of silica synthesized from rice husk.

4.2.5 X-ray diffraction pattern (XRD):

The mineralogical composition of samples was analyzed by X-ray diffraction (XRD) by Bruker D8 Advance X-ray Diffractrometer with θ to 2θ geometry with copper emission lines (CuKα → λ = 1.5418 Å). The experimental conditions were: 40 kV, 40 mA, 2θ scanning range from 5 to 90º, angular step of 0.05º. Peaks were identified using the software X1Pert High Score 2.0a. The **(figure 4.2.5)** shows the X-Ray diffractrogram with 4 distinct and sharp peaks at 20.02˚, 22.0˚, 57.5˚ and 58.04˚**),** which confirms that the silica nanoparticle sample was amorphous in nature and also

indicated the absence of any form of crystalline or any disordered structure (**Kalapathy *et al.*, 2000**). This confirmed the purity and the amorphous structure of silica nanoparticles synthesized from the rice husk. The results of the present study confirm previous findings, since the silica nanoparticles synthesized from rice husk led to the generation of predominantly amorphous silica. The amorphous nature of the silica nanoparticles makes it advantageous to be used in agricultural system

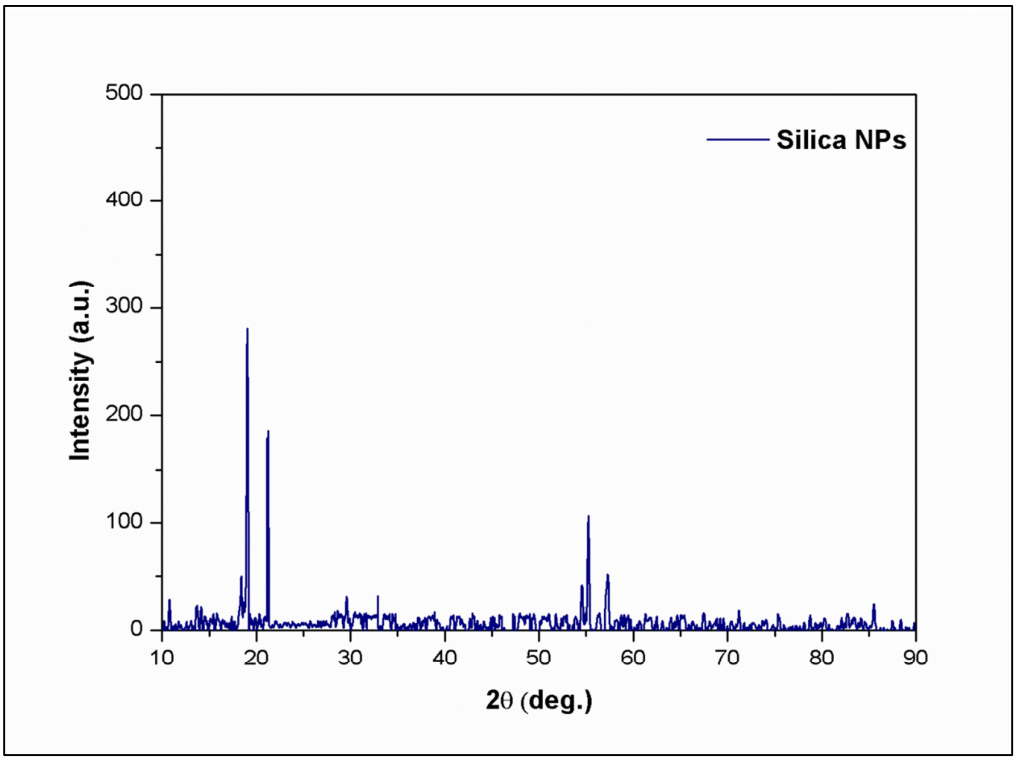


Fig. 4.2.5: X-ray diffraction pattern of silica nanoparticles synthesized from rice husk.

4.2.6 UV-vis absorption spectroscopy:

Amorphous silica does not absorb photons in the visible portion of the spectrum and large silica colloids will elastically scatter light (known as Rayleigh scattering). In silica nanoparticles, the optical phenomena is mainly due to the presence of different defects due to incomplete formation of Si– O– Si tetrahedral network at the silica surface, such as oxygen and silicon vacancies. As shown in the **(figure 4.2.6)** the absorbance peak is observed at ~220 to 230 nm wavelength which represents a size of the silica nanoparticles in the range of 5.4 to 12.2 nm **(Qasim *et al.*, 2014)**.

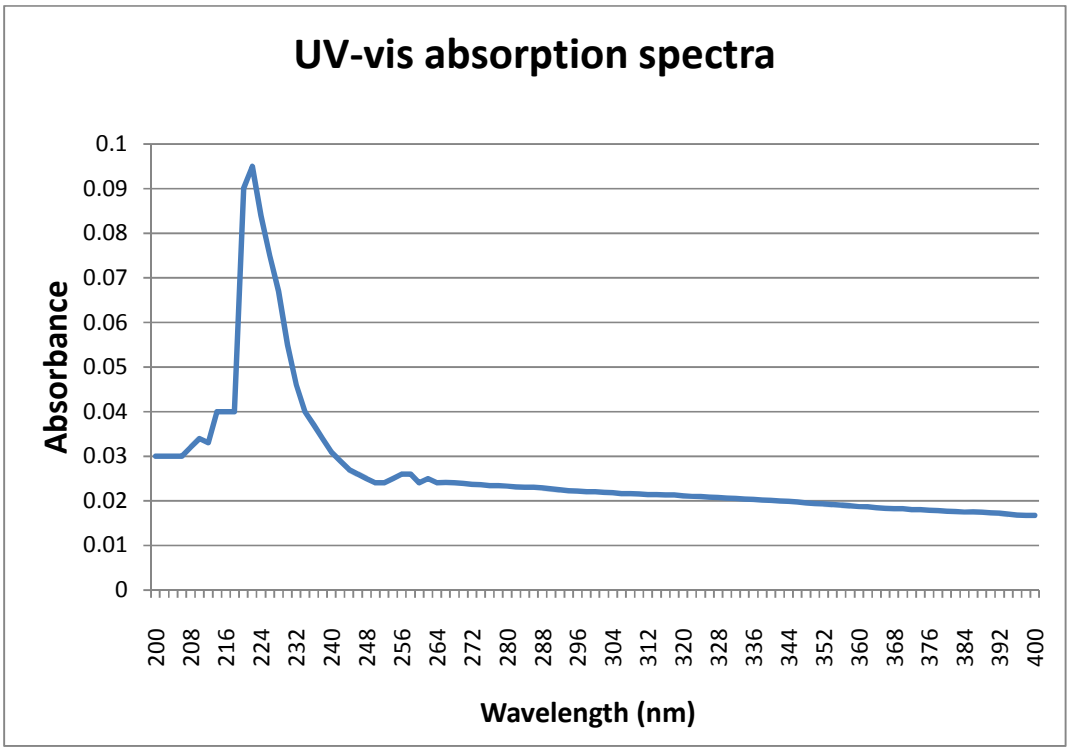


Fig. 4.2.6: UV-vis absorption spectra of silica nanoparticles synthesized from rice husk.

4.3 Phenotypic assessment of the effect of drought on finger millet in presence of silica nanoparticles

The **(figure 4.3.a- f, 4.3.A- F)** describes the experimental set up for the study that was undertaken. The pots in which is labeled drought (+) represent the plants that were under water stress condition. The pots that are marked drought (-) represents the plants that are well watered. The purpose of maintaining well-watered plant pot of each silica nanoparticle concentration treatment was to compare the damaging effect of drought on plants. In the **(figure 4.3.a - 4.3.c)** it was observed that there is no significant difference in between the PRM-6107 plants treated with particular concentration of silica nanoparticles and its respective control plants, on the 1st day of drought imposition. Same results were observed in case of PES-400 as shown in **(figure 4.3.d - 4.3.f).** With the increasing days of drought the effect of silica nanoparticle treatment is observed on the two genotypes. A marked increase in the drought enduring ability of PES-400 was observed in plants treated with silica nanoparticles (25mg/L and 50mg/L). The treated plants were greener, stouter and erect as compared to the plants without any treatment **(figure 4.3.D)**. There was less number

of leaves that dried and died as an effect of 5 days of drought, in the treated plant when compared to the plants with no treatment.

The phenotypic observations were later confirmed by different morphological and physiological analysis of drought stress.

4.4 Morpho-physiological parameters studied to evaluate the effect of drought stress:

4.4.1 Shoot Length

The shoot length was measured from soil layer up to the base of the flag leaf. Height of the shoot from three randomly selected plants was measured for all the silica nanoparticles treated and non-treated plants of PRM-6107 and PES-400 subjected to drought stress. Height of plants under drought stress and treated with silica nanoparticles were found to increase when compared with the control plants in which no silica nanoparticles was applied. However the shoot length of PRM-6107 plants that were treated with 50mg/L was found to be highest as compared to the PRM-6107 in which no silica nanoparticles was applied. The length of PRM-6107 which was treated with 50mg/L was closely followed by PRM-6107 plant which was treated with 25mg/L silica nanoparticles. PES-400 without any silica nanoparticles treatment and subjected to water stress had the shortest shoot length amongst all plants. This shows that PRM-6107 is more capable to tolerate drought than PES-400 **(figure 4.4.1)**. **Kramer (1969)** reported that growth and development in morphological traits is severely hampered as a result of moisture stress. He described this as a typical response of crop plants when exposed to drought conditions. **Inamullah *et al*. (1999)**, reported about significant reduction in plant height under moisture stress when compared to control conditions. The decrease in height under water deficit conditions is often either due to inhibition of cell division or cell expansion. Drought stress impacts severely the morphology of a grass plant. Cell enlargement is more drastically affected compared to cell division. The most important effects are exhibited in the form of decreased growth rate and reduced leaf area **(Pande and Singh, 1985)**.

Plants treated with silica nanoparticles were found to cope up with drought more effectively than the plants not treated with silica nanoparticles of the same genotypes. PES-400 treated with silica nanoparticles showed marked increase in the

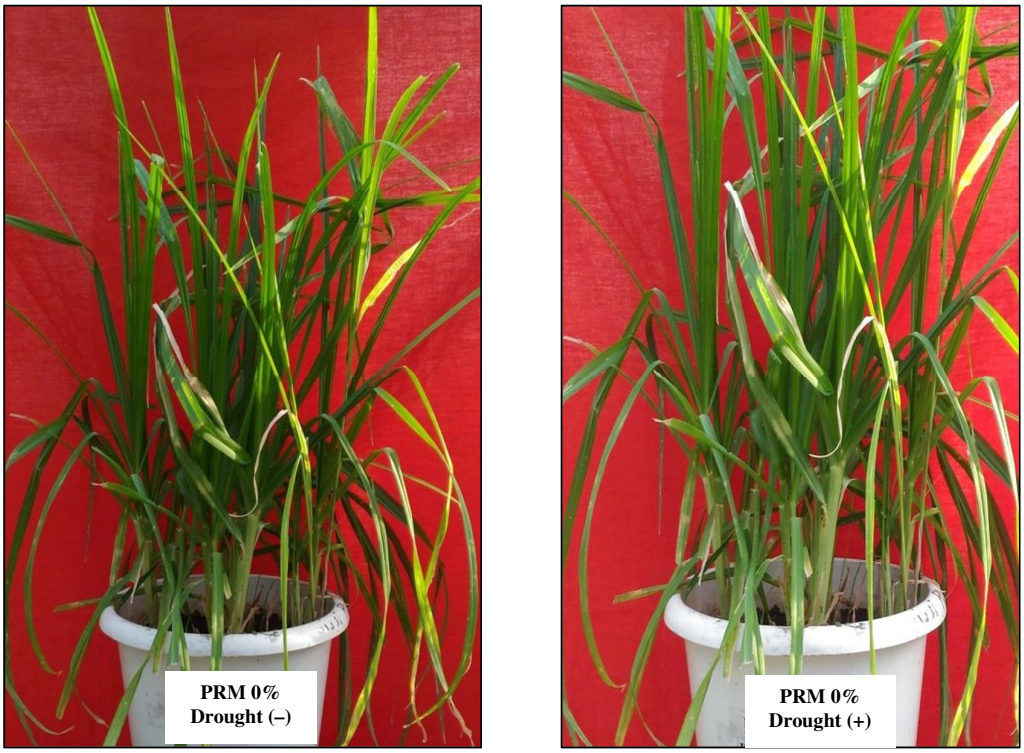


Fig. 4.3.a. PRM-6107 having no silica nanoparticles treatment (1st day of drought)

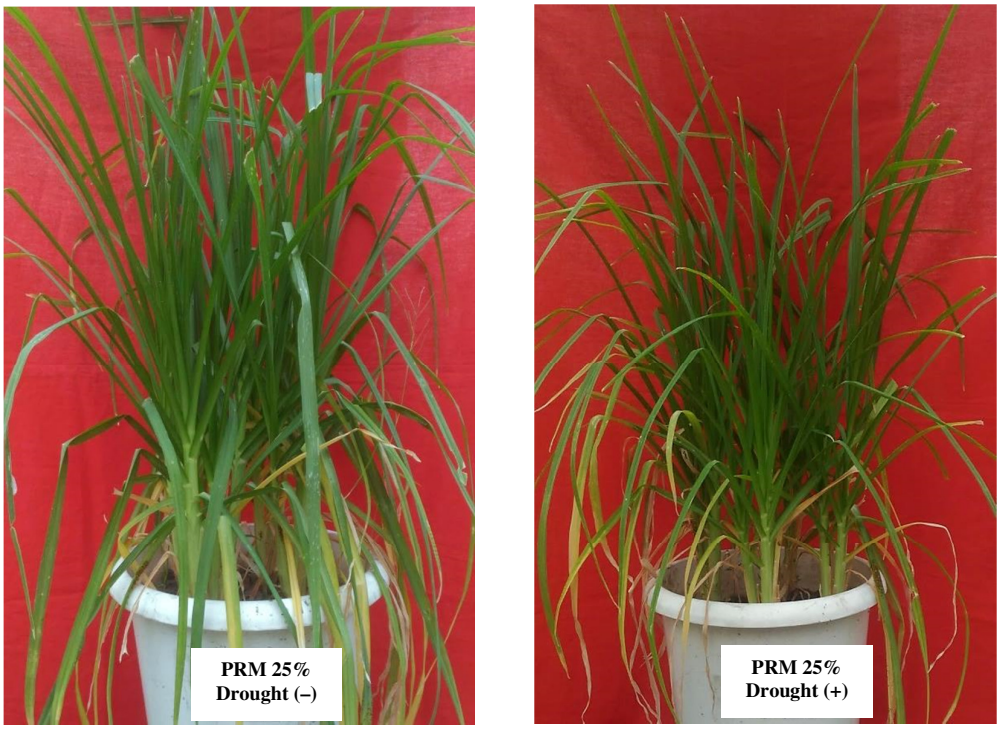


Fig.4.3.b. PRM-6107 having 25mg/L silica nanoparticle treatment (1st day of drought)

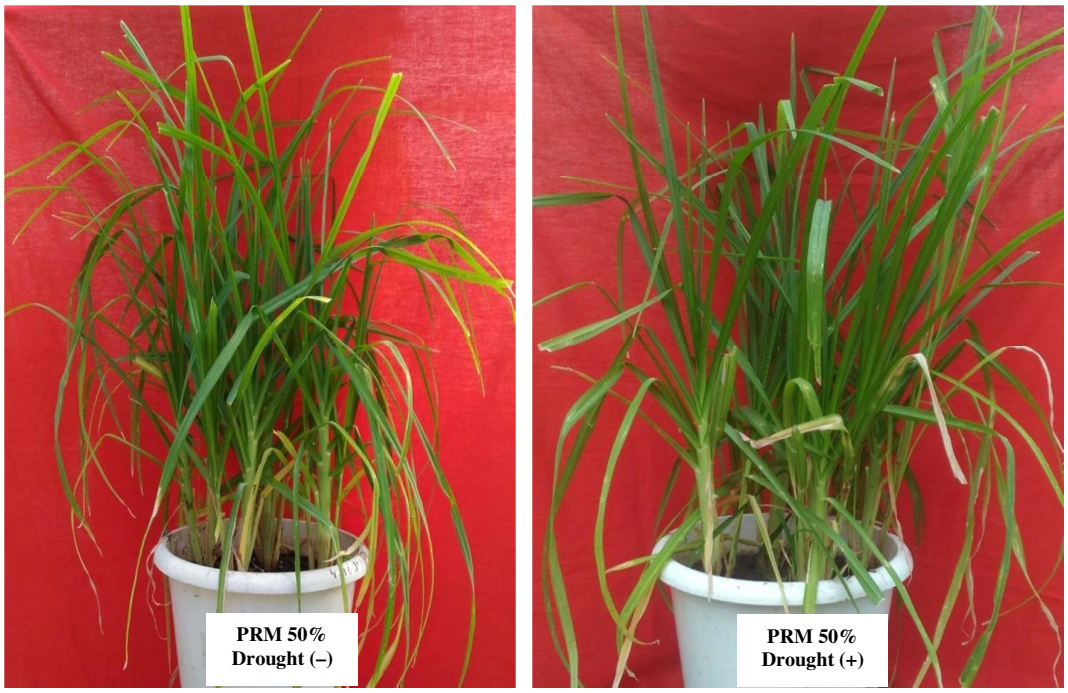


Fig.4.3.c. PRM-6107 having 50mg/L silica nanoparticle treatment (1st day of drought)

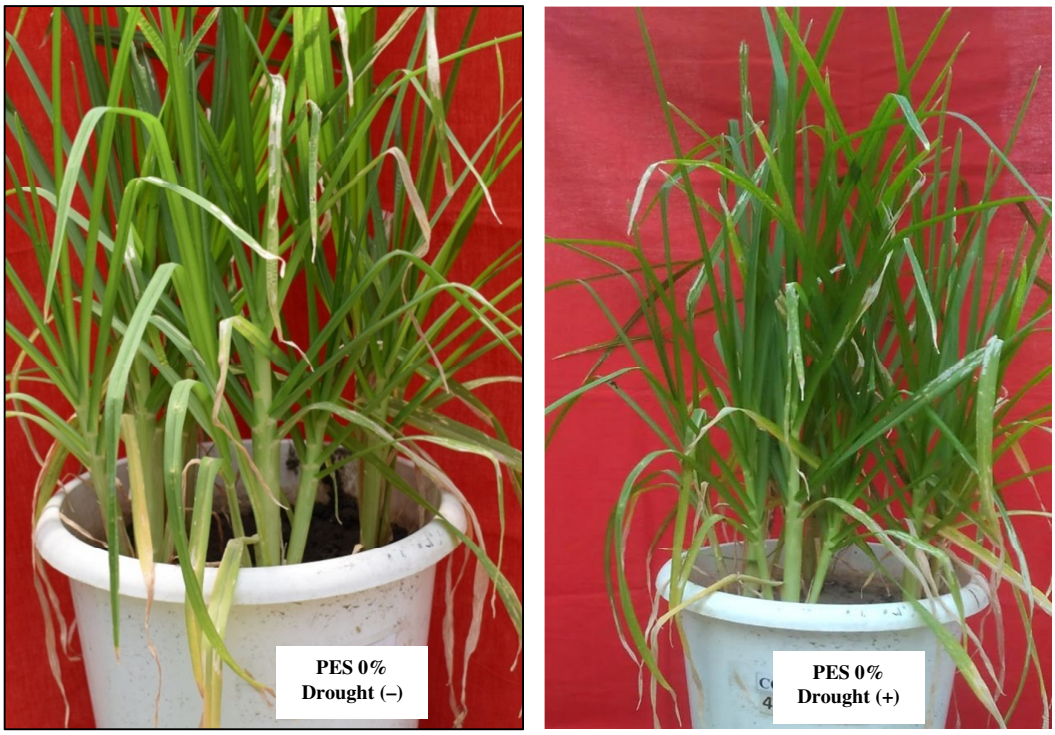


Fig.4.3.d. PES-400 having no treatment of silica nanoparticles (1st day of drought)

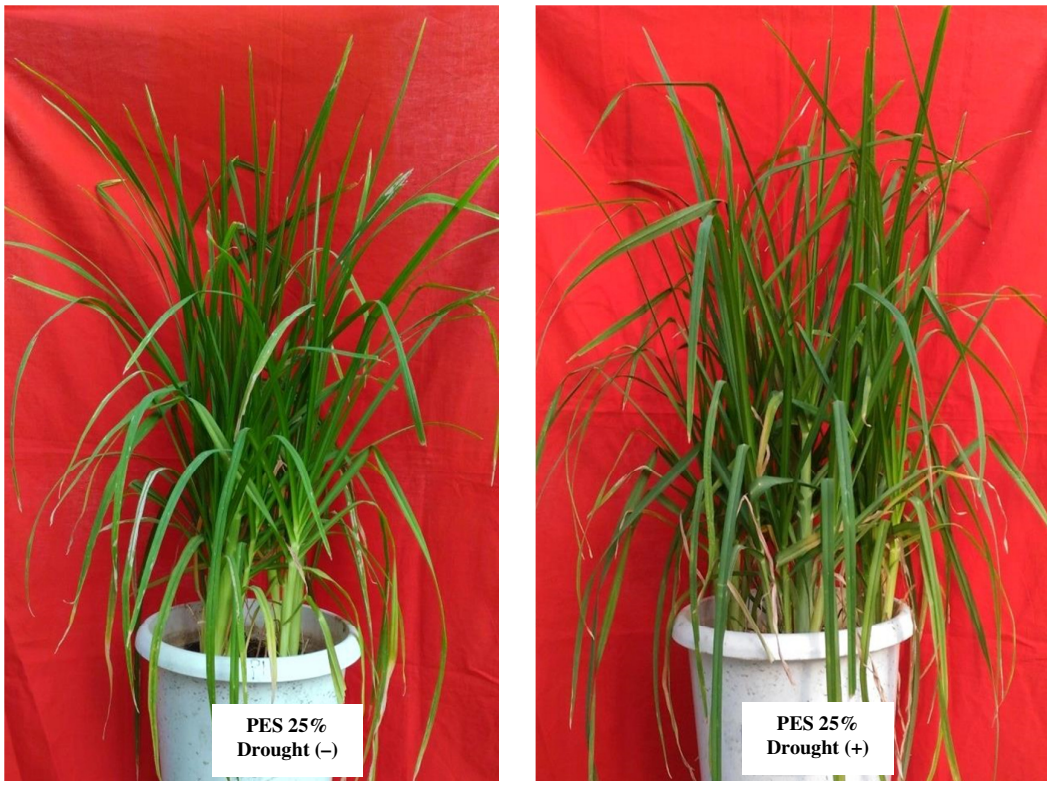


Fig.4.3.e. PES-400 having 25mg/L treatment of silica nanoparticles (1st day of drought)

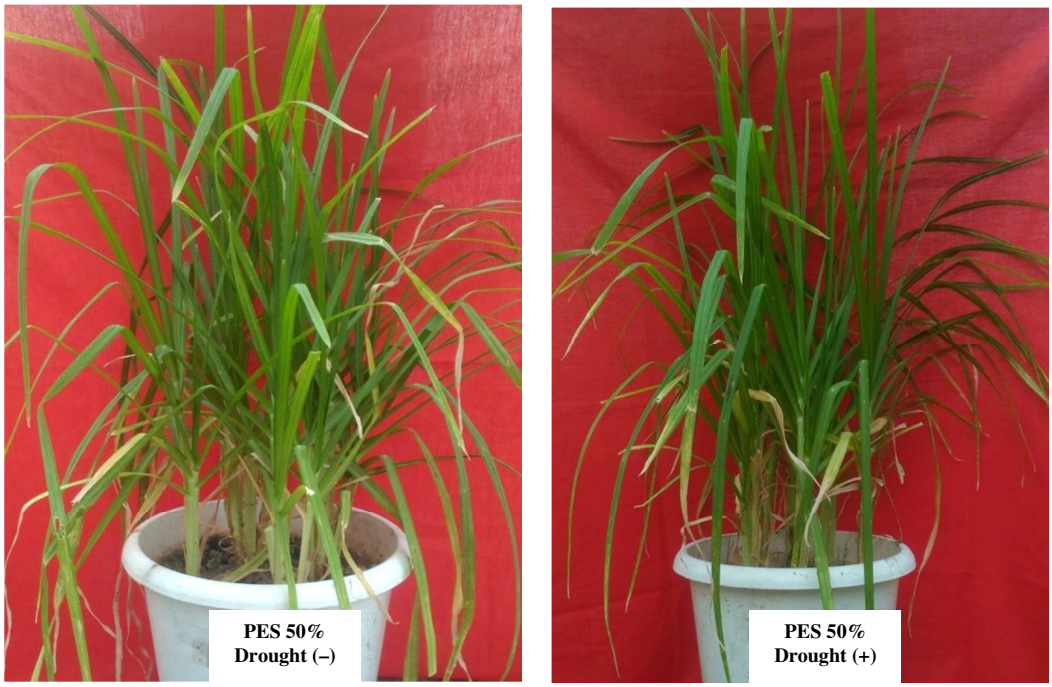


Fig.4.3.f. PES-400 having 50mg/L treatment of silica nanoparticles (1st day of drought)

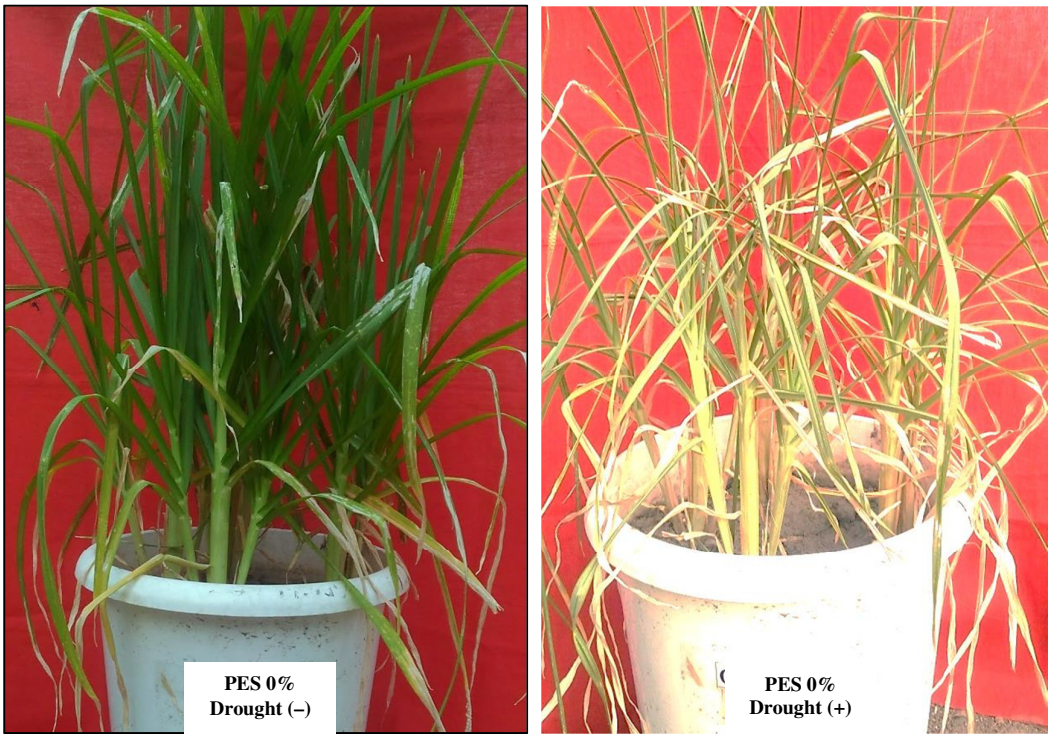


Fig.4.3.A.PES-400 having no treatment of silica nanoparticles (5th day of drought)

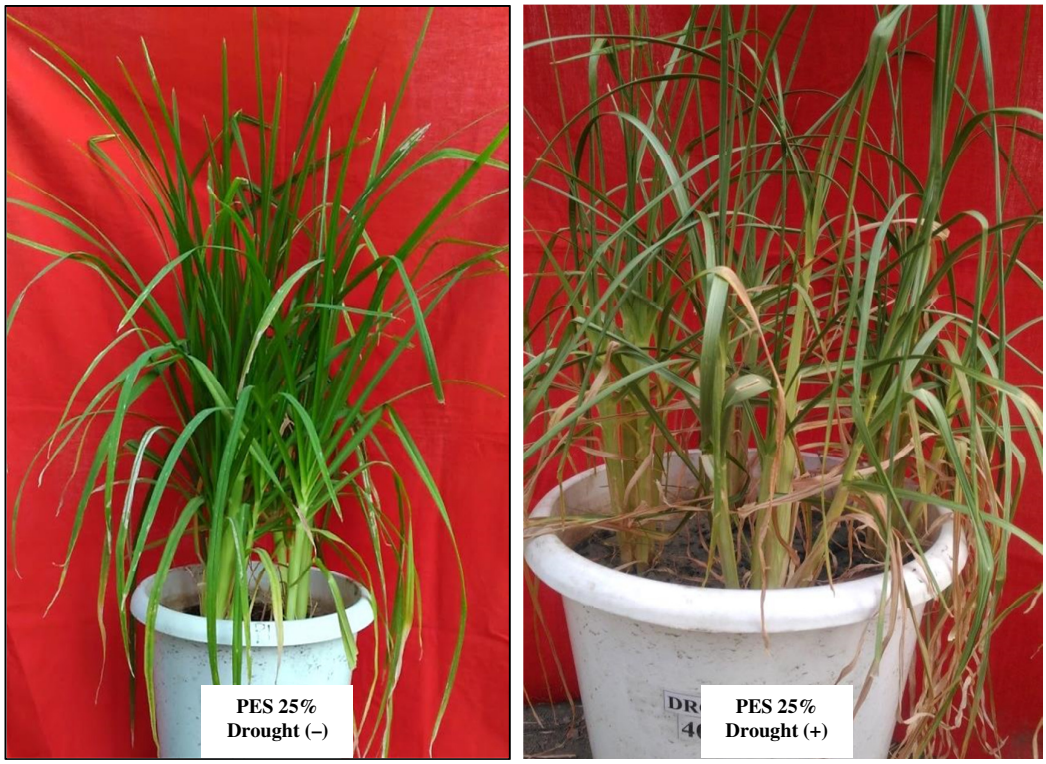


Fig.4.3.B. PES-400 having 25mg/L treatment of silica nanoparticles (5th day of drought)

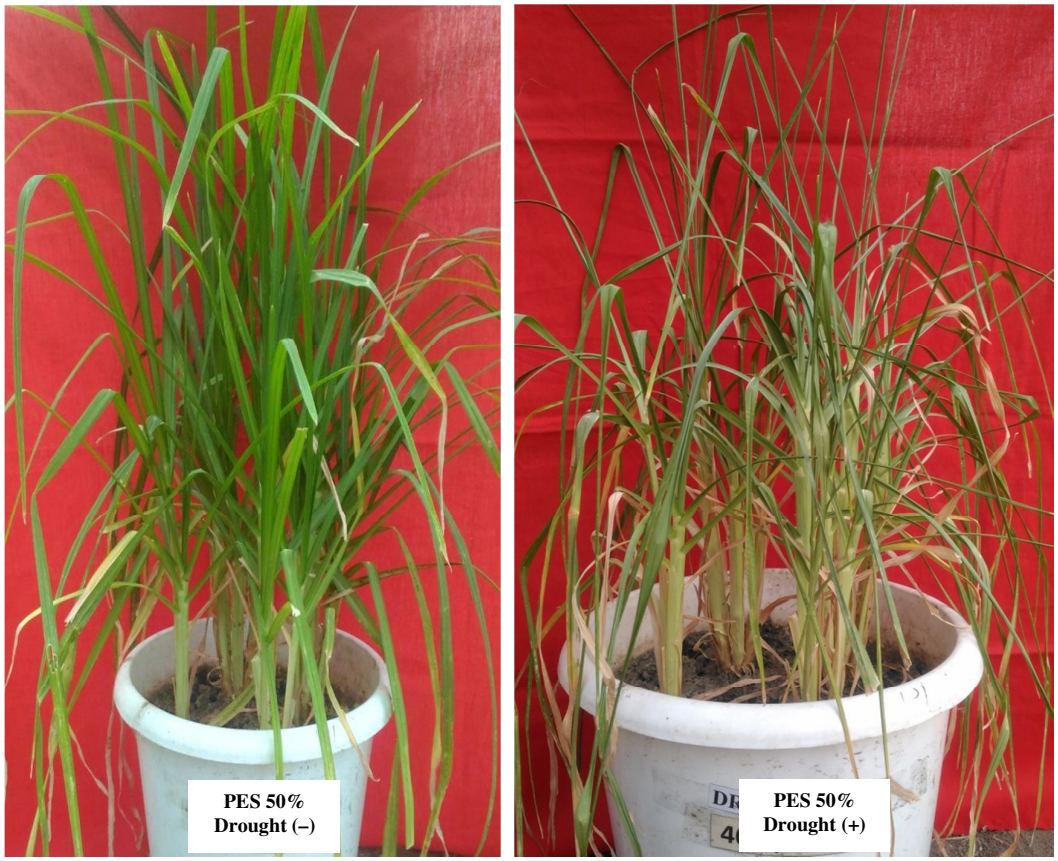


Fig.4.3.C. PES-400 having 50mg/L treatment of silica nanoparticles (5th day of drought)

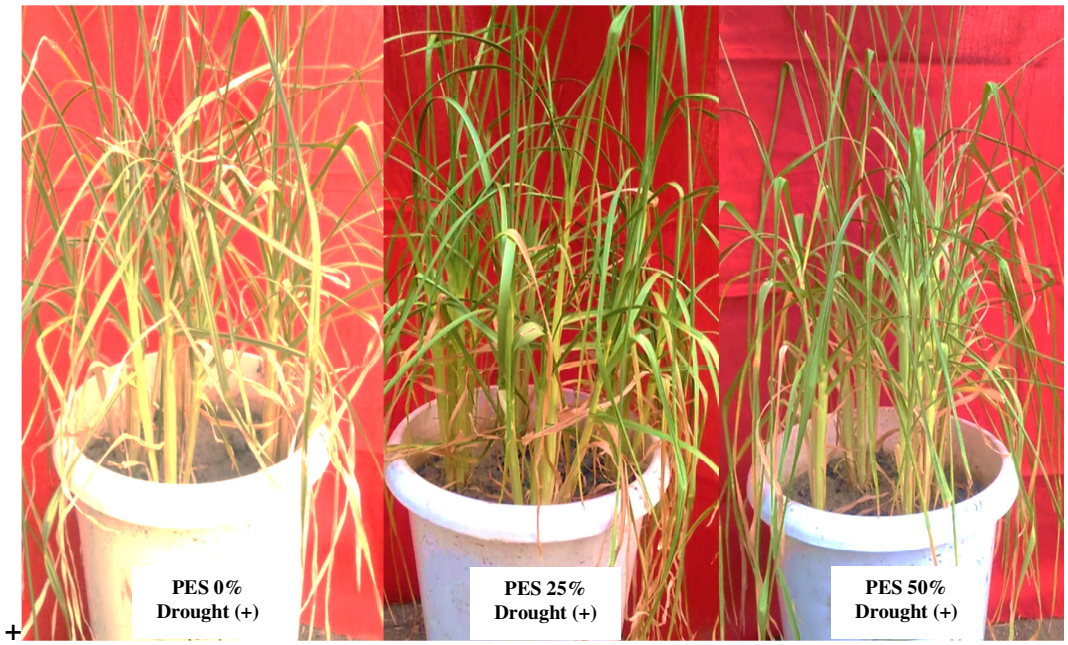


Fig.4.3.D. PES-400 having different concentration of silica nanoparticles (5th day of drought)

Fig.4.3.E. PRM-6107 having no silica nanoparticles treatment (5th day drought)

Fig.4.3.F. PRM-6107 having 25mg/L silica nanoparticles treatment (5th day drought)

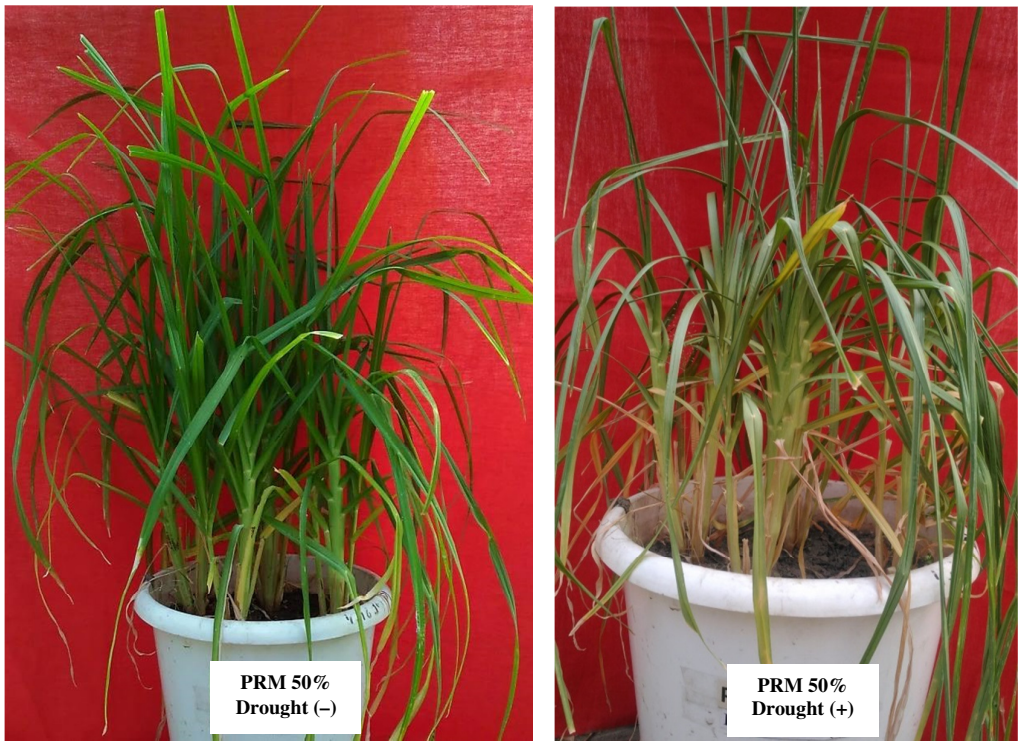


Fig.4.3.G. PRM-6107 having 50mg/L silica nanoparticles treatment (5th day drought)

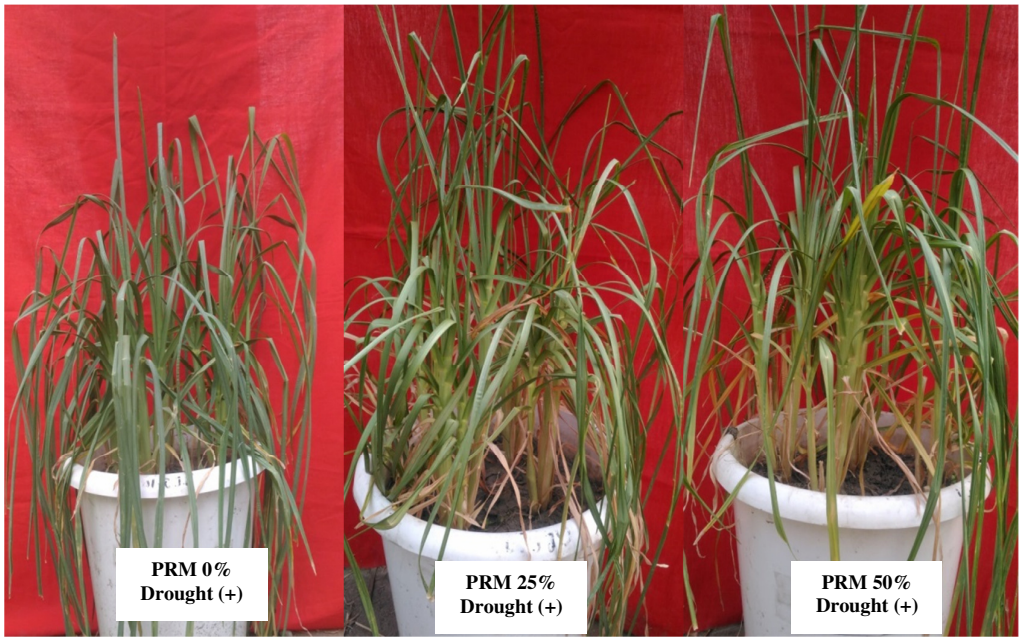


Fig.4.3.H. PRM-6107 having different silica nanoparticles treatment (5th day drought)

shoot length thus conferring the fact that it became more capable to tolerate drought. The percentage increase in the shoot length of PES-400 was 18% and 20% in the plants which was treated with 25mg/L and 50mg/L silica nanoparticles respectively as compared to the shoot length of the plants that was given no treatment of silica nanoparticles. This shows that the drought tolerance property was boosted in PES-400

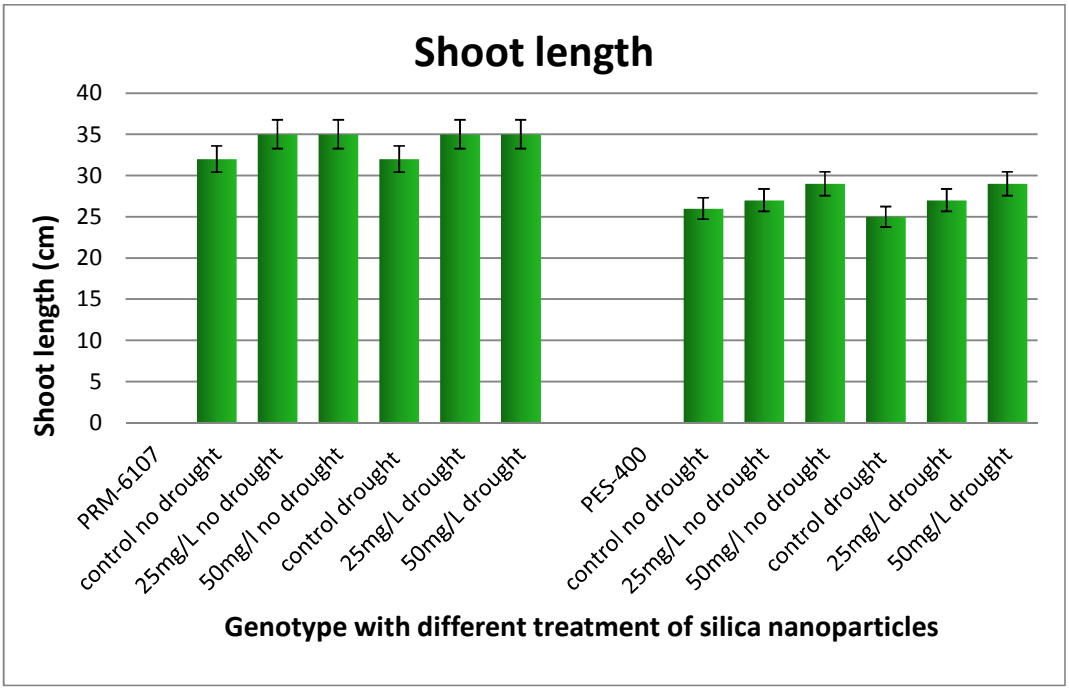


Fig 4.4.1: Effect of different concentration of silica nanoparticles on the shoot length in PRM-6107 and PES-400

4.4.2 Root Length

PRM-6107 with 50mg/L concentration treatment of silica nanoparticles had the maximum root length amongst the drought treated plants, and it was closely followed by PRM-6107 with 25mg/L concentration treatment of silica nanoparticles. In fact both the PRM-6107 and PES-400 plants which were treated with different concentration of silica nanoparticles and was subjected to drought conditions exhibited a higher root length than the plants that were not treated with silica nanoparticles **(figure 4.4.2.a, 4.4.2.c)** after the 5 days drought period **(figure 4.4.2).**

Traditionally root length density and depth are regarded as an important criterion for determining drought tolerance of plant root system **(Kashiwagi *et al.*, 2006).** There is report that total root length is a major contributor to root architecture

variation in different genotypes and can be used as an important selection criterion for drought tolerance at the seedling stage **(Li *et al*., 2015)**. The root system functions not only by supporting the above ground parts of a plant, but also by accessing water sources far below into the soil profile and obtaining water for the plant. The first organ that sense water shortage is the root. Its morphological and physiological properties largely determine the ability and rate of water and nutrient uptake. **(Boyer 1982)** observed that deep rooted plants are better adapted to counter drought conditions since they face very little water stress. **(Nahar *et al*., 2011)** worked on 7 tomato cultivars and observed that the cultivars that had a higher root length performed better in drought stress conditions than the others.

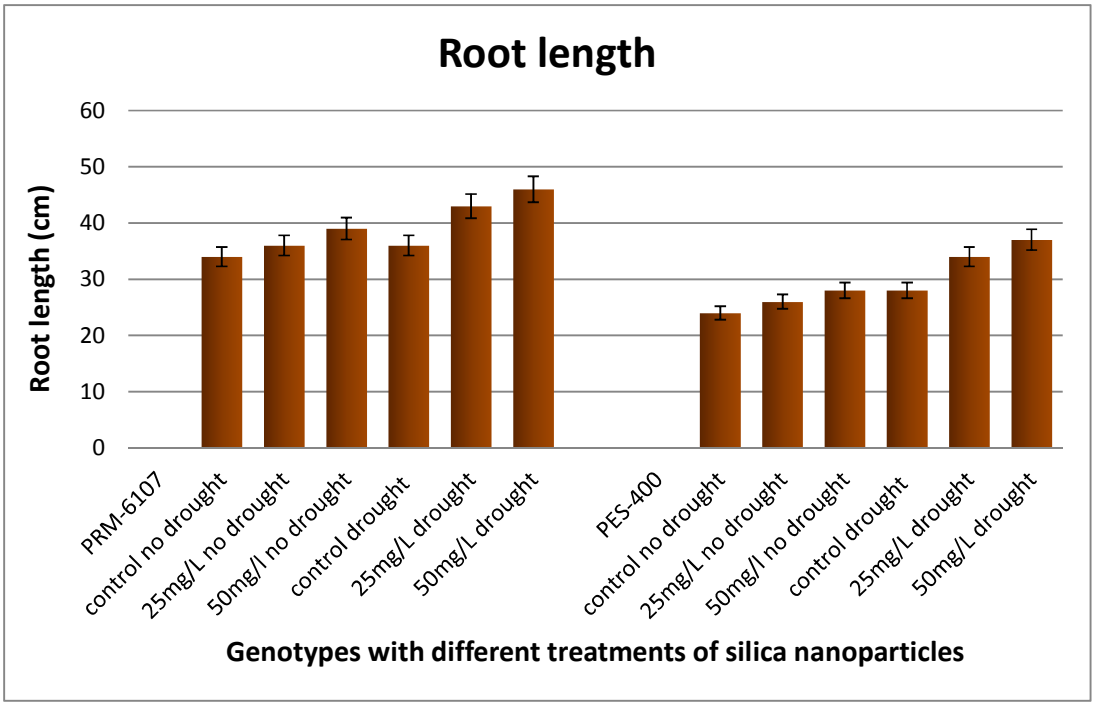


Fig 4.4.2: Effect of different concentration of silica nanoparticles on the root length in PRM-6107 and PES-400

Tomar *et al.* (2016), based on the study of drought tolerant cultivars of wheat reported that drought tolerant cultivars had a compact root system with higher depth. Similar observation was recorded in the present study in PRM-6107 and PES-400 in both the cases the plants treated with silica nanoparticles and exposed to drought **(figure 4.4.2.b, 4.4.2.d)**. While sensitive genotype had lesser depth and higher horizontal root spread as was observed with PES-400 which was not treated with silica

Fig.4.4.2.a Root length of PRM-6107 with no water stress (control) condition with 0mg/L; 25mg/L; and 50mg/L silica nanoparticles treatment respectively.

Fig.4.4.2.b. Root length of PRM-6107 under water stress condition with 0mg/L; 25mg/L; and 50mg/L silica nanoparticles treatment respectively.

Fig.4.4.2.c. Root length of PES-400 with no water stress (control) condition with 0mg/L; 25mg/L; and 50mg/L silica nanoparticles treatment respectively.

Fig.4.4.2.d. Root length of PES-400 under water stress condition with 0mg/L; 25mg/L; and 50mg/L silica nanoparticles treatment respectively

nanoparticles and exposed to water stress, but in this study a remarkable increase in the depth of the roots were observed in the plants of PES-400 which was treated with silica nanoparticles

The root length of PRM-6107 increased by 19% and 38% in the plants which was treated with 25mg/L and 50mg/L silica nanoparticles respectively as compared to the roots of the plants that was given no treatment of silica nanoparticles **(figure 4.4.2.b).** The percentage increase in the length of the roots of PES -400 was 21% and 40% in the plants which was treated with 25mg/L and 50mg/L silica nanoparticles respectively as compared to the roots of the plants that was given no treatment of silica nanoparticles **(figure 4.4.2.d)**. Nearly similar results were obtained in both the genotypes. With the increase in the concentration of the silica nanoparticles the ability to endure water stress also increased as higher root penetration was observed in 50mg/L treatment plants as compared to 25mg/L treatment. This shows that the silica nanoparticles treated plants possessed the capability to bring out drastic morphological alterations with regard to root system architecture in response to drought stress.

4.4.3 Root to Shoot Length Ratio

The mean readings of root shoot ratio was obtained when the root length was divided by the corresponding shoot length for the two genotypes under different treatment of silica nanoparticles to obtain the root to shoot length ratio of the control and plants subjected to water stress.

PRM-6107 treated with 50mg/L silica nanoparticles and subjected to water stress was found to have the highest root to shoot length ratio, closely followed by PES-400 treated with 50mg/L silica nanoparticles and subjected to water stress was found to have the almost highest root to shoot length ratio. PES-400 which was not treated to silica nanoparticles and was subjected to drought stress had the least root to shoot length ratio, which indicates PES-400 plants lacked the capability to reallocate its metabolites from the shoot to the root system. In case of higher root to shoot ratio the roots could grow into deeper soil profiles in search of water **(figure 4.3.3)**.

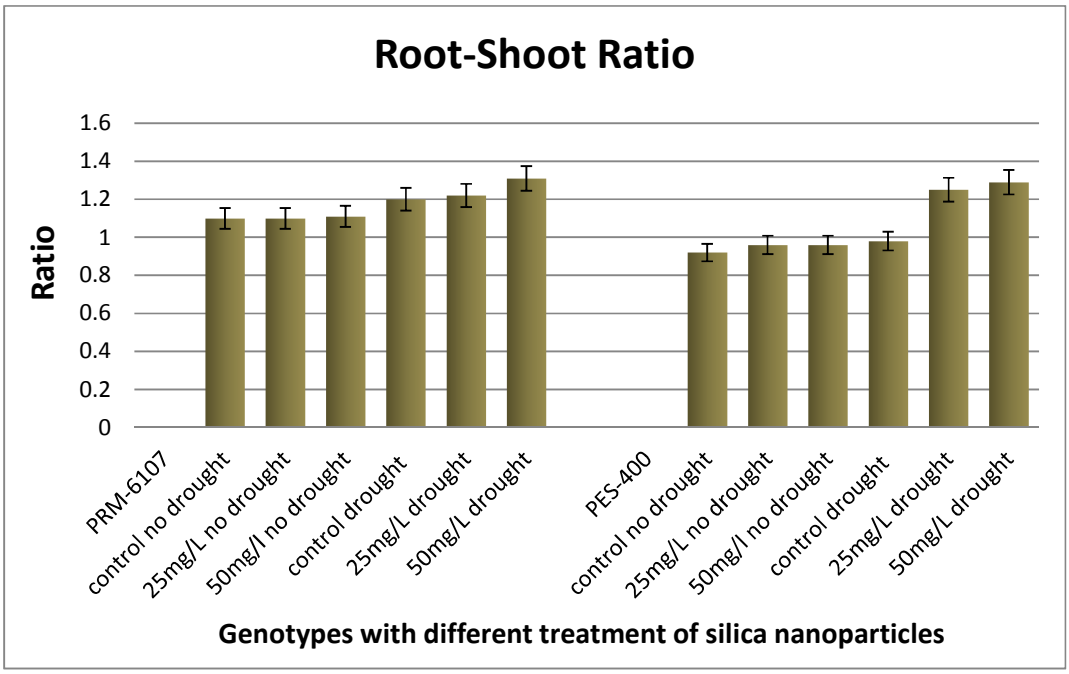


Fig 4.4.3: Effect of different concentration of silica nanoparticles on the root-shoot ratio in PRM-6107 and PES-400

This is important for plants to survive in case of prolonged drought stress conditions **(Rich and Watt, 2013; Xu *et al.*, 2010)** reported that the increased root to shoot ratio under drought stress indicates that carbohydrates were distributed more to roots than to shoots. **Hammond and White (2011)**, proposed though their study on drought tolerant plants that sucrose present in leaves (source organ) is used as a transportable substance to sink organs, such as roots, where it is hydrolyzed either by invertases or sucrose synthase in order to provide carbon and energy for growth and storage reserves, and as signals for the growth of roots.

The ratio of root to shoot length is used as an important parameter to evaluate drought tolerance in plants because deep rooted systems extract more water while relatively smaller shoots transpire less **(Srividya *et al.*, 2011)**. So the increase in the root shoot ratio in the drought sensitive genotype PES-400. This showed that the application of silica nanoparticles confers the drought tolerance property even in the drought sensitive genotype of PES-400.

The root shoot ratio of PRM-6107 increased by 9% and 18% in the plants which was treated with 25mg/L and 50mg/L silica nanoparticles respectively as compared to

the control plants . The percentage increase in the relative water content of PES -400 was 27% and 31% in the plants which was treated with 25mg/L and 50mg/L silica nanoparticles respectively as compared to the relative water content of the plants that was given no treatment of silica nanoparticles. Thus this showed that silica nanoparticles help in enduring drought stress even in the drought sensitive genotype. The results indicate a significant increase in root shoot ratio of PES-400 on treatment with silica nanoparticles. This helped the sensitive genotype the capacity to increase the drought stress enduring property.

4.4.4 Leaf number

Leaves from three randomly selected plants were counted for all the silica nanoparticles treated and non-treated plants of PRM-6107 and PES-400 in both the drought stress condition as well as fully watered conditions. Leaf growth is highly affected by the water stress condition. PES-400 plants which were subjected to drought stress condition without any silica nanoparticles treatments were found to have the least number of leaves **(figure 4.4.4)**. Drought stress have the least effect on the plants of PRM-6107 with regard to leaf growth because there was very little difference between the leaf number of plants that were subjected to drought condition and in well water condition. From the results it is evident that new leaf production is extremely reduced due to reduction in soil moisture availability since the plants that were watered possessed higher number of leaves than the plants exposed to water stress.

PRM-6107 the plants treated with silica nanoparticles and exposed to drought, as well as the plants that were not treated with silica nanoparticles and exposed to drought had higher number of leaves as compared to PES-400 plants, but with the increase in the concentration of the silica nanoparticles the ability to endure water stress also increased. While sensitive cultivars had lesser number of leaves as was observed with PES-400 which was not treated with silica nanoparticles and exposed to water stress, but in this study a remarkable increase in the number of leaves were observed in the plants which was treated with silica nanoparticles and with the increase in the concentration of the silica nanoparticles the ability to endure water stress also increased as higher number of leaves was observed in 50mg/L treatment plants as compared to 25mg/L treatment.

The number of PRM-6107 that died decreased by 6.6% and 25% in the plants which was treated with 25mg/L and 50mg/L silica nanoparticles respectively as compared to the control plants . The percentage decrease in the number of leaves that died of PES -400 was 38% and 53.8% in the plants which was treated with 25mg/L and 50mg/L silica nanoparticles respectively as compared to the number of leaves that died in the plants which was given no treatment of silica nanoparticles and exposed to drought stress . Thus this showed that silica nanoparticles confer drought tolerance property even in the drought sensitive genotype. The results showed prominent decrease in number of leaves of PES-400 that died during drought stress on treatment with silica nanoparticles.

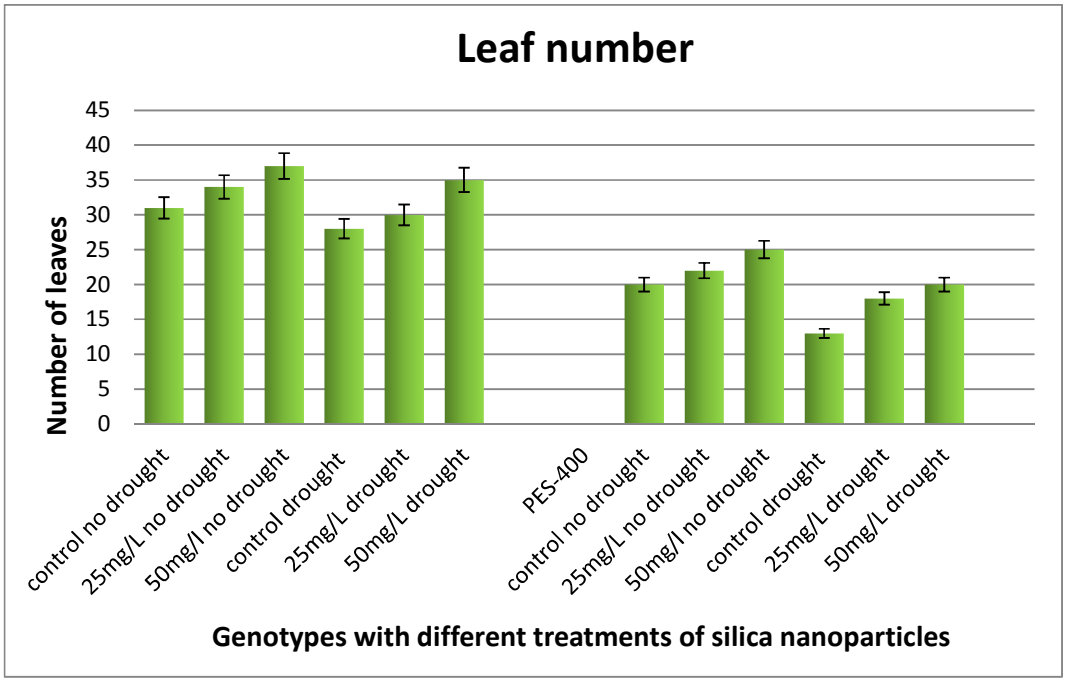


Fig 4.4.4: Effect of different concentration of silica nanoparticles on the number of leaves in PRM-6107 and PES-400

4.4.5 Leaf Area

Leaf area has direct relation with the photosynthetic capacity of a plant **(Potter and Jones, 1977)**. But drought stress severely affects the leaf area of plants. **Pande and Singh (1985)** reported that cell expansion is severely affected in case of prolonged drought stress, which manifests in the form of reduced leaf area. Hence the plants that are able to maintain their leaf area despite drought conditions ought to be considered more drought tolerant than others.

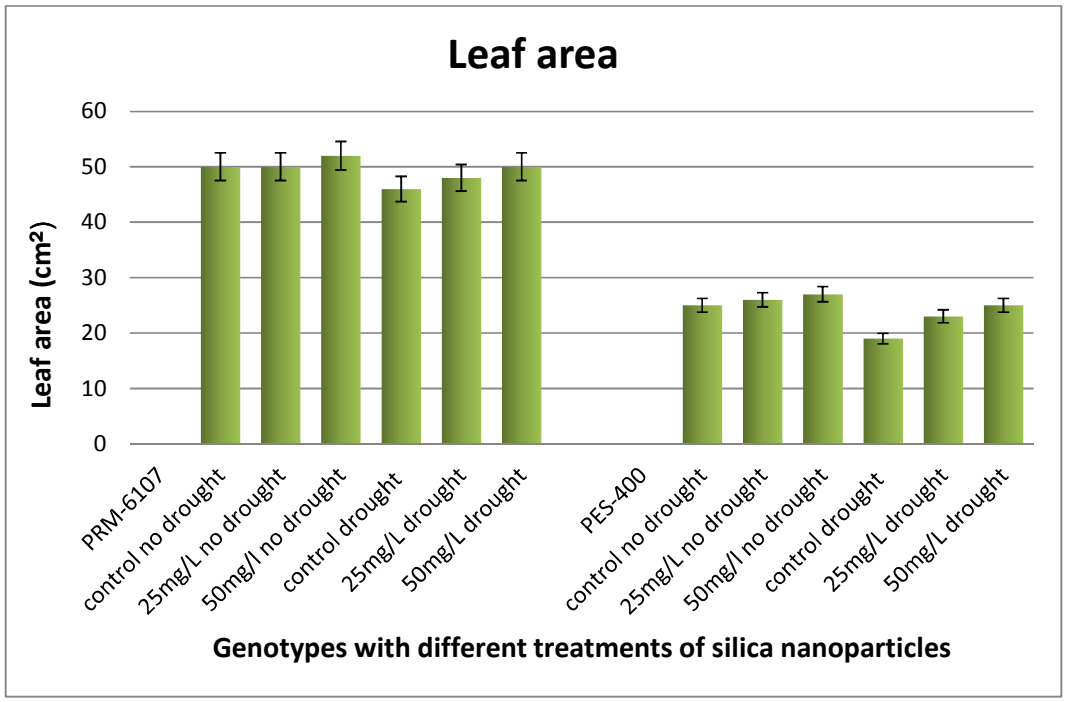


Fig 4.4.5: Effect of different concentration of silica nanoparticles on the leaf area in PRM-6107 and PES-400

In this study it was observed that leaf area of plants of both genotypes under study was highly reduced under drought stress. But compared to PES-400, PRM-6107 exhibited higher leaf area. This indicates they are better able to maintain their leaf area and hence physiological processes like photosynthesis than others. PES-400 which was not treated with silica nanoparticles exhibited least leaf area than others and so can be considered sensitive according to this parameter **(figure 4.4.5)**. However the PES-400 plants that were treated with silica nanoparticles showed increased leaf area as compared to its control plant. Thus it showed that application of silica nanoparticles provides drought tolerance ability of the PES-400 which is sensitive to water stress.

The leaf area of PRM-6107 increased by 4% and 8% in the plants which was treated with 25mg/L and 50mg/L silica nanoparticles respectively as compared to the control plants under drought condition. The percentage increase in the leaf area of PES -400 was 21% and 31% in the plants which was treated with 25mg/L and 50mg/L silica nanoparticles respectively as compared to the leaf area of the plants that were given no treatment of silica nanoparticles.

4.4.6 Leaf rolling and drying

Higher number of green leaf retention is an important characteristic feature of drought stress tolerant plant **(Blum *et al*., 2005)**. Leaves roll and dry as a result of moisture deficit conditions. Leaves roll to minimize the leaf surface area in order to reduce the amount of water lost by evapotranspiration. Rolling induced just at the onset of moisture stress is generally displayed by drought sensitive plants. Leaf rolling in such a case is induced as a result of poor osmotic adjustments and loss of turgor **(Hsiao *et al*., 1984)**.Thus the area for photosynthesis gets severely reduced. Amongst the treatment plants least leaf rolling and drying was observed in PRM-6107 indicating they are the most tolerant to drought stress with regard to leaf rolling and drying, while PES-400 is the most sensitive as it showed the highest rolling and drying in leaves when exposed to drought stress **(Table 4.1)** However the PES-400 plants that were treated with silica nanoparticles showed decrease in the leaf rolling and drying as compared to its control plant which was not treated with silica nanoparticle.

Table 4.1: Effect of drought stress on leaf rolling and drying

Sl.	Genotypes	Rolled and Dried Leaves
	PRM-6107	
1.	control no drought	No leaf rolling
2.	25mg/L no drought	No leaf rolling
3.	50mg/l no drought	No leaf rolling
4.	control drought	+
5.	25mg/L drought	++
6.	50mg/L drought	+
	PES-400	
7.	control no drought	No leaf rolling
8.	25mg/L no drought	No leaf rolling
9.	50mg/l no drought	No leaf rolling
10.	control drought	++++
11.	25mg/L drought	+++
12.	50mg/L drought	++

Legend + , Upto 4 Leaves Rolled and Dried ++, Upto 6 Leaves Rolled and Dried
+++, Upto 10 Leaves Rolled and Dried ++++, Upto 20 Leaves Rolled and Dried

4.4.7 Relative Water Content

Relative water content (RWC) is the appropriate measure of plant water status in terms of the physiological consequence of cellular water deficit **(Barrs and Weatherley, 1962)**. It estimates the current water content of the sampled leaf tissue relative to the maximal water content it can hold at full turgidity. RWC changes during the day as irradiance and temperatures change. **(Larbi, 2004)** reported that relative water content can be used as an effective screening method to determine the level of drought tolerance in crops.

Top-most fully expanded leaves were sampled. As the plant water status is influenced by the solar radiation and temperature, sampling was performed at a fixed time of the day, between 11AM –12 noons. The results indicate that drought stress resulted in the reduction in the relative water content in all the genotypes. However, PRM-6107 was able to maintain a higher RWC status as compared to PES-400 genotype. This shows that there is a better maintenance of water status in PRM-6107, owing to osmotic adjustments as a result of drought stress. This indicates that PRM-6107 has a better ability to counter drought conditions by bringing out osmoregulatory alterations in the plant tissue. PES-400 which was not treated with silica nanoparticles turned out to be the most sensitive genotype based on RWC, since it was not able to maintain its water status in response to drought stress **(figure 4.4.6)**. But the plants treated with silica nanoparticles were able to maintain the water status in response to drought stress.

The relative water content of PRM-6107 increased by 20% and 33%, in the plants which were treated with 25mg/L and 50mg/L silica nanoparticles respectively as compared to the control plant under water stress condition . The percentage increase in the relative water content of PES -400 was 66% and 90% in the plants which was treated with 25mg/L and 50mg/L silica nanoparticles respectively as compared to the relative water content of the plants that was given no treatment of silica nanoparticles. Thus this showed that silica nanoparticles confer drought tolerance property even in the drought sensitive genotype. The results indicate a significant increase in RWC of PES-400 on treatment with silica nanoparticles. This helped the sensitive genotype to cope up with drought stress as observed in the plants which were more green and erect compared to control **(figure 4.3.1.D).**

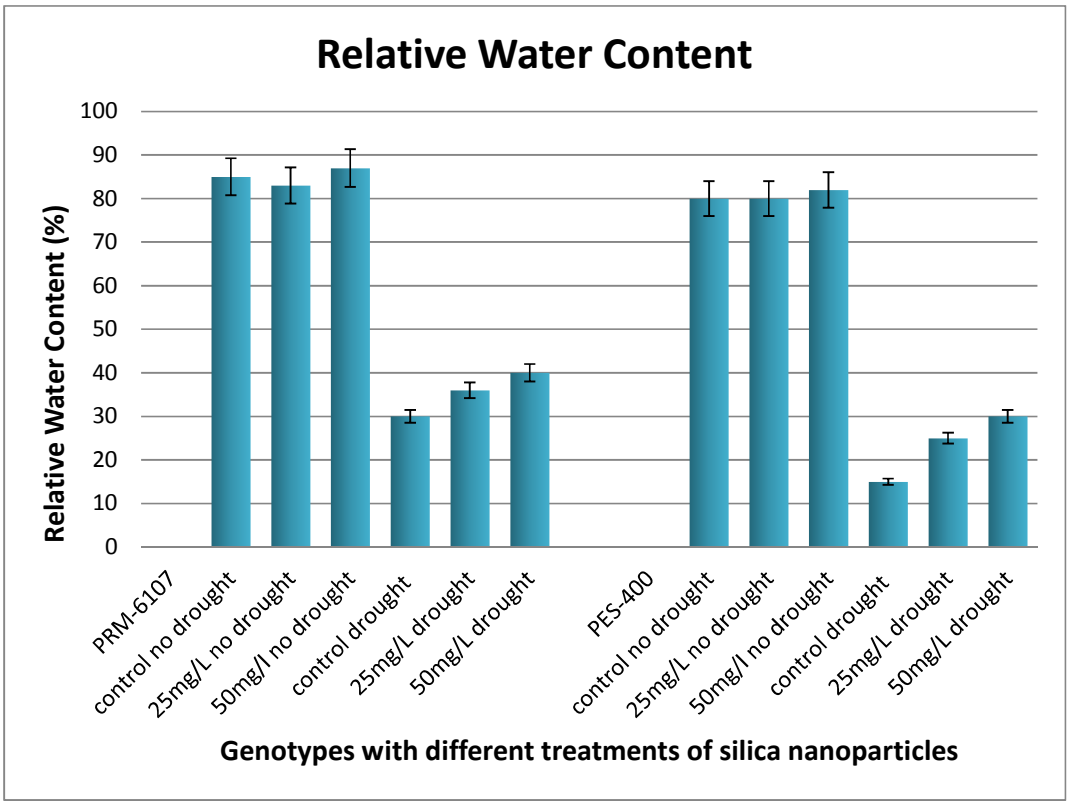


Fig 4.4.6: Effect of different concentration of silica nanoparticles on the Relative Water Content in PRM-6107 and PES-400

4.4.8 Total chlorophyll content

Leaves were collected randomly from both the genotypes of the different treatment of silica nanoparticles from the well watered and drought stress pots in order to estimate the total chlorophyll content. The values for chlorophyll A and chlorophyll B content were calculated and then added to obtain the total chlorophyll content of the leaves. It is evident from the result that the pigment apparatus of both the genotypes exhibited differential sensitivity to moisture stress (**figure 4.4.7**). **Shmat'ko *et al.*, (1977)** reported that drought tolerant genotypes show little change in the chlorophyll content compared to drought susceptible genotypes.

Huge reduction in the chlorophyll content was observed in the leaves of both the genotypes which was exposed to drought stress when it was compared to the plants that were well watered. PRM-6107 which was treated with 50mg/L silica nanoparticles was found to have the highest total chlorophyll content, followed by PRM-6107 which was treated with 25mg/L silica nanoparticles. PES-400 plants that were exposed to

drought stress exhibited least chlorophyll content as compared to the plants of PES-400 that was treated with 25mg/L and 50mg/L silica nanoparticles **(figure 4.4.7)**. **Shmat'ko *et al*., (1977)** reported that drought tolerant genotypes show little change in the chlorophyll content compared to drought susceptible genotypes.

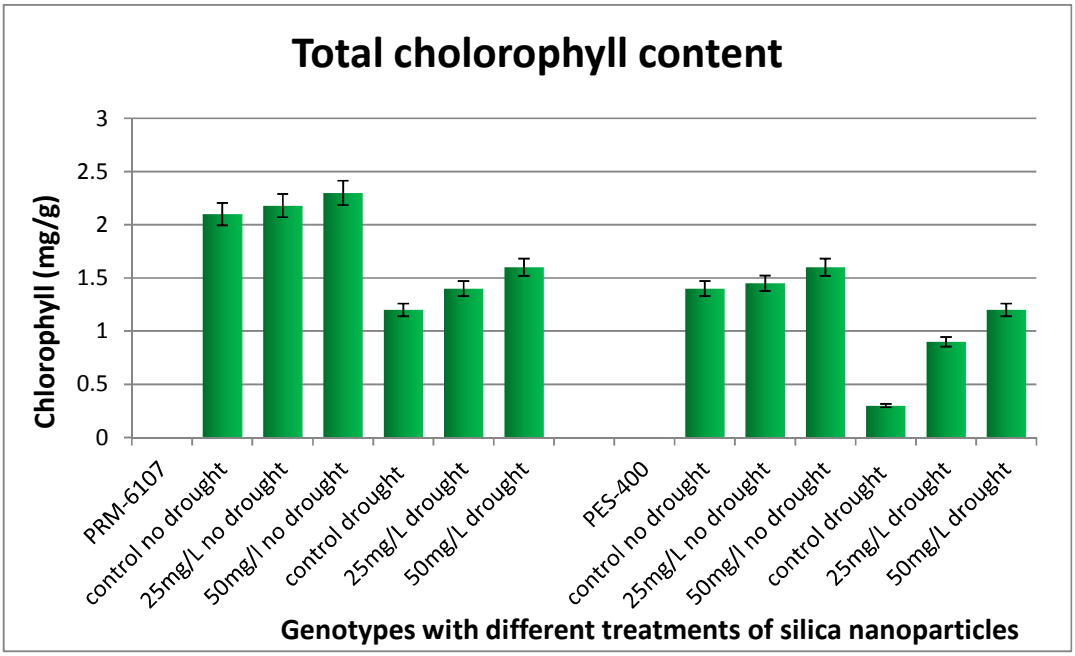


Fig. 4.4.7: Effect of different concentration of silica nanoparticles on total chlorophyll content in PRM-6107 and PES-400

Leaf chlorophyll decline as a consequence of water stress is a common observation **(Chakraborty *et al*., 2001; Kar, 2002)**. The reduction in chlorophyll content might be due to generation of excessive reactive oxygen species under drought condition. Moisture stress results in formation of reactive oxygen species like superoxide radical, hydrogen peroxide, singlet oxygen, and hydroxyl radical which disrupt cellular structure and thus damage the mitochondria and the chloroplast **(Singh *et al*., 1987)**. The other causes may be increased chlorophyllase enzyme activity or the suppression of such enzymes that catalyze the formation of chlorophyll in the leaves **(Sreenivasulu *et al*., 1999)**.

Green leaf retention under drought conditions is considered to be an important parameter to select for drought tolerance **(Datta *et al*., 1988)**. The results obtained convey that PRM-6107 is more capable to maintain its total chlorophyll content and hence perform photosynthetic processes in a better way than others.

The total chlorophyll content of PRM-6107 increased by 16% and 38% in the plants which was treated with 25mg/L and 50mg/L silica nanoparticles respectively as compared to the total chlorophyll of the plants that was given no treatment of silica nanoparticles. The percentage increase in the total chlorophyll content of PES -400 was 30% and 40% in the plants which was treated with 25mg/L and 50mg/L silica nanoparticles respectively as compared to the total chlorophyll content of the plants that was given no treatment of silica nanoparticles **(figure 4.3.1.D)**. This shows that the drought tolerance enduring capacity of PES-400 was increased. This shows that the application of silica nanoparticles will greatly enhance the photosynthetic efficiency of the plants.

4.4.9 Total carotenoid content

Carotenoids are large isoprenoid molecules which are synthesized *de novo* by all the photosynthetic organisms and a number of non-photosynthetic organisms. They are divided into xanthophylls, typified by lutein, and carotenes like beta carotene and lycopene.

Total carotenoid content from randomly collected leaves of all genotypes was calculated. It was found drought stress caused reduction in the carotenoid content of all genotypes. However, PRM-6107 that was treated with 50mg/L silica nanoparticles has the highest total carotenoid content amongst the treatment plants, followed by PRM-6107 that was treated with 25mg/L of silica nanoparticles. PES-400 that was treated with no silica nanoparticles was the most severely affected and showed the lowest total carotenoid content **(Fig. 4.4.8)**.

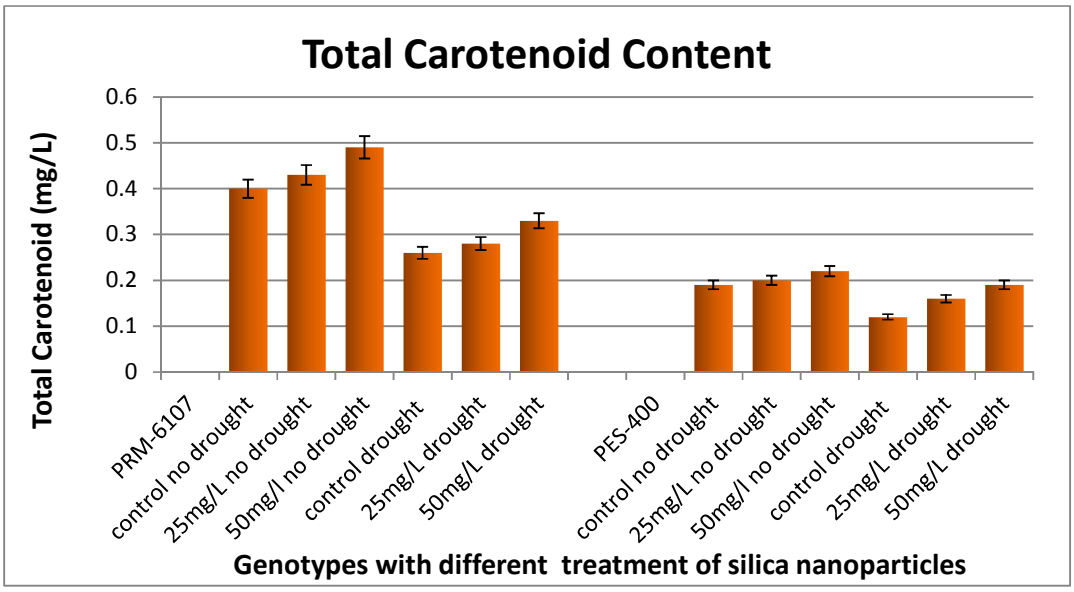


Fig 4.4.8: Effect of different concentration of silica nanoparticles on total carotenoid content in PRM-6107 and PES-400

Carotenes perform an important role in the plant antioxidant defense system. Beta carotene plays a significant role in safeguarding photo chemical processors and sustaining them by acting as an effective antioxidant, in addition to its function as an accessory pigment. **Farooq *et al.* (2009)** reported that beta carotene might be performing a major protective role in the photosynthetic tissue buy directly quenching the triplet chlorophyll and thus preventing the formation of singlet oxygen

The total carotenoid content of PRM-6107 increased by 7% and 26% in the plants which was treated with 25mg/L and 50mg/L silica nanoparticles respectively as compared to the total carotenoid content of the plants that was given no treatment of silica nanoparticles. The percentage increase in the total carotenoid content of PES -400 was 33% and 58% in the plants which was treated with 25mg/L and 50mg/L silica nanoparticles respectively as compared to the total carotenoid content of the plants that was given no treatment of silica nanoparticles. The carotenoids exhibit multifarious roles in drought conditions which involve light harvesting and oxidative damage protection. Thus an increased carotenoid content in a genotype is an important indicator of stress tolerance.

4.5 Biochemical parameters studied to study the effect of drought stress:

To study the biochemical parameters, the leaf samples were taken randomly from the silica nanoparticles treated and control plants under drought stress condition.

4.5.1 Proline content

As water stress is given to the plants a cascade of events, involving several biochemical changes is observed. Among the events following water stress the most significant and immediately detectable change is the accumulation of free proline In the plants .PES-400 and PRM-6107 exhibited low free proline content in the control plants. After the 5 day drought stress, both the genotypes showed a significant rise in their free proline content, with highest in PRM-6107 (50mg/L), followed by PRM-6107 (25mg/L) and PRM-6107 in which no silica nanoparticles was applied. PES-400 plants in which no silica nanoparticles were applied exhibited the lowest proline content **(figure 4.5.1)**. The application of silica nanoparticles resulted in the increase of free proline content. With the increase in the concentration of the silica nanoparticles the free proline content also increased in the two genotypes. In this experiment it was observed that drought stress caused significant increase in the proline content

The free proline content of PRM-6107 increased by 4% and 8% in the plants which was treated with 25mg/L and 50mg/L silica nanoparticles respectively as compared to the free proline content of the plants that was given no treatment of silica nanoparticles. The percentage increase in the total proline content of PES -400 was 25% and 42% in the plants which was treated with 25mg/L and 50mg/L silica nanoparticles respectively as compared to the free proline content of the plants that was given no treatment of silica nanoparticles. The proline accumulation observed makes it evident that increased accumulation of proline is an adaptive response of plants to counter drought stress in confirmation with the findings of **(Sultan *et al*., 2012)**.

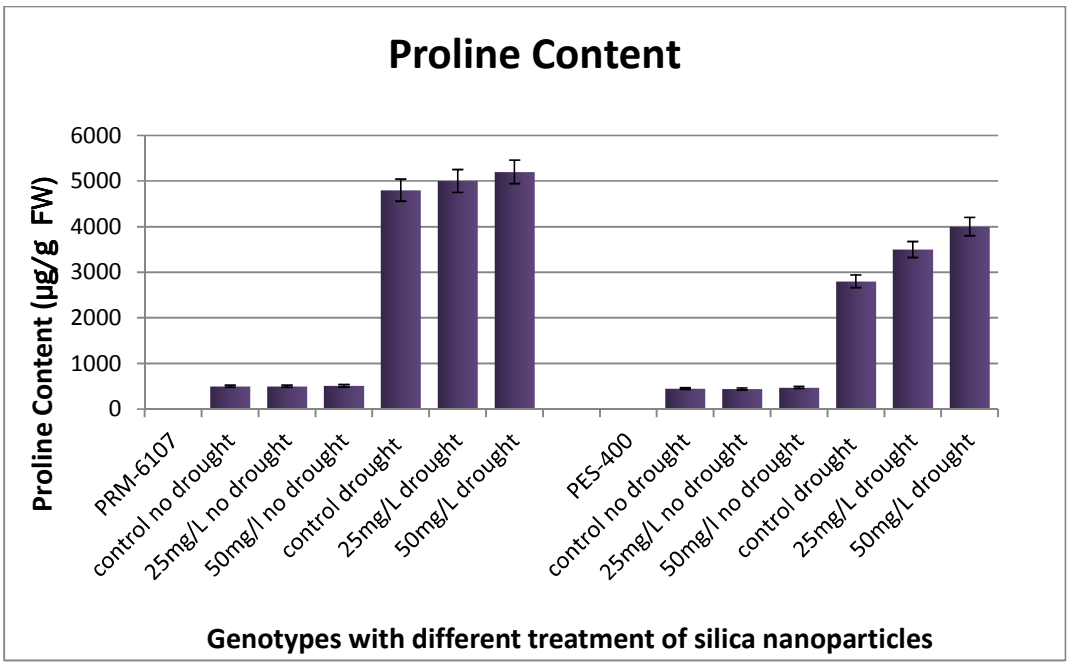


Fig. 4.5.1: Effect of different concentration of silica nanoparticles on proline content in PRM-6107 and PES-400

4.5.2 Malondialdehyde content:

The cell membrane instability under prolonged drought stress can be determined by the malondialdehyde accumulation levels in plants. Detection of the degradation of poly unsaturated fatty acid products such as malondialdehyde is considered as an important criterion for determining the degree of stress effect in response to moisture deficit conditions in plants **(Jain *et al*., 2001)**. In this study it was observed that two genotypes showed different responses with respect to the MDA content when exposed

to drought conditions. The genotype PES-400 that was not treated with silica nanoparticles has showed the highest MDA accumulation indicating that it has lower ability to maintain the cell membrane integrity while PRM-6107 which was treated with 50mg/L silica nanoparticles showed the least MDA content indicating it has a better ability to counter oxidative stress generated as a result of drought conditions **(figure 4.5.2)**.

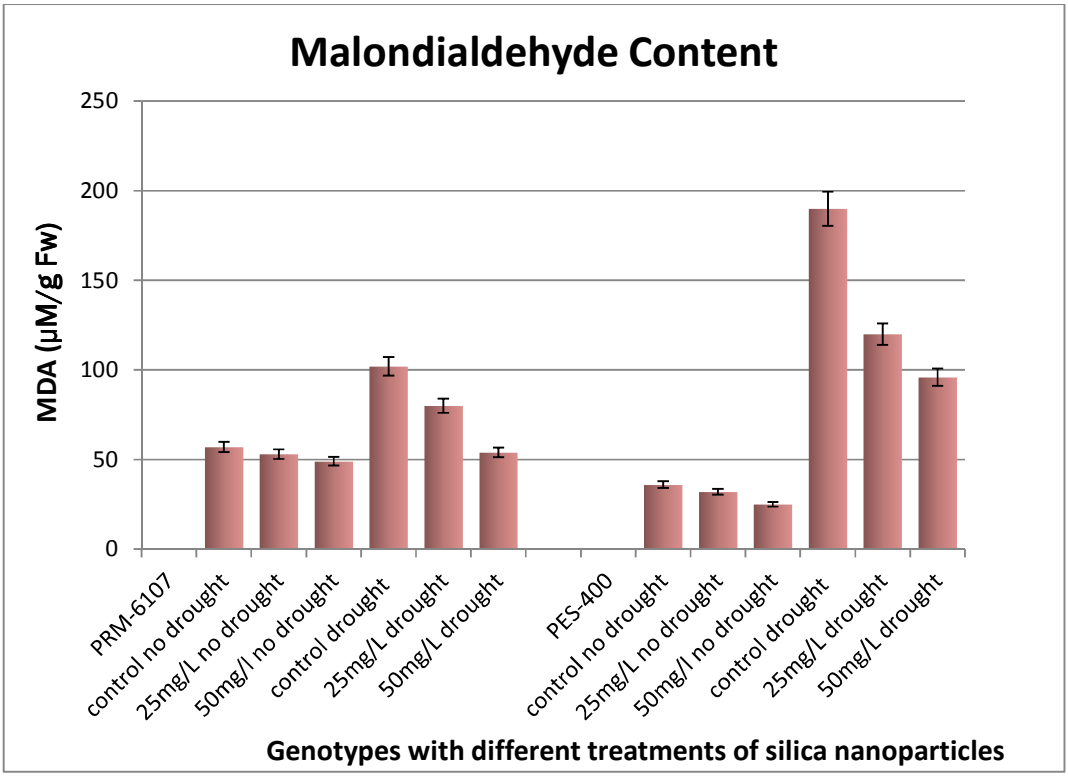


Fig. 4.5.2: Effect of different concentration of silica nanoparticles on malondialdehyde content in PRM-6107 and PES-400.

The total MDA content of PRM-6107 decreased by 21% and 47% in the plants which was treated with 25mg/L and 50mg/L silica nanoparticles respectively as compared to the MDA content of the plants that was given no treatment of silica nanoparticles. The percentage decrease in the MDA content of PES -400 was 36% and 49% in the plants which was treated with 25mg/L and 50mg/L silica nanoparticles respectively as compared to the MDA content of the plants that was given no treatment of silica nanoparticles. This showed that the silica nanoparticles are protecting the sensitive genotype from the deleterious effect caused by drought stress by reducing the MDA content.

- It can be concluded from the result obtained that PRM-6107 is a superior drought stress tolerant genotype that is a potential material for drought areas. With the application of silica nanoparticles a very noteworthy increase in the proline content of PES-400 was observed. Increased accumulation of free proline is an adaptive response of the plants to counter drought stress. Maintenance of the green color of the leaves during drought conditions is considered to be an important parameter for a plant to be considered as drought tolerant; this was observed in the plants that were treated with different concentration of silica nanoparticles as compared to the control plants when both were given water stress. This was due to increase in the total chlorophyll level, which ultimately increased the photosynthetic efficiency of the plants. With the application silica nanoparticles the MDA content decreased even in the drought sensitive genotype PES-400 resulting in a protecting the plants from the deleterious effect caused by drought stress. RWC was also increased to a pronounced extent by the application of Silica nanoparticles. There was very significant increase in the total carotenoid content of the plants of the PES-400 treated with silica nanoparticles as compared to the control plant under drought condition. The carotenoid content plays direct role in harvesting of light during photosynthesis and protecting the plants from oxidative damage caused by the singlet oxygen produced during drought stress.

The above discussion showed that silica nanoparticles conferred drought tolerance property even in the drought sensitive genotype and silica nanoparticles play an important role in maintaining critical morpho-physiological and biochemical function in finger millets under drought stress conditions.

Summary and Conclusions

Chapter 5 SUMMARY AND CONCLUSION

Drought stress can be defined as insufficient soil water availability to the plants to achieve their maximum genetic potential. Plants experience drought when the evapotranspiration demand exceeds the soil water availability to the plants. Interactions of climate, soil type, soil water (storage & precipitation) determines the occurrence, intensity and duration of drought. Thus irrigation is required, to avoid plant water deficit. In order to overcome this problem an important approach is to enhance drought tolerance capacity of the crop, by utilizing silica nanoparticles.

The term “millets and minor cereals " embrace ten genera and at least fourteen species, all of which are members of the grass family Gramineae. Collectively the millets have a wide range of adaptation and use. There are types to fit almost every situation. Individual millet species or varieties frequently possess some unusual characters for adaptation or use, like tolerance to drought or water stress.

The present study was conducted in the Department of Molecular Biology and Genetic Engineering, College of Basic Sciences and Humanities, G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand with the following objectives:

1. To synthesize silica nanoparticles from rice husk (major agricultural waste).
2. Characterization of silica nanoparticles.
3. Study of morpho-physiological characters in different genotypes of *Eleusine coracana* treated with silica nanoparticles and subjected to experimental drought stress.
4. Study of biochemical parameters (Chlorophyll, Malondialdehyde, proline content) in contrasting genotypes (one drought tolerant and another drought sensitive) of *Eleusine coracana* treated with silica nanoparticles and subjected to experimental drought stress.

The effect of 5 days drought stress treatment on various genotypes of finger millet was studied at the vegetative stage. The genotypes were collected from the Department of Molecular Biology and Genetic Engineering, College of Basic Sciences and Humanities,

G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand. In order to screen the different genotypes for tolerance to drought they were subjected to silica nanoparticles treatments at three different concentration (0, 25 and 50 mg/L) for 45 days and exposing it to water stress by withholding water supply for a period of 5 days. Soil moisture content of the treatment pots was determined and was observed to be around 20-25% in all the drought imposed pots. Drought stress was imposed on 45 day old plants and based on various morphological, physiological and biochemical parameters they were evaluated for drought tolerance. The control plants were evaluated in parallel. Drought stress was found to have an effect on various morpho-physiological and biochemical characters like shoot length, root length, root to shoot length ratio, tiller number, leaf number, leaf area, leaf rolling and drying, relative water content, chlorophyll content, carotenoid content, proline content and malondialdehyde content.

Results obtained from the present work are summarized below:

- Amorphous silica nanoparticles of 80nm sizes, nearly spherical in shape, non-conducting in nature (i.e. having no surface charge) has been successfully synthesized by a modified method using rice husk as the starting material. The nanoparticle was of nano-size was very evident from the different characterization methods such as SEM, STM, FT-IR, EDX, XRD, UV-vis spectroscopy. Silica nanoparticles with the correct size distribution (80nm) was prepared without the use of any surfactant as catalyst
- The results showed that the two genotypes of finger millet responded differently with regard to tolerance to moisture stress.
- The plants treated with silica nanoparticles showed increase in the root shoot ratio when drought stress was imposed. The increase in this ratio suggests that the plants can efficiently relocate the water and minerals from the soil to the different parts of the shoot even in the stress condition.
- There was a decrease in the MDA content of PES -400 plants which was treated with silica nanoparticles as compared to the MDA content of the control plants under drought stress condition. This showed that the silica nanoparticles had a protecting effect on the sensitive genotype. The deleterious effect caused by drought stress was reduced by reduction in the MDA content.

- RWC was also affected to a great extent by the application of Silica nanoparticles. This showed that silica nanoparticles conferred drought tolerance property even in the drought sensitive genotype. The results indicate a significant increase in RWC of PES-400 on treatment with silica nanoparticles.
- The free proline content was less in the non-treated plants as compared to the plants that were treated with Silica nanoparticles and was exposed to drought stress condition. A very significant increase in the proline content of PES-400 treated with silica nanoparticles was observed. Increased accumulation of free proline is an adaptive response of the plants to counter drought stress.
- Green leaf retention under drought conditions is considered to be an important parameter to select for drought tolerance; this was observed in the plants that were treated with different concentration of silica nanoparticles as compared to the control plants when both were given water stress. This proves that the retention of the green leaves even during drought condition was due to the application of silica nanoparticles as it led to the increase in the total chlorophyll level.
- There was tremendous increase in the total carotenoid content of the plants of the PES-400 treated with silica nanoparticles as compared to the control plant under drought condition. The carotenoid content has direct role in harvesting of light and protecting the plants from oxidative damage caused by the singlet oxygen produced during drought stress.
- It has been observed from the above results that exogenous application of silica nanoparticles play an important role in maintaining critical morpho-physiological and biochemical function in finger millets under drought stress conditions.

Future prospects:

1) The current study opens many opportunity for expanding the utilization of silica nanoparticle to elevate the drought tolerance property in the sensitive varieties of crops.

2) The results were very promising with the two concentration of silica of nanoparticles ie.25mg/L and 50mg/L in alleviating drought stress in finger millet. So further studies should be done by increasing the concentration of silica nanoparticles to 75mg/L and 100mg/L. In order to determine at what concentration the effect of the nanoparticles becomes steady and after which no further increase is observed in increasing the drought tolerance property.

3) For obtaining more pronounced effect of silica nanoparticles in drought tolerance field trials must be planned and carried out. As a significant increase in the drought tolerance property in the sensitive genotype was observed in the pot experiment.

4) The silica nanoparticles synthesized from the rice husk was uncharged, thus having the property to aggregate. So further techniques could be searched for giving the silica nanoparticles surface some quantity of charge. As all the nanoparticles would be of the same charge so they will repel each other and the problem of aggregation could be solved.

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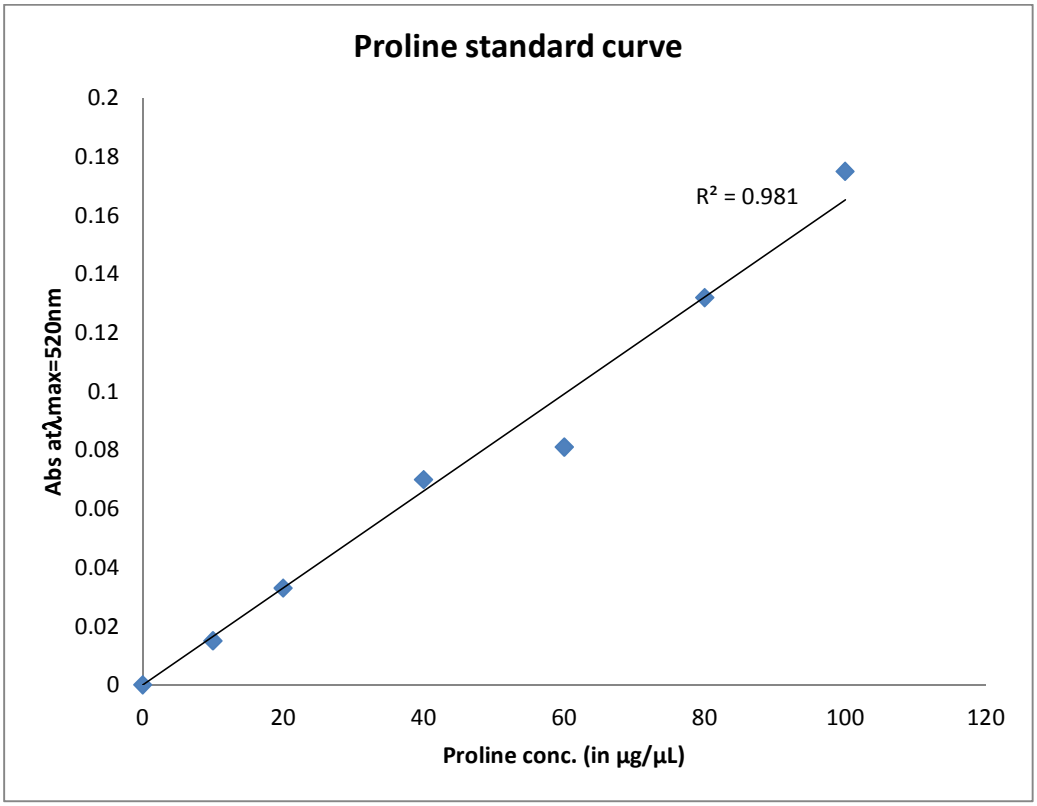
Appendices

A.1. Standard curve of Proline



VITA

The author, Sikha Snehal, belongs to the Dhanbad province in the state of Jharkhand. She completed her secondary education from De-Nobili school C.M.R.I., Dhanbad and higher secondary education from De-Nobili school C.M.R.I., Dhanbad. After passing the Higher Secondary examination, she joined Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh and graduated with a B.Sc. (Hons.) Agriculture degree in May, 2016 with 88.8%.

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ABSTRACT

Name : SIKHA SNEHAL **Id. No.** : 51105

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Thesis title : **"Study on synthesis, characterization and effect of silica nanoparticles on drought tolerance in *Eleusine coracana*"**

Advisor : Dr. Pushpa Lohani

Drought is a major abiotic stress factor limiting crop productivityworldwide. Finger millet (*Eleusine coracana*) is a hardy crop that can withstand variant degree of drought stress. The physiological and biochemical responses of finger millets to the different concentrations of silica nanoparticles (0, 25, and 50 mg/L) and two soil moisture treatments (without stress and severe stress) were investigated. Application of the SNPs was done for 45 days before exposing them to5 days of drought stress. Morpho-hysiological parameters and biochemical parameters such as malondialdehyde (MDA), relative water content (RWC), chlorophyll, carotenoid, and proline content were determined. Under drought conditions, the effect of silica nanoparticles on the morpho-physiological and other biochemical was evident. A decrease in ROS production and an enhancement in the antioxidant system for Silica nanoparticles applied plants may account for their increased tolerance to abiotic stresses. Application of silica nanoparticles decreased $O2^{-}$, H_2O_2, and MDA concentrations in finger millets leaves by increasing the activities of ascorbate peroxidase (APX) dehydroascorbatereductase (DHAR), or glutathione (GSH) reductase (GR) under water stress. A significant increase in the relative water content (RWC), chlorophyll, carotenoid, and proline content was observed in the PES-400 genotype in which silica nanoparticle was applied. Hence, it was concluded from this study that application of silica nanoparticles confers drought tolerance property even in the drought sensitive variety of finger millet PES-400.

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सारांश

नाम	:	शिखा स्नेहल	परिचयां :	५११०५
सत्र एवं प्रवेश वर्ष	:	प्रथम, २०१६-१८	उपाधि :	स्नातकोत्तर (कृषि)
मुख्य विषय	:	आणविक जीव विज्ञान एवं जैव प्रौद्योगिकी		
विभाग	:	आणविक जीव विज्ञान एवं अनुवंशिक अभियांत्रिकी		
शोध शीर्षक	:	''सिलिका नैनोकण के संशलेषण विशेषता और प्रभाव का रागी सुखा सहिष्णुता का अध्ययन''		
सलाहकार	:	डा0 पुष्पा लोहनी		

दुनिया भर में सुखा तनाव फसल उत्पादकता सीमित कने का प्रमुख कारक है। मडुवा एक सूखा प्रतिरोधक फसल है जो सुखा का सामना करने में सक्षम है। सिलिका नैनोकण (०,२५ और ५० मिलीग्रम/लीटर) और दो मिट्टी नमी उपचार (बिना तनाव और गंभीर तनाव) के विभिन्न सांद्रता संयोजन का मडुवा के शारिरिक और जैव रासायनिक प्रतिक्रियाओं की जाँच की गई। सूखा तनाव देने से पहले उनमें ४५ दिनों के लिए सिलिका नैनोकण का प्रयोग किया गया। रूपात्मक, दैहिक क्रियात्मक और जैवरासायनिक प्रशिक्षणों जैसे मैलोन्डिलडेहाइड, सापेक्ष जल स्तर, कलोरोफिल, कैरोटिनाइड और प्रोलीन स्तर निर्धारित की गई थी। सूखे की स्थिति के तहत, रूपात्मक, दैहिक क्रियात्मक और अन्य जैव रासायनिक प्रशिक्षणों पर सिलिका नैनोकणों का प्रभाव स्पष्ट था। सिलिका नैनोकण का प्रयोग आर0 ओ0 एस0 में कमी, एंटी ऑक्सीडेंट सिस्टम में वृद्वि करने में सक्षम है, और अजैविक तनावों में सहिष्णुता के लिए जिम्मेदार है। सिलिका नैनोकण के प्रयोग ने मडुआ में एम0 डी0 ए0 सांद्रता में कमी लाई है। सिलिका नैनोकण पानी तनाव के तहत एस्कॉर्बेट परोक्साइड, डी हाड्रोस्कोर्बेट परोक्साइडस या ग्लूटाथियोन रेडक्टेज की गतिविधियो को बढ़ाता है, जो कि पौधों के लिए हानिकारक है। सिलिका नैनोकण के प्रयोग ने मडुआ के पी0 ई0 एस0–४०० प्रजाति में सापेक्ष जल स्तर, कलोरोफिल, कैरोटिनाइड और प्रोलीन के स्तर को भी बढ़ाया है। इस लिए यह निष्कर्ष निकाला गया है कि इस अध्ययन में सिलिका नैनोकण ने मडुआ के सुखे सहिष्णुता के गुण को पी0 ई0 एस0–४०० सूखे संवेदनशील प्रजाती में भी प्रदान किया है।

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