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RESPONSE OF RICE TO NANOSILICA: PHYSIOLOGICAL AND LEAF SURFACE ANATOMICAL CHARACTERS UNDER DROUGHT STRESS

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ABSTRACT
The aim of this study was to assess the effects of nanosilica on drought imposed rice plants and to evaluate the impact of different concentrations of nanosilica on growth, anatomical, physio-biochemical parameters and yield characters of rice under drought conditions. The experimentation was carried out during the summer 2020-2021 at the Rice Department of Tamil Nadu Agricultural University (TNAU), Coimbatore. Different concentrations of nanosilica formulation were used as foliar spray in this present experiment viz., 200, 400, 600, 800 and 1000 ppm under drought condition. In this present field experiment, application of 400 ppm nanosilica formulation increased leaf area and specific leaf weight with14.3 and 15.3 percent under drought stress. Application of 400 ppm nanosilica increased up to 12.5 percent in terms of Menbrane Stability Index (MSI); meanwhile in Chlorophyll Stability Index (CSI) increased up to 20.4 percent. The proline content wasdecreased up to 26.9 percent by the application of nanosilica (400 ppm) in drought-stressed plants. Trichome length and the length of the silica bodies were significantly increased by about 17.4 and 9.1 percent over the control respectively. Application of 400 ppm nanosilica resulted in a maximum increase of 68.9 percent and 29.4 percent in terms of trichome and silicon bodies' length over the drought treatment, respectively. Stomatal structures were reduced significantly with a mean reduction of 43.5 percent compared to the control in both the rice varieties. Application of 400 ppm nanosilica increased the mean stomatal size by 65.5 percent in drought stressed plants. Comparing the two varieties, CO54 expressed a greater response to 400 ppm of nanosilica treatments in terms of leaf area, specific leaf weight, Menbrane Stability Index (MSI), Chlorophyll Stability Index (CSI), proline and leaf surface characters under drought.

KEYWORDS: Rice, Nanosilica, Drought, Physiology, leaf surface characters

INTRODUCTION
Rice (Oriza sativa L.) is considered as one of the most important staple food crops for the world`s population. It is grown under irrigated ecosystems, while in some parts of India and Tamil Nadu it is cultivated under rainfed ecosystems as well. Rice is also known as 'Global grain' because it is cultivated in more than 100 countries. Asian countries are the leaders in the production and consumption of rice (Samal et al., 2018). According to Pandey and Shukla (2015), rice is the most important staple food and most of the world's population depends on its consumption and income generation. The major constraints in rice production is climate change such as drought and flooding, which generally affects the hydrological fluctuation levels and ultimately agriculture, mostly in developing countries (Turral et al., 2011). Drought is the major limiting factor for rice production (Nelson et al., 2014). Therefore, enhancing rice yield with climate change andwater limitation is the first approach for enhancing rice production to meet out the food demand in future, expected from the probable increasing in world population. Silicon is considered as non-essential nutrient because, silicon cannot fulfil the "essential criteria for a nutrient" given by Arnon and Stout (1939). So, until 1994 silicon considered as non-essential element. After that, Epstein (1994) given the astonishing review on silicon in 'The anomaly of silicon in plant biology' change the prospects of silicon in the agricultural field. Several studies reported that beneficial effects of silicon. 'International Plant Nutrition Institute' promote the silicon from non-essential to beneficial element (IPNI, 2015).Because small number of plant species requires silicon particularly in rice plant. McLarnon etal. (2017) reported that, silicon requirements increased during stress and silicon actively absorbed in some grass species. In this present study

framed with the objectives of enhancing the drought tolerant capacity in rice through foliar application of different concentrations of nanosilica.

Justification for Drought Intensity Selection

Drought is a major constraint to rice cultivation in Tamil Nadu, with agricultural drought occurring frequently, particularly during the southwest monsoon or Kharif season (June–September). The state experiences varying intensities of drought, and approximately 64% of its area is considered drought-prone.

Drought Intensity and Impact

During drought years, Tamil Nadu experiences a water deficit of about 39% (approximately 694 mm compared to the normal 1035 mm). Drought can lead to a significant reduction in mean grain yield, with losses of up to 85.7% observed in some rice genotypes due to cumulative stress. Yield variability in these drought-prone regions ranges from 44% to 60%.

Drought intensity is highest during the maximum tillering stage, although stress during the reproductive stage is also highly detrimental. The districts most affected by drought include Ramanathapuram, Sivagangai, Thiruvallur, Coimbatore, and Kanyakumari.

MATERIALS AND METHODS

The experimentation was conducted during 2020-2021 at Rice department, TNAU, Coimbatore. Geographically, the experimental site was situated in the zone of western agro-climatic of Tamil Nadu at an altitude of 426.72 meters above mean sea level with 11° Nlatitude and 77° E longitude. The total duration of the experiment was five months from January to May, 2021-2022. During the experiment period, the average of maximum and minimum temperature was 32.2 °C and 22.6 °C, respectively. The total amount of rainfall received during the crop period (2021-2022) was 71.3 mm. However, there is no rain fall received at the time of stress imposition and foliarapplication of nanosilica. The weather parameters data was collected from the ACRC, TNAU, Coimbatore. The two rice varieties of CO53 and CO54 having short duration were collected from the Rice department, TNAU, Coimbatore. Nanosilica (SiO_2) materials were prepared and collected from the Nano science and Technology Department, TNAU, Coimbatore.

Table 1. Characters of varieties

Variety	Parentage	Duration (days from Sowing to Harvest)
CO 53	PKM(R) x Norungan	115-120
CO 54	CB04110 x CB05501	115-120

Preparation of nanosilica formulation

Nanosilica emulsion was prepared from pure nanosilica and it was mixed with tween 80 and water (1:3). Then solution was subjected to sonication at 50% amplification for 30 minutes with 10:10 plus rate. Solutions were well homogenized. Based on the requirement ofexperiment different concentration viz., 200, 400, 600, 800 and 1000 ppm of nanosilica has been prepared and used for this field study as foliar spray.

Step 1. Mixing of nanosilica with tween 80 + water (1:3 ratio)

Step 2. Sonication for 30 minutes

Step 3. Collect the nanosilica formulation from sonicator for foliar spray

The drought was imposed by withholding of irrigation for all treatments except T1 (Irrigated) from 12 days before flowering and 10 days after flowering. The total period of the stress was 22 days. The irrigation was controlled by bund and the water leakage wasvetoed by placing of plastic sheets. The water drained out by buffer channels in drought imposed plots. The application of nanosilica as foliar spray at 50 % flowering stage (mid of the drought period) **(Fig 1).** Totally seven treatments were choocen for this experiment viz., T1 – Irrigated, T2 – Drought, T3 – Drought + Nanosilica (200 ppm), T4 – Drought + Nanosilica (400 ppm), T5 – Drought + Nanosilica (600 ppm), T6 – Drought + Nanosilica (800 ppm) and T7 – Drought + Nanosilica (1000 ppm).

Justification for Using the Sonication Approach in Nanosilica Formulation Preparation
Improved Dispersion and Stability:
Nanosilica tends to aggregate in aqueous solutions. Sonication effectively breaks these aggregates, resulting in a more stable colloidal suspension. This stability, often indicated by zeta potential measurements, leads to smaller and more uniform particle sizes, typically achieved within 5–15 minutes of sonication.
Enhanced Foliar Uptake: Sonicated nanosilica (e.g., 30–50 nm) can more effectively penetrate the stomata and cuticular wax layers of rice leaves compared to aggregated or bulk silica particles.
Non-toxic and Beneficial at Low Concentrations: At low concentrations (typically 50–200 ppm or 0.10–0.15%), nanosilica is generally beneficial for rice, a known silicon-accumulating crop. It promotes plant growth, increases chlorophyll content, and enhances antioxidant enzyme activities such as superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT).
Mitigation of Abiotic Stress: Foliar application of sonication-prepared nanosilica enhances rice tolerance to abiotic stresses such as salinity, drought, and heavy metal contamination (e.g., cadmium and lead).
Experimental Plot size
Plot sizes for drought screening in rice typically range from 1.5 m × 2 m to 3 m × 3 m, depending on whether the screening is conducted under field conditions or within rainout shelters. A commonly used plot size for field trials is 3 m × 3 m. Planting is generally done with a spacing of 20 cm × 15 cm or 20 cm × 20 cm.
Application of Nano Silica as Foliar Spray in Rice
Nano silica was applied as a foliar spray using a hand-operated sprayer with a tank capacity of 16 liters. The sprayer nozzle was of 02 yellow size, equipped with 8 holes to ensure uniform spray distribution. The total spray volume used was 500 liters of water per hectare for effective foliar application in rice crops.
Observations recorded
Plant sampling
The growth anatomical, physiological, biochemical and yield parameters were recorded in each treatments with three plants per replications per treatments were collected atseven days after application of nanosilica in 50 percent flowering stage and physiological maturity stage for measuring all the observation.

Growth attributes
Leaf area (cm^2)
Leaf area was measured from three randomly selected plants from each plot by usingleaf area meter (LI-3100 Area Meter) and expressed in cm^2.
Specific leaf weight
Specific leaf weight was calculated from leaf weight of the plant and leaf area of the plant and it was expressed in dry weight per unit leaf surface area. It is an indirect measurementof width and thickness of the leaf and expressed in mg cm^{-2}.

Leaf weight of the plant (mg)

$$Specific\ leaf\ weight = \frac{}{\text{Leaf area per plant (cm}^2\text{)}}$$

Leaf anatomical characters (SEM measurements)

Stomatal characters

Leave bits are collected from plants of each treatment at seven days after the application of nanosilica and the length and breadth of the stomata are measure under ScanningElectron Microscope (SEM) available at Nano science and Technology Department, TNAU, Coimbatore.

Leaf surface characters

Trichomes characters and silicon bodies of two rice varieties were viewed and measured under the scanning electron microscope (SEM) at seven days after foliar applicationof nanosilica.

Physiological and biochemical analysis

Membrane stability index (MSI)

Take 0.3g of freshly collected physiologically active flag leaf sample was weighed andplaced in a test tube containing 30 ml of double distilled water and kept in water both with 30^{o} C temperature for 2 hours, after that the initial electric conductivity was measured. Then keptin boiling water for 15 mins then measured the final electrical conductivity (EC) value. Membrane stability index was calculated by following the formula adopted by Bajji et al. (2002).

$$MSI = \frac{\text{EC1 (Initial)}}{\text{EC2 (final)}} \times 100$$

Chlorophyll stability index (CSI)

Murthy and Majumdar (1962) derived procedure has been followed for estimation of CSI. Take 500 mg of physiologically active leaf sample in two test tubes. Add 20 ml of distilled water in control test tube and add 20 ml of hot water (55°C) in another set of test tubes. Then both the test tubes were kept in hot water both for 30 mins. The total chlorophyll content (TCC) was estimated by following the procedure developed by Hiscox and Isrealstam (1979).

$$CSI = \frac{\text{TCC (treated)}}{\text{TCC (control)}} \times 100$$

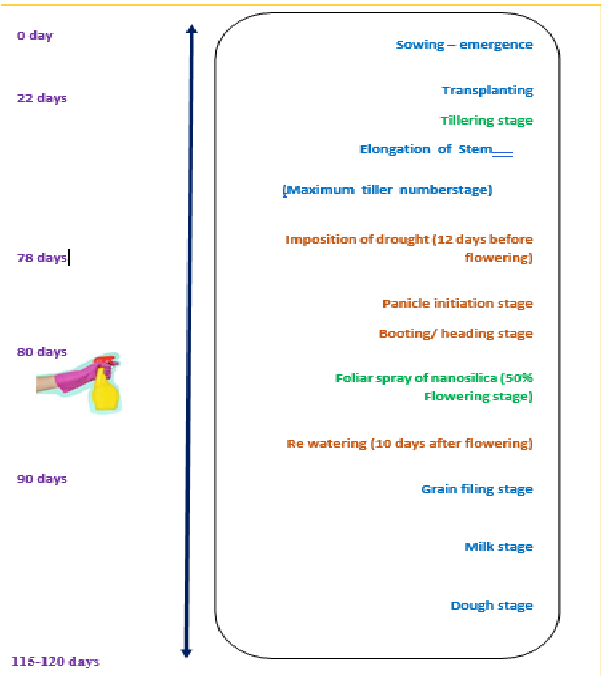


Figure 1. Flow cart for drought imposing period in

Proline content

Bates et al. (1973) derived procedure has been followed in proline estimation. 500 mgof leaf samples was weighed, and then macerated with 10 ml of 3 % sulpho salicylic acid. After that, centrifuged the solution at 3000 rpm for 10 minutes and 2ml of supernatant was taken and 2 ml of acid ninhydrin, 2ml of glacial acetic acid, 2 ml of 6M orthophosporic acid were added in the test tube and kept it in hot water bath for one hour. Later the solutions were transferred into separating funnels and 4 ml toluene was added, then shaked uniformly for 30secs. The colourless solution was discarded and upper pink colour solution was collected, thenoptical density measured at 520 nm and expressed in mg g^{-1} of fresh weight.

Statistical analysis

The data were collected and subjected to statistical examination in factorial randomizedblock design (FRBD) through ANOVA and a post hoc test (Gomez and Gomez., 1984).

RESULTS AND DISCUSSION

Leaf area

The effect of silica nanoformulations in leaf area are described in Table 2. The result explained that an increasing trend in control and declined trend in both drought alone and drought with silica nanoformulation applied plants. Among the treatments, control plants hadmaximum leaf area of 1814.4 cm^2 $plant^{-1}$ and 1744.6 cm^2 $plant^{-1}$ in CO53 and CO54respectively. Comparing the two varieties, CO53 had maximum leaf area of 1616.5 cm^2 $plant^{-1}$ than CO54 (1441.3 cm^2 $plant^{-1}$) under drought alone condition. The results revealed that a significant variation could also be observed between drought alone and drought with silica nanoformulation treatments. Among the nanosilica treatments, T4 (Drought + 400 ppm) recorded maximum leaf area of about 1787.8 cm^2 $plant^{-1}$ and 1699.0 cm^2 $plant^{-1}$ in CO53 andCO54 under drought condition respectively. Whereas, the treatment T3 (Drought + 200 ppm) recorded the leaf area of about 1705.2 cm^2 $plant^{-1}$ and 1660.5 cm^2 $plant^{-1}$ under drought conditions. The treatment T4 and T3 are on par with each other. Leaf is considered as a most important functional organ of plant. Many physiological and biochemical reaction are carried out inside the leaf and it is the effective in the yield underabiotic stress condition (Wang et al., 2005). It is main source of carbon fixation by photosynthesis and maintains the leaf temperature by transpiration through stomata (Buckley, 2015). The reduction of leaf area surface is the first response of drought adoption to reduce the transpiration losses (Larcher, 2003). According to Kafi et al. (2021) who stated that, the inhibition of cell division and elongation due to drought. In the present filed experiment shows, drought minimise the leaf area in both varieties. Comparing the two varieties, maximum reduction of leaf area are observed in CO54 (17.4 %) than CO53(11.0 %) under drought (Fig. 5). Application 400 ppm of nanosilica in drought affected plantsincreases the leaf area up to 14.25 % in both the rice varieties. This may be due to silicon increases the water use efficiency (Ma et al., 2006) and maintain the higher relative water content in the leaf, it helps leaf expansion (Da Silva Lobato, 2020). Present study was agreement with Shen et al. (2010) who opined that silicon improve the growth characters including leaf area. The same kind of results reported by Gong et al. (2008) insisted that siliconimprove the leaf growth under drought condition.

Specific leaf weight

The effect of silica nanoformulation on specific leaf weight in rice displayed in Table 2.Comparing seven treatments, the increasing trend in specific leaf weight were noticed in control plants, besides declining trend was observed in drought and nanosilica applied plants. comparing the two varieties CO53 had maximum specific leaf weight (5.58 mg cm^{-2}) over thedrought alone treatments (CO53- 4.42 mg cm^{-2}; CO54-3.86 mg cm^{-2}). Among the different concentration of nanosilica treatments, T4 (Drought + 400 ppm) differed significantly an increasing of specific leaf weight under drought in both varieties (CO53-5.30 mg cm^{-2}; CO54-
4.15 mg cm^{-2}) which was followed by T3 (CO53-4.80 mg cm^{-2}; CO54-4.62 mg cm^{-2}) treatments. Both are on par with each others. Specific leaf weight (SLW) represent the measure of leaf thickness. It has been foundto have a strong positive correlation with leaf carbon assimilation, which leads to increasing photosynthetic index and indirectly denoted the density of the leaf in rice (Sarkar et al., 1996). Leaf thickness and succulence of the plants had strong connection and improve the drought tolerance (Bussoti et al., 2002). These evidence are in agreement with the present study found that, CO54 had maximum specific leaf weight reduction of 26.8 percent than CO53 (20.7%) under drought alone condition. Reduction of specific leaf weight shows that, CO53 registered more resistance to drought than CO54. Foliarapplication of 400 ppm of nanosilica on drought induced plants, slightly alleviate the adverseeffect of drought; besides higher recovery percent of 26.0 and 20.0 percent registered by CO54and CO53 respectively. Drought reduce the leaf mass, thickness and area for minimize the transpiration loss. These results are agreement with Siddique et al. (2015), opined thatapplication of silica improve the water status, it helps the cells to enlarge and enhance the photosynthesis in the plants also silicon helps to maintaining the turgidity of the cells (Isa et al., 2010). The same

findings were in agreement with Gong et al. (2005) and Wang et al. (2019) insisted that, silicon improving the number of mesophyll cells and mesophyll cell enlargement, which leads to enhance the photosynthetic efficiency in the rice under drought.

Leaf proline

The results on proline content showed a smaller reduction were noticed in drought with nanosilica treated plants (Table 5.). CO54 had maximum proline content when compared to the CO53 (291.8 µg g^{-1}) under drought alone condition. Comparing the different nanosilica treatments, T4 (Drought + 400 ppm) recorded the least proline content of about 216.1 µg g^{-1} to 242.0 µg g^{-1}, which was followed by T3 (Drought + 200 ppm) treatment (239.86 µg g^{-1} to 270.51 µg g^{-1}) under drought condition. Proline is an imino acid (proline) acts as best osmolytes which will help to keep up the more plant tissueΨw under water deficit conditions. According to Mc neil et al., (1999) stated that, the osmoprotected iminoacid (proline) resides in the cytoplasm and scavenging of free radicals that are produced under drought (Delauney and Verma, 1990). In the present study, drought adversely affect the protein degradation and significantly increases the proline content in both varieties. High proline accumulation could be noticed in CO54 (87.0 percent over the control) than CO53 (54.2 percent over the control). Application of silicon has the capacity to curtail the impact of water deficit by plummeting the degradation of protein and also reduces the proline production (Gunes et. al., 2008). Among different nanosilica treatments of nanosilica application, 400 ppm of nanosilica had 26.5 percent decreased proline accumulation over the drought alone treatment. These evidences are in agreement with this present study found that, nanosilica application as foliar spray during mid of the drought period had the capacity to diminish the ROS production through stir up the proline synthesis. Samekind of results was reported Agarie et al. (1998) in rice under drought.

Catalase activity

A rising trend was observed in nanosilica treated plants under drought and reduction of catalase activity in control alone treatment. Highest catalase activity of 235.1 µg of H_2O_2 g^{-1} min^{-1} found in CO53 than CO54 (195.4 µg of H_2O_2 g^{-1} min^{-1}) under drought alone condition (Table 5.). Control alone treatment plants showed lowest catalase activity of 159.6 µg of H_2O_2 g^{-1} min^{-1} registered by CO54; whereas CO53 had (189.3 µg of H_2O_2 g^{-1} min^{-1}). In the present study, silicon treated plants had highest catalase activity under drought condition. Among the nanosilica treatments, Drought + 400 ppm treatment (T4)had lowest catalase activity of 310.2 µg of H_2O_2 g^{-1} min^{-1} recorded in CO53; CO54 had 272.1µg of H_2O_2 $g^{-1}min^{-1}$ under drought condition. Catalases is second most abundant antioxidant enzyme and the ability of catalase underdrought is to curtail the activity of H_2O_2 produced in peroxisome then detoxified in to water and oxygen (Vendemiale et al., 1999). Catalase prevent the lipid peroxidation, cell membranedamage and chlorophyll degradation (Uchida et al., 2012). Luna et al. (2012) reported that, drought increases the catalase activity to alleviate negative effects of hydrogen peroxide. Thesefindings are in agreement with present study explained that, drought induced plants had highestcatalase activity of 24.1 percent (CO53), whereas CO54 had (22.4 percent) over the control. Application of nanosilica significantly increases the catalase activity in drought induced plants. Foliar application of 400 ppm nanosilica had maximum increases of catalase activity of about 40.8 percent in CO54 and 31.9 percent in CO53 under control. These results are in agreement with the result of Liang et al. (2003) in barley. Ahmad et al. (2011) who opined that, silicon increases the antioxidant enzymes like catalase (CAT) peroxidise (POX), superoxide dismutase (SOD) under drought condition. The application of silicon in rice couldenhance drought tolerance capacity by altering activity of antioxidative enzymes and also induces the cation binding capacity of the cell wall (Sivanesan and Park, 2014).

Silicon content (%) (SEM-EDAX)

The silicon content were measured under scanning electron microscope by following the energy dispersive x-ray analysis (EDAX)method and tabulated in Table 3. Highest silicon content could observed in CO53 (10.12 %) than CO54 (6.82%) under control alone treatment (T1) (Fig 3.). An increasing trend could also be observed in drought alone and drought with nanosilica treated plants. Among the different concentration of silica nanoformulations treatments under drought, T7 had maximum silicon content of about 16.66 % registered by CO53 than CO54 (13.66 %), which was followed by T6(Drought + 200 ppm) (CO53-14.23%; CO54-12.12%). However, the treatment drought alone registered lesser amount of silica content of 12.1% (CO53) and 8.81% (CO54) respectively than other nanosilica treatments. Besides; this drought alone (T2) treatment had higher silica content than the control (T1) treatment.

Leaf surface characters

Trichome length

The data on trichome length of different nanosilica treatments are presented in Table 4. An increasing trend was observed in nanosilica treated plants and reduction of trichome lengthobserved in control alone treatment. Between two varieties, highest trichome length of 284.27µm registered by CO53 than CO54 (222.27 µm) under drought alone condition (Fig 4.). Control alonetreatment plants showed lowest trichome length of 184.35 µm in CO54 and CO53 had 246.53µm. In the present study, silicon treated plants had highest length of trichomes. Among the nanosilica treatments, Drought + 400 ppm treatment (T4) recorded lowest trichome length of 522.70 µm in CO53 than CO54 (342.10 µm) under drought condition. Trichomes are unicellular unicellular (or) multi cellular branched hair like structure arises from the single (or) multi cell of protodermal cells (Hulskamp et al., 1994). Trichome contains essential oils, secondary metabolites and act as salt gland under salinity stress. Underdrought condition trichomes are very use full trait for resistance against water deficient. Trichomes involved in the production of secondary metabolites like terpenes for drought tolerance (Kennedy, 2003). Guntwer et al. (2012) opined that density and length of the trichome are increased under drought conditions. These results are agreement with present study, drought stress had 19.3 percent increases of trichome length in CO54 and 15.15 percentin CO53 over the control (Table 4 & Fig. 4). Application of silicon increases the trichome lengthof 68.85 percent over the drought. Silicon improve the plant trichome length under drought. Present study results are agreement with Rostkowska et al. (2016), who opined that, silicon deposited in the inside the trichomes and it helps to improve the length of the trichomes. Samekind of results observed by Ahmad et al. (2011) in Mentha piperita. Takeda et al. (2013) reported that silicon deposited in the trichome increases the infra-red light use efficiency in plant.

Stomatal size

The results on stomatal size illustrated in the Table 4. The observation on stomatal size shows an increasing trend in control and declined trend in drought and silica nanoformulation applied plants. The table shows that control plant had maximum stomatal size(31.34 μm^2) and drought plants shows lowest stomatal size of (15.71 μm^2) (Fig 5.). Between the two varieties, CO54 recorded maximum stomatal size of 31.34 μm^2 than the CO53 (25.9 μm^2) under control. A significant variation was observed between drought and drought with nanosilica applied plants. Among the different nanosilica treatments, T4 (Drought + 400 ppm)registered maximum stomatal size of about 30.25 $\mu m^2$and 20.00 μm^2in CO54 and CO53 respectively, with lesser reduction over control. Which was closely flowed by T3 (Drought + 200 ppm) treatment in both varieties (CO54- 29.69 μm^2; CO53-19.57 μm^2). Surface area of the rice leaf covered by cuticle layer. All surface cells are not identicaleach other same degree of changes were noticed based on the nature specie. Some surface cellsdiffer from the outer epidermal cell they are kidney shaped specialised cell for gas exchange purpose (opening and closing of stomata) that cells called guard cells drought alter the development and differentiation of pore size, stomatal length and breadth, density of the Stomata. Wang et al. (2019) reported that size of the stomata reduced under drought. Droughtincreases the density of the stomata (stomatal frequency) are increases, while stomata size were reduced which leads rapid stomatal conductance for increasing the photosynthetic rate. The results are agreement with present study, drought stress significantly decreases the stomatal size in both varieties. CO54 had highest present decreases of about 47.7 percent thanCO53 (39.3 percent) over the control (Table 4 & Fig 5). Application of 400 ppm nanosilica had 68.85 percent increases in stomatal size in rice over the control. These results are agreement with Verma et al. (2020) in sugarcane. Vandegeer et al. (2020) opined that, application of silicon helps to maintain the water content of the guard cell and improve the stomatal conductance to increases the photosynthetic rate in tall fescue.

Silicon bodies length

The data (Table 4.) represent the silicon bodies present in the leaf surface. Results revealed that nanosilica treatment had increasing trend and drought alone and control plants had declining trend. Comparing the two rice varieties, CO54 had minimum silicon body lengthof 13.02 µm than the CO53 (12.34 µm) under control alone condition. However, a considerableincrement could also registered in silicon body length due to drought. Maximum silica bodieslength recorded in CO53 (14.10 µm) than CO54 (13.54 µm) under drought alone condition. Asignificant increasing trend could also be noticed in nanosilica treatment. Among the differentsilica treatments, T4 had maximum silicon bodies length of 18.70 µm than all the other treatments. In T4 treatment, the varieties CO53 and CO54 both on par with each other. Silicon absorbed from the lateral roots and deposited in stems, lead sheath, and leaf blade in the form of silica bodies (Okuda and Takahashi, 1961). It is essential for logging resistance, then biotic and abiotic stress tolerance in rice (Yoshida et al., 1969). Under droughtcondition activate the silicon transporters to active adsorption of silicon. Application of silicondirectly correlated with the size and number of silicon bodies in the leaf surface (Agarie et al., 1998), these results are agreement in present study. Drought condition size of the silicon bodiesare increased. CO53 had maximum size increases of 14.2 percent than CO54 (4 percent) overthe control (Table 4). Application of silicon 400 ppm of nanosilica increase the maximum percent of 32.7 percent of silico bodies size in CO53 than CO54 (26.14 percent) over the drought. Application of silicon increases increase the silicon bodied. It may be due toapplication of silicon deposited in the silico bodies, which increases the size (Agarie et al., 1998). Isa et al. (2010) reported that silicon may be deposited in the motor cells of leaf, it helpsto maintain the water status of the leaf.

CONCLUSION

Leaf area had 10.9 per cent reduction under drought condition.Foliar application of 400 ppm nanosilica had increase in leaf area and leaf area index of 14.25 per cent in drought induced plants over the unsprayed nano silica drought stressed rice plant. Among the different nano silica concentrations, 400 ppm of nanosilica concentration had significantly increased in specific leaf weight under drought of about 19.9 to 26.4 per cent over the drought alone conditions. Drought tolerance trait like relative water content, chlorophyll stability index and membrane stability index had significant reduction of 15.7, 17.25 and 14.65 per cent overthe control in rice under drought condition. These drought tolerant traits are increased by10.6, 18.6 and 10.7 per cent over the drought in rice varieties due to foliar application of 400 ppm of nano silica under drought over the drought alone condition. Proline content was increased due to drought. Maximum proline accumulations of 87.0 per cent were noticed in CO54; meanwhile, CO53 had 54.2 per cent increment. Application of 400 ppm of nanosilica had mean reduction in proline content of 33.9 per cent in both varieties when compared to drought over the drought alone and control. The length of the trichome and silica bodies were measured under scanning electron microscope (SEM) and a significant increase in terms of length of the trichome and silicabodies by 17.4 and 9.1 per cent due to application of 400 ppm of nano silica under drought.The same results were observed in stomatal characters where an increment of 27.3 per cent registered by application of 400 ppm nanosilica under drought than the drought aloneconditions. Hence, the concentration of 400 ppm nanosilica as foliar spray can be efficiently alleviating the effect of drought at reproductive stage in rice.

Table 2. Effect of silica nanoformulations on leaf area (cm^2) and specific leaf weight (mg cm^{-2}) in rice under drought condition.

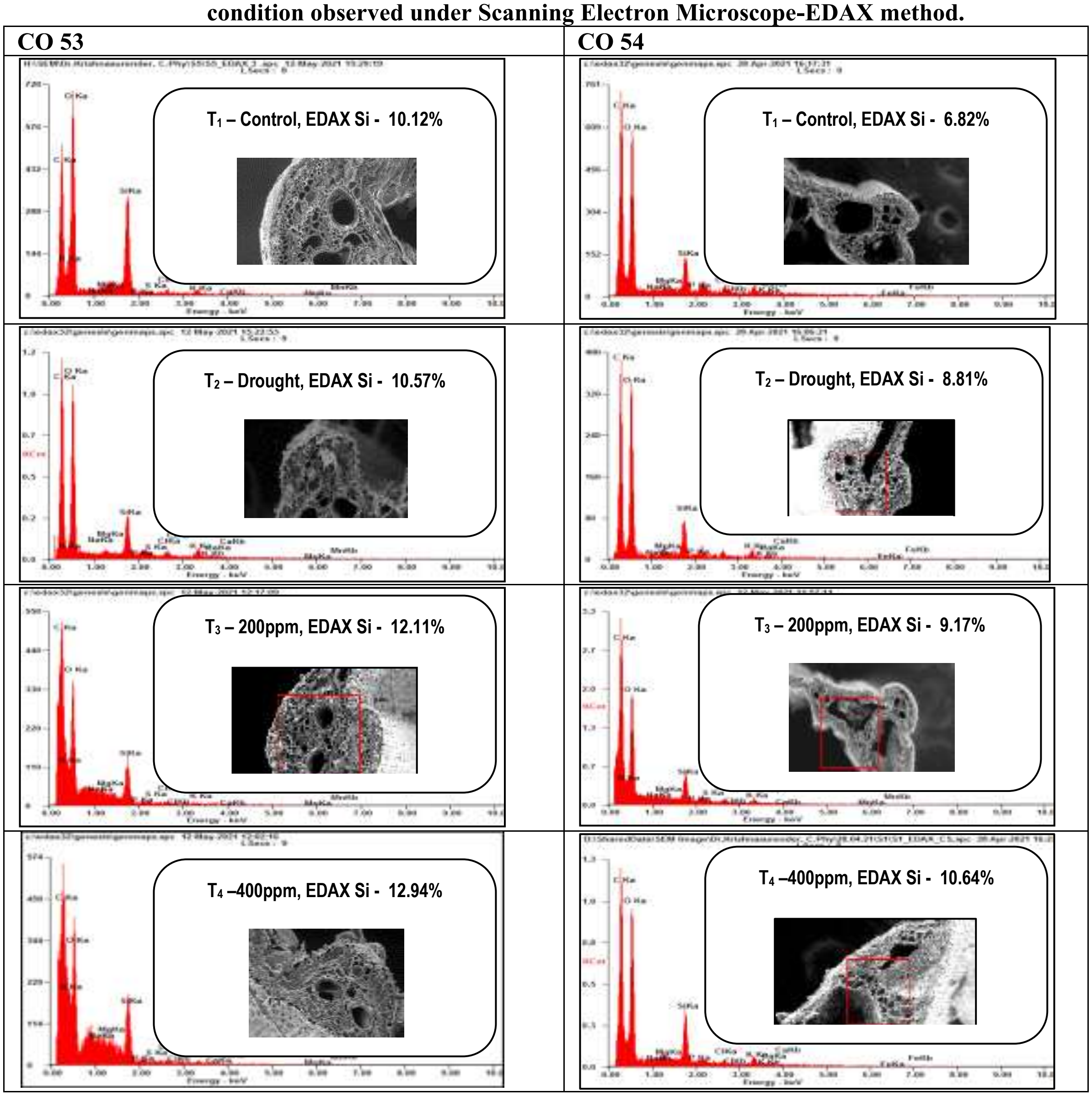
	Leaf area (cm^2)		**Specific leaf weight (mg cm^{-2})**	
Treatments	**CO54**	**CO53**	**CO54**	**CO53**
T1 – Control	1744.6	1814.4	5.28	5.58
T2 – Drought	1441.3	1616.5	3.86	4.42
T3 - Drought +200ppm	1660.5	1705.2	4.62	4.80
T4 - Drought +400ppm	1699.0	1787.8	4.88	5.30
T5 - Drought +600ppm	1619.9	1680.4	4.43	4.61
T6- Drought +800ppm	1568.3	1654.7	4.25	4.58
T7 - Drought +1000ppm	1550.1	1636.4	4.06	4.52
Mean	**1612.0**	**1699.3**	**4.48**	**4.83**
	SEd	**CD (0.05)**	**SEd**	**CD(0.05)**
V (Variety)	35.38	72.71*	0.195	0.400
T (Treatment)	66.18	136.04**	0.364	0.768*
V x T	93.59	192.38	0.515	1.059
	* Significant at 5 per cent level		** Significant at 1 per cent level	

Table 3. Effect of silica nanoformulations on silica content (%) (SEM-EDAX) inrice under drought condition.

Silica content (%)		
Treatments	**CO54**	**CO53**
T1 - Control	6.82	10.12
T2 - Drought	8.81	12.11
T3 - Drought +200ppm	9.17	12.57
T4 - Drought +400ppm	10.64	12.94
T5 - Drought +600ppm	11.05	13.50

T6 - Drought +800ppm	12.12	14.23
T7 - Drought +1000ppm	13.66	16.66
Mean	**21.1**	**23.1**
	SEd	**CD(0.05)**
V	0.104	0.215**
T	0.196	0.402**
V x T	0.277	0.568**
* Significant at 5 per cent level		** Significant at 1 per cent level

Fig. 3. Effect of silica nanoformulations on solica content (%) in rice under drought condition observed under Scanning Electron Microscope-EDAX method.



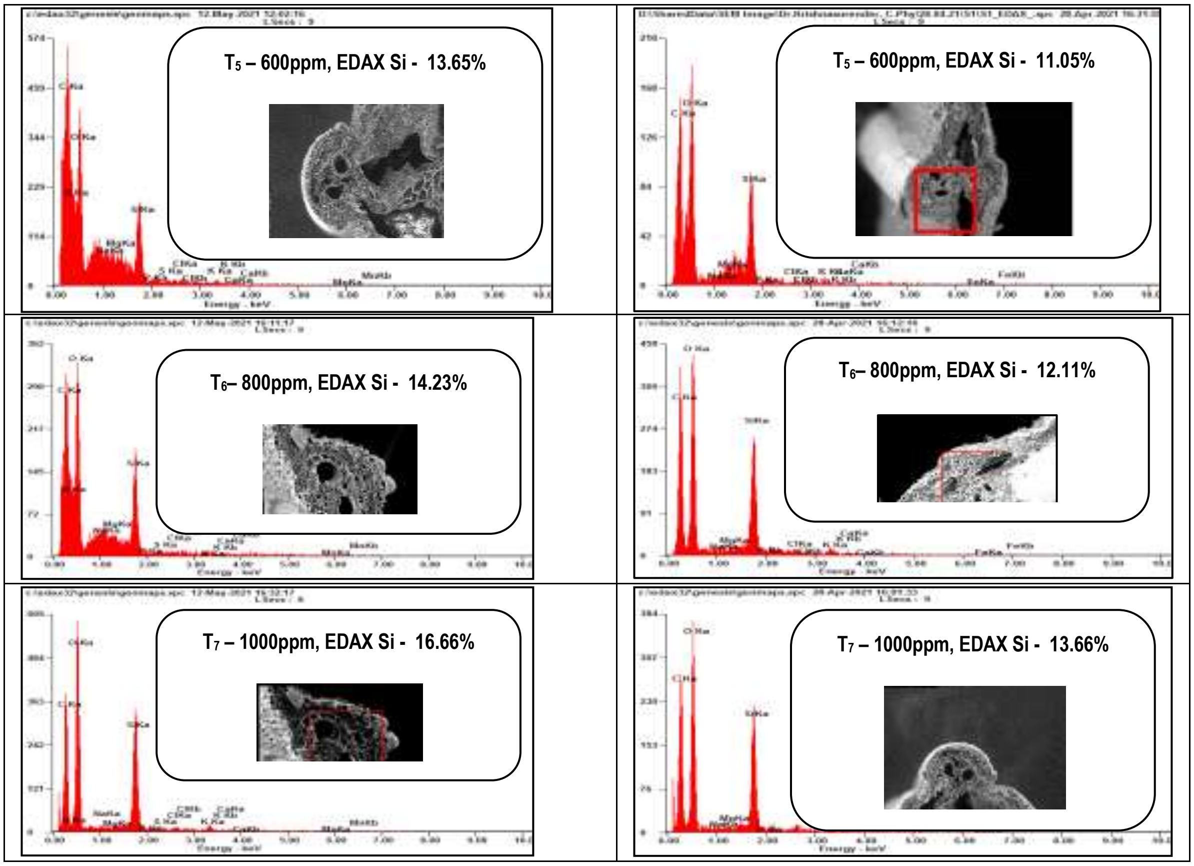
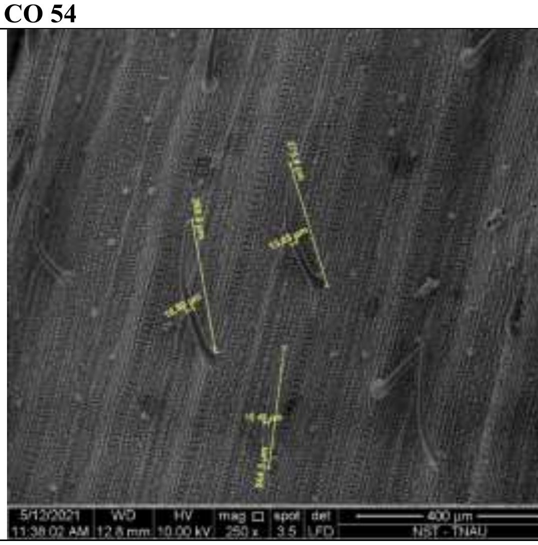
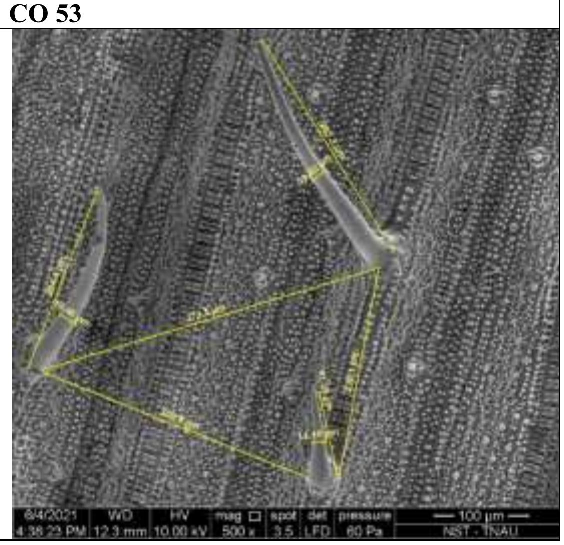


Fig. 4. Effect of silica nanoformulations on trichome (µm) in CO54 and CO53 n rice under drought condition observed under Scanning Electron Microscope-EDAX method.

	CO 54	CO 53
CONTROL		

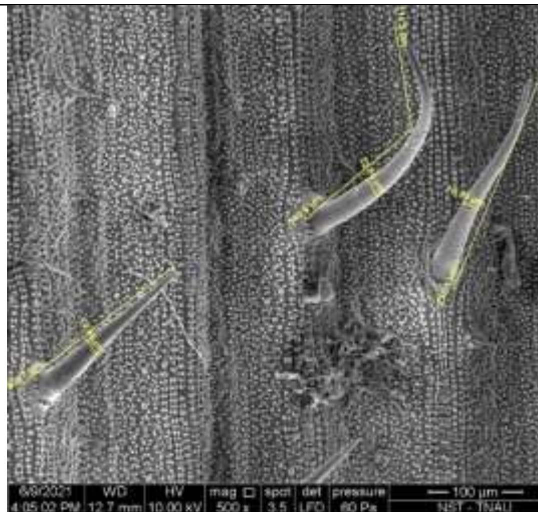
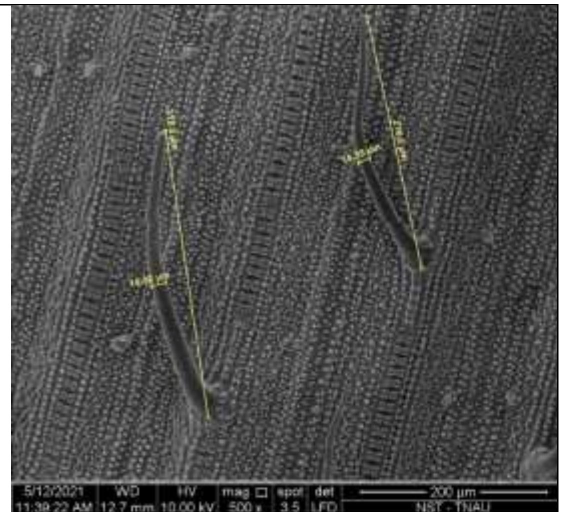
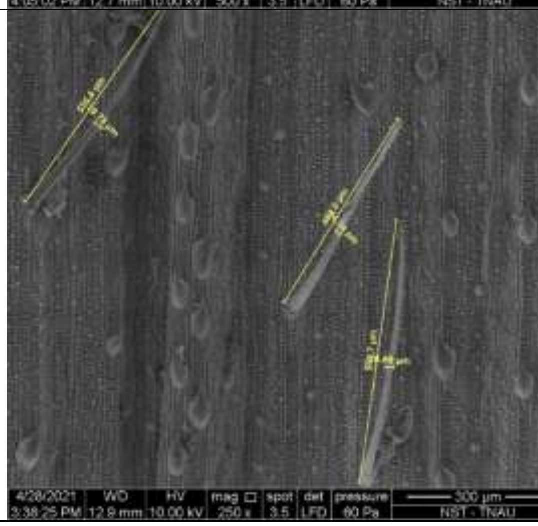
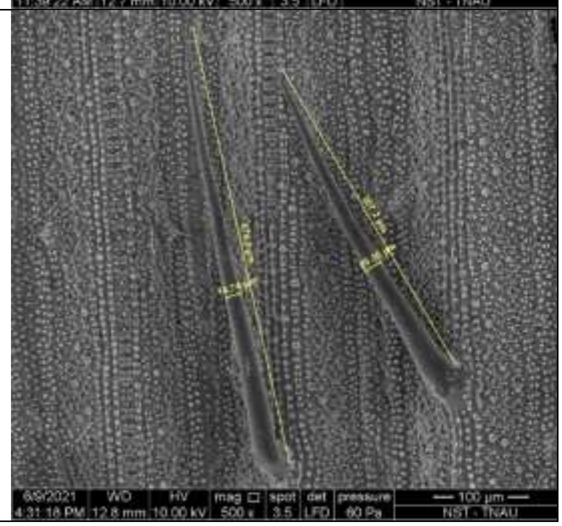
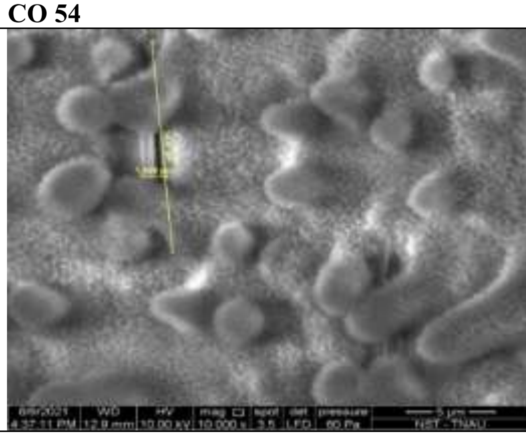
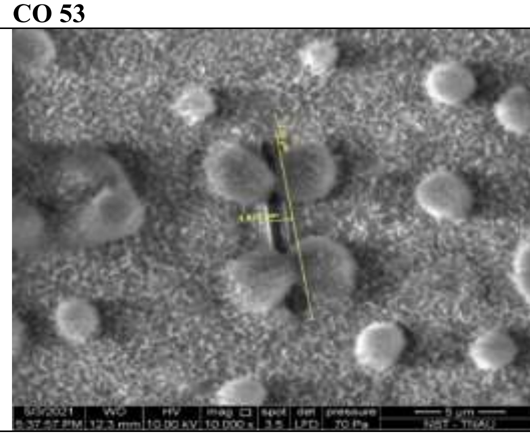
DROUGHT		
Drought + 400ppm Nanosilica		

Fig. 5. Effect of silica nanoformulations on stomatal size (µm^2) in CO54 and CO53 n rice under drought condition observed under Scanning Electron Microscope-EDAX method.

	CO 54	CO 53
CONTROL		

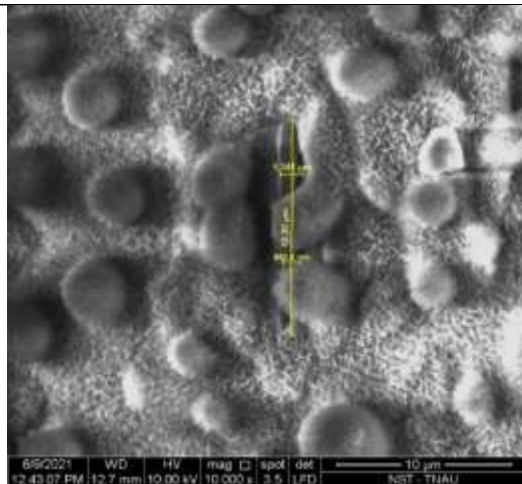
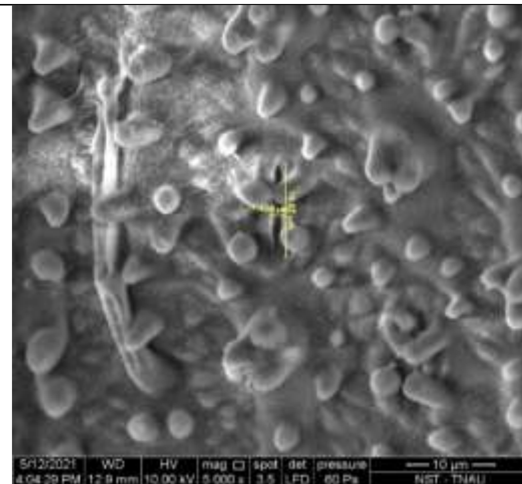
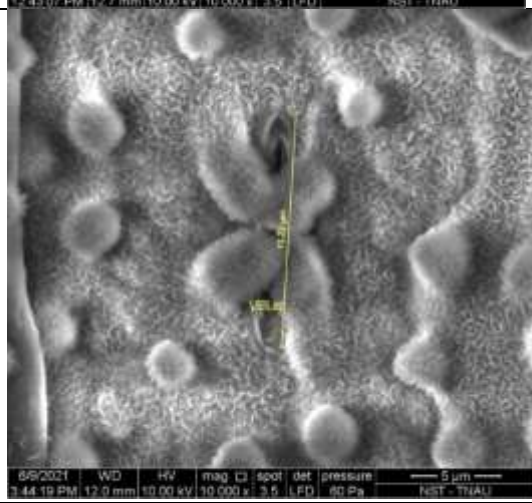
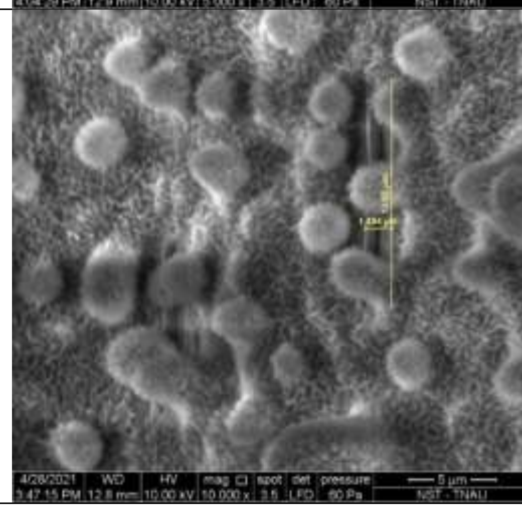
DROUGHT		
Drought + 400ppm Nanosilica		

Table 4. Influence of silica nanoformulations on trichome length (µm), stomatal size (µm^2) and silica bodies (µm)

Trichome length (µm)			**Stomatal size (µm^2)**		**silica bodies (µm)**	
Treatments	**CO54**	**CO53**	**CO54**	**CO53**	**CO54**	**CO53**
T_1 – Control	186.35	246.53	31.34	25.90	13.02	12.34
T_2 – Drought	222.27	284.27	16.39	15.71	13.54	14.10
T_3 - Drought +200ppm	305.23	497.30	29.69	19.57	16.12	16.12
T_4 - Drought +400ppm	342.10	522.70	30.25	20.00	17.08	18.70
T_5 - Drought +600ppm	273.47	424.93	25.28	17.23	15.87	15.43
T_6- Drought +800ppm	263.70	418.27	24.87	17.03	15.51	15.26
T_7 - Drought +1000ppm	260.93	300.43	20.00	16.60	15.20	14.30
Mean	264.86	384.92	25.40	18.86	15.19	15.18
	SEd	**CD(0.05)**	**SEd**	**CD(0.05)**	**SEd**	**CD(0.05)**

V (Variety)	30.827	63.366**	1.755	3.608**	0.249	0.511
T (Treatment)	57.672	118.548**	3.284	6.750**	0.4654	0.957**
V x T	81.561	167.653	4.644	9.546	0.658	1.353
		* Significant at 5 per cent level		** Significant at 1 per cent level		

Table 5. Influence of silica nanoformulations on proline (µg g^{-1}) and catalase activity (µg of H_2O_2 g^{-1} min^{-1}) in rice under droughtcondition.

	Leaf proline (µg g^{-1})		**Catalase activity (µg of H_2O_2 g^{-1} min^{-1})**	
Treatments	**CO54**	**CO53**	**CO54**	**CO53**
T_1 – Control	177.0	188.2	161.3	190.2
T_2 – Drought	331.2	292.3	196.1	234.9
T_3 - Drought +200ppm	270.5	238.8	242.7	290.7
T_4 - Drought +400ppm	242.0	216.1	271.7	311.2
T_5 - Drought +600ppm	285.4	261.8	232.5	275.1
T_6- Drought +800ppm	311.3	238.4	218.2	255.4
T_7 - Drought +1000ppm	317.9	273.9	208.1	245.9
Mean	276.47	244.21	218.66	257.63
	SEd	**CD(0.05)**	**SEd**	**CD(0.05)**
V (Variety)	7.850	16.136**	2.780	5.714**
T (Treatment)	14.685	30.188**	5.200	10.690**
V x T	20.769	42.693	7.355	15.118
	* Significant at 5 per cent level		** Significant at 1 per cent level	

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