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Influence of Nanosilica on Physio-biochemical and antioxidative enzymes in rice under drought

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Abstract
Rice is an important food crop in India, feed more than 60 percent of the population drought causes severe effect on rice during reproductive phase that causes more than 60 percent yield loses. Silicon is a quasi-essential element for rice. External application of silicon gives tolerance to abiotic stresses. Compared with silicon, silicon nano particle have more proficient in inducing the drought tolerance capacity. In the present study, drought was imposed at 12 days before flowering to 10 days after flowering; followed by normal irrigation. Different concentrations of silica nanoformulation *viz*., 200ppm, 400ppm, 600ppm, 800ppm, 1000ppm were applied foliarly to know the effect of silica nanoformulation under water deficit stress condition. The application of nanosilica had correlated positively with drought tolerance traits in CO54 and CO53 varieties. Foliar application of silica nanoformulation at the rate of 400 ppm had maximum value on total chlorophyll content, soluble protein content, relative water content, chlorophyll stability index, membrane stability index and antioxidant enzymes of catalase, proline accumulation (decreased) compare with drought alone in rice (CO54 and CO53).

Keywords: Rice, drought, nanosilica, physiological traits, antioxidant enzymes

1. Introduction

Global warming is a serious issue in all over the world which had a major impact on plants, animals and humans. Global warming leads to climate change. Climate change causes frequent occurrence of drought. According to United Nations Office for Disaster Risk Reduction (UNDRR) 2021, special report stated that severe drought impacts on total GDP of India by 2 to 5 percent. Rice is an important cereal crop and staple food for 4 billion people all around the world. Drought is recognized as a major constraint in the production of rice (Hsiao, 1982) [24]. It requires larger amount of water throughout its life cycle and water requirement of rice is 1386 mm (Vibhute *et al.*, 2017) [50]. Where, 40% of drought causes yield losses for about more than 60 percent (Venkatesan *et al.*, 2005) [48]. Especially, drought stress during the flowering stage causes 64% yield reduction in upland rice (Venuprasad, 2007) [49]. Mild drought stress in reproductive stage causes yield losses for 28 percent (Yang *et al.*, 2019) [52]. In rice, silicon is a quasi-essential element that plays an important role in lodging resistantance and abiotic stress tolerance. Plants uptake the silicon in the form of silicic acid ($Si(OH)_2$). The effect of drought stress on plants is mitigated by the application of silicon in the field (Maghsoudi, 2015) [33]. Silicon acts as key element in stress signalling and it induces resistance to abiotic stress (Fauteux *et al*., 2005) [17].

Silicon helps in the formation of stomata in tissue culture (Soundararajan *et al*., 2017) [43]. Silicon have several complex mechanisms to tolerate abiotic stresses by regulating the physio-biochemical, and antioxidant metabolism (Prabhakaran *et al.*, 2017) [38]. Application of silicon on sorghum improved the photosynthetic rate and dry matter production in drought conditions (Hattori *et al.*, 2005) [22]. Nano particles having unique physical and chemical activity compared with normal chemical compounds. So, it is having a lot of scope in various industry, especially in agriculture industry. Nano-pesticide, nano-coated fertilizers are the emerging areas in agriculture (Siddiqui *et al.*, 2015) [42]. Application of nanosilicon increases germination percentage, root length in tomato seedlings under abiotic stresses (Almutairi, 2016) [6]. Under abiotic stress condition, application of silicon dioxide improves the photosynthetic pigments, carotenoids and maintain the epicuticular wax structure in strayberry and increases the accumulation of antioxidant enzymes like proline contents are reduced in silicon treated plants (Avestan *et al.*, 2019) [7].

Nanosilicon application improves the dry matter production, plant water content and greenness of the plants under drought in *Tanacetum parthenium* L. (Esmaili *et al.*, 2021) [15] and change the leaf thickness and epicuticular wax coating in plants (Avestan *et al.*, 2019) [7]. The application of silica nano particles (SNPs) alleviated the effect of drought stress on xylem water potential. RWC is a complement of xylem water potential to assess the water status of plants (Zarafshar *et al*., 2015) [54]. Antioxidant enzymes maintains the plant in stress free condition. Foliar application of nano Se/SiO_2 increases the antioxidant enzymes like proline, catalase, ascorbate peroxidase (APX), guaiacol peroxidase (GPX), super oxide dismutase (SOD) (Zahedi *et al.*, 2020) [53]. With this background, we used the silica nanoformulation to know the effect on physiological and biochemical characteristic of rice for drought tolerance.

2. Materials and Methods

The field experiment was carried out at Department of rice, Tamil Nadu Agricultural University, Coimbatore, during 2021 (Late *Rabi* season). The recently released TNAU rice varieties (CO53 and CO 54) were used for this experiment. The seeds were sown in the nursery and transplanted to the main field during late *Rabi* season, which makes crop reproductive stage coincide with summer season. The package and practices were followed as per the TNAU recommendation. In this field study, Drought was imposed at 12 days before flowering up to 10 days after flowering; followed by normal irrigation. The total duration of drought stress (withholding water) period was 22 days. In this field trial experiment, Seven treatments were tested, each in three replication *viz*., Control (T_1), Drought (T_2), Drought+ 200 ppm (T_3), Drought+ 400 ppm (T_4), Drought+ 600 ppm (T_5), Drought + 800 ppm (T_6), Drought+ 1000 ppm (T_7) to meet out the objectives of this study. The silica nanoformulation was prepared from pure nano sand silica *i.e.*, mixed with tween 80 and water in the ratio of 1:3. The formulation was subjected to sonication at 50% amplification for 30 mins with 10:10 plus rate. Solutions (Tween 80 and Water) are well homogenized with nanoslica. The physiological and biochemical parameters like total chlorophyll content, soluble protein content, relative water content, chlorophyll stability index, membrane stability index, were measured and estimated by following the method of Hiscox and Isrealstam (1979) [23], Lowry's, *et al.* (1951) [31], Barrs and Weatherley (1962) [9], Murthy and Majumdar (1962) [37], Bajji *et al*. (2001) respectively. The osmolyte of proline and antioxidant enzyme of catalase activity were estimated by adopting the procedure of Bates *et al.* (1973) [10] and Volks and Ferierabend (1989) [51] respectively. The data were analysed by using Factorial Randomized Block Design (FRBD).

3. Results and Discussion

3.1 Soluble Proteins

The data on soluble protein illustrated in Table 1. The result shows an increasing trend in control and declined in drought and silica nano particle treated plants. The value shows that, the control treatment had maximum amount of soluble protein (16.8 mg g^{-1}) then the other treatments. A significant variation was observed between drought and nanosilica treated plants. Between two varieties, CO54 with the foliar application of 400ppm of silica nanoformulation (T_4) registered maximum amount of soluble protein content (15.0 mg g^{-1}) than CO53 (14.7 mg g^{-1}). Which was followed by T_3 (Drought+200ppm) recorded 14.4 mg g^{-1} and 14.1 mg g^{-1}. Soluble protein is important for inducing RuBisCO activity also maintaining the osmolytes content in plants under water deficit condition. Silicon application increases the soluble protein level and also involved in the protein production in moisture stress condition (Karunakaran *et al*., 2013) [25]. According to Liang *et al*. (2003) [28], who reported that, silicon directly or indirectly involved in the membrane stability under abiotic stress conditions. Water deficit condition reduce soluble protein content through diminution of RuBisCO activity in plants (Li *et al.*, 2010) [27]. These evidence in agreement with the present study shows that application of nanosilicon will increases the soluble protein content under drought. Silicon indirectly involved in the stability of the lipid membranes by decreasing ROS production and increase in soluble protein level under water deficit condition (Agarie *et al*., 1998) [1]. The results are in accordance with the findings of Gong *et al.* (2005) [20] in wheat, Zhu *et al.* (2004) [55] in cucumber and Crusciol *et al.* (2009) [11] in potato under drought.

3.2 Total Chlorophyll Content

The data on total chlorophyll content represented in the Table 1. Results show on total chlorophyll content, increasing trend in control plants but opposing trend in the drought and silica nano formulation applied plants. Based on the total chlorophyll content, control plant of CO 54 (3.47 mg g^{-1}) had maximum amount of total chlorophyll content, which was followed by CO53 (2.93 mg g^{-1}). There is significant variation observed at 400 ppm of silica nanoformulation treated plant had maximum amount of chlorophyll content of 2.63 mg g^{-1} in CO54 which was followed by 2.36 mg g^{-1} in CO 53. These results are closely related with control (T_1) and T_3 treatment. Amount of chlorophyll present in the chloroplast tissue are directly represents the rate of the photosynthesis. However, water deficient condition, decreases the total chlorophyll content due to loss of photosynthetic pigments also increasing chlorophyllase enzyme activity which leads to reduction of leaf photosynthetic rate (Farooq *et al*., 2009) [16]. This evidence in agreement with the present study noticed that, application of nanosilica alleviate the negative effect of drought. Because addition silicon improved plant antioxidant system to detoxify reactive oxygen species, which in turn helped in increasing chlorophyll content during abiotic stresses (Al-aghabary *et al.*, 2005) [5] and maintain the chlorophyll pigments under water deficit conditions (Lobato *et al*.,2009) [30]. Similar results was reported by Ghorbanpour *et al*. (2020) [19] in barley. According to Zahedi *et al.* (2020) [53] who found that application of nanosilica enhances the chlorophyll content in strawberry plant under water deficit condition.

3.3 Proline Accumulation

The results on proline content showed an increased trend in drought alone (T_2) over control; besides a smaller reduction were noticed in drought with nanosilica treated plants (Table 2). Between the two varieties expressed that, CO54 had higher proline (330.70 µg g^{-1}) content than the CO53 (291.75 µg g^{-1}). Comparing the five nanosilica treatments, Drought+400ppm of nanosilica treated plants recorded the least proline content, about 215.69 µg g^{-1} to 241.75 µg g^{-1}, which was followed by Drought + 200ppm of nanosilica treatment (239.86 to 271.42 µg g^{-1}). Proline is one of the imino acid act as best osmolytes to maintain the plant tissue water potential under water deficit conditions. Proline, as an important osmoprotectant, it's resides in the cytoplasm (Mc neil *et al*., 1999) [36]. Proline play major role in the scavenging of free radicals produced under water deficit stress conditions (Delauney and Varma, 1993) [13]. Application of silicon will decreases the impact of water deficit stress by reducing the protein degradation, which in turn, decreases the production of proline content (Gunes *et. al.,* 2007) [21]. This evidence in agreement with this study noticed that, application of nanosilica was decreased the ROS production through inducing the proline activity. Same kind of results observed in rice by Agarie *et al*. (1998) [1] under water deficit conditions and Shen *et al*. (2010) [41] also observed in soybean.

3.4 Catalase Activity

The data for catalase activity of different silica nanoemulsion treatments are illustrated in Table 2. An increasing trend observed in control plants and reduction of catalase activity observed in drought. Compare with two varieties, highest catalase activity of 11.45 µg of H_2O_2 g^{-1} min^{-1} recorded in CO54 and followed by 9.75 µg of H_2O_2 g^{-1} min^{-1} in CO53 under drought alone treated plants. Control plants shows lowest catalase activity of 5.44 µg of H_2O_2 g^{-1} min^{-1} in CO54 followed by 5.78 µg of H_2O_2 g^{-1} min^{-1}. In present study, silicon improve the catalase activity and among the treatments, Drought+400 ppm treatment (T_4) shows lowest catalase activity of 6.80µg of H_2O_2 g^{-1} min^{-1} in CO54 fallowed by 6.46 µg of H_2O_2 g^{-1} min^{-1} recorded in CO53. Catalases is second most abundant antioxident enzyme and act on H_2O_2 (produced in peroxisome) to detoxify into water and oxygen (Vendemiale *et al*., 1999) [47]. Catalase prevents the lipid peroxidation, cell membrane damage and chlorophyll degradation (Uchida, 2003) [44]. This evidence were in agreement with the result of Liang *et al.* (2003) [28] in barley.

3.5 Chlorophyll Stability Index

The data on chlorophyll stability index exhibited an increased trend in control treatment with a drastic reduction in drought and drought with nanosilica treated plants (Table 3). Comparison of two varieties revealed that, CO53 recorded higher chlorophyll stability index of 76.71% than CO54: 70.98% under drought. Among the nanosilica treatment T_4 (Drought+400ppm) registered significantly higher chlorophyll stability index percentage of about 89.58% and 85.48% also had lesser reduction when compared to control at seven days after application of nanosilica under drought. This was closely followed by T_3 (Drought+200 ppm) 79.92% and 83.25% respectively. A more chlorophyll stability index improve water deficit stress tolerance for plants through higher availability of chlorophyll pigment which helps increased photosynthetic rate, more dry mater and higher yield. In this present study observed that, the deceased chlorophyll stability index were noticed under water deficit stress conditions due to reduction in chloroplast membrane integrity, besides it was associated with enhanced the role and activity of chloroplast membrane phosphatase activity (Da silva *et al.*,2013) [12]. These evidence were agreement with this present study revealed that the variety CO53 showed a lesser reduction in chlorophyll stability index in response to water deficit stress than CO54. According to Sairam *et al.* (1997) [40], who opined that, under water deficit stress conditions the chlorophyll stability was decreased due to chlorophyll double membrane was degraded by free radicles. Also application of nanosilica reducing the level of hydrogen peroxideproduction peroxisome. Silicon involved in the improving the chlorophyll stability index by reducing the chlorophyll degradation (Siddiqui *et al.*, 2015) [42]. Similar results was observed by Maghsoudi *et al.* (2016) [34] and Ur Rahman *et al.* (2021) [46] in wheat.

3.6 Membrane Stability Index

The effect of different concentration nanosilica on membrane stability index was described in Table 3. The results on membrane stability index showed an increasing trend in the control (T_1) alone treatment and decline in the drought alone treatment (T_2) as well as drought with nanosilica application. Between the two varieties, CO53 had rank first to maintain the higher membrane stability index CO53 (90.19%) then the CO54 (88.88%). Comparing the different nanosilica treatments, T_4 (Drought+400ppm) registered higher membrane stability index value of 84.06% to 85.17% which was higher than the T_2 (Drought) (74.73% to 78.23%). A considerable lesser reduction in membrane stability index also were noticed due to drought interaction with nano silica application. Silica has many beneficial physiological role in crop performance and life (Epstein, 1999). According to Gunes *et al.* (2007) [21], who opined that, the application of silica is able to reduce the membrane damage in tomato and spinach. Also, silica application is able to overcome the oxidative damage in wheat under drought (Gong *et al.*, 2005 and Rémus-Borel *et al.*, 2008) [20]. Water stress caused water loss from the plants cell organelles and plant tissues which leads to seriously damage membrane structure and functions. Plant cell membrane is one of the important physiological targets of drought and the ability of rice plant to maintain integrity of the membrane under drought is what determines tolerance toward drought (livitt, 1972) [26]. In this present experiment results from electrolyte leakage measurement in rice leaf showed that, the variety CO53 had higher membrane integrity was maintained and had less electrolyte leakage due to drought also by the application of silica nanoparticles at the concentration of 400 ppm. These findings were agreement with the report of Zhu *et al*. (2004) [55] and Liang *et al*.(2005), who stated that, the application of silica protects the plants from drought (water deficit) by supplying enormous stable lipids and proteins present in the cell membrane. According to Desmuukh *et al*. (1991) [14] who opined that the higher electrolyte leakage of the water deficit stresses plants was positively correlated with the huge amount production of reactive oxygen species activity, which leads to desiccation of cell due to water deficit (Ajithkumar *et al.*, 2014) [4] and application of silicon reduce the lipid peroxidation to support the increases the membrane stability (Liang *et al*., 1996) [29]. Agarie *et al.* (1998) [1] observed that, the membrane stability increased in rice plant in drought stress. Ahmed *et al*. also found that application of Si could decrease sorghum leaf membrane permeability.

3.7 Relative Water Content

The data on relative water content (RWC) revealed that, an increasing trend were observed in control than the drought treatments (Table 3). Comparing the two varieties CO54 registered higher relative water content of 91.12% than the CO53 (88.15%). Among the silica nanoformulation treatments, T_4 (Drought+ 400 ppm) recorded more relative water content about 87.67% (CO54) and 86.80% (CO53) under drought, which was followed by T_3 (Drought+200 ppm) 84.1% (CO54) to 82.32% (CO53). However, a considerable reduction could also be noticed in RWC due to drought. RWC is an alternative measure of plants water status and metabolic activity of leaf (Flower and Ludlow, 1986) [8]; besides it has been used as an important physiological index to identifying the drought tolerance capacity of the plant (barrs and weatherly,1962) [9]. Silicon play a major role in rice to reduce the water loss and allows the plant to withstand the drought conditions (Ma, 2004) [32]. This evidence in concordance with the present study shows that, silica nanoformulation application as foliar spray improve the RWC of the rice (CO54 and CO53) under drought. In this experiment, drought (T_2) causes significantly reduce the RWC (CO54:73.60%; CO53:77.46%) over the control (CO54:91.12%; CO53:88.15%). Among the different concentration of nanosilica application, the treatment T_4 (Drought+400ppm) had higher RWC of about 87.67 to 86.80% in CO54 and CO53, which was followed by the treatment of T_3 (Drought+200ppm) under drought. These finding were agreements with the results of Mali and Aery (2008) [35], who found that a positive correlation between reduction in transpiration losses and silicon deposition in the epidermal cells in wheat.

Table 1: Effect of silica nanoformulations on leaf soluble protein (mg g-1) and total chlorophyll content (mg g-1) in rice

	Leaf Soluble Protein (mg g^{-1} of fresh weight)		Total Chlorophyll Content (mg g^{-1} of fresh weight)	
Treatments	CO54	CO53	CO54	CO53
Control	16.88	15.95	3.47	2.93
Drought	11.73	12.84	2.15	1.44
Drought+200ppm	13.92	14.17	2.63	2.36
Drought+400ppm	14.54	14.89	2.93	2.60
Drought+600ppm	13.13	13.65	2.42	1.91
Drought+800ppm	12.78	13.30	2.36	1.74
Drought+1000ppm	12.57	13.01	2.29	1.67
Mean	13.65	13.9	2.61	2.09
	SED	CD(0.05)	SED	CD(0.05)
V	0.229	0.470	0.108	0.223**
T	0.428	0.880**	0.203	0.417**
V x T	0.606	1.245	0.287	0.589

Table 2: Effect of silica nanoformulations on proline (µg g^{-1}) and catalase activity (µg of H_2O_2 g^{-1} min^{-1}) in rice.

	Leaf Proline (µg g^{-1})		Catalase activity (µg of H_2O_2 g^{-1} min^{-1})	
Treatments	CO54	CO53	CO54	CO53
Control	176.78	189.18	159.23	210.57
Drought	330.70	291.75	195.56	232.58
Drought +200ppm	271.42	239.86	244.68	289.62
Drought +400ppm	241.75	215.69	272.78	310.25
Drought +600ppm	284.82	261.75	232.13	274.17
Drought +800ppm	312.43	272.72	217.31	257.75
Drought +1000ppm	318.19	280.9	209.36	245.75
Mean	276.59	250.25	8.55	7.98
	SED	CD(0.05)	SED	CD(0.05)
V	7.850	16.136**	2.780	5.714**
T	14.685	30.188**	5.200	10.690**
V x T	20.769	42.693	7.355	15.118

Table 3: Effect of silica nanoformulations on relative water content (%), chlorophyll stability index (%) and membrane stability index (%) in rice

	Relative Water Content (%)		Membrane Stability Index (%)		Chlorophyll Stability Index (%)	
Treatments	CO54	CO53	CO54	CO53	CO54	CO53
Control	91.12	88.15	88.88	90.19	87.42	91.05
Drought	73.60	77.46	74.73	78.23	70.98	76.71
Drought +200ppm	84.1	82.32	81.01	83.43	79.92	83.25
Drought +400ppm	87.67	86.80	84.06	85.17	85.48	89.58
Drought +600ppm	78.62	80.31	79.21	81.01	78.43	81.23
Drought +800ppm	77.89	79.56	78.47	80.08	74.08	79.53
Drought +1000ppm	75.76	78.43	77.62	78.73	72.89	77.31
Mean	81.25	81.86	80.57	82.38	78.46	82.67
	SED	CD(0.05)	SED	CD(0.05)	SED	CD(0.05)
V	2.548	5.237	1.997	4.106	2.891	5.942
T	4.767	0.799*	3.737	7.682*	5.408	11.118*
V x T	6.741	13.857	5.285	10.864	7.649	15.723

4. Conclusion

This study shows nanosilica emulsion induces the antioxidant enzyme like catalase activity and it is important for detoxifying the production of H_2O_2 in peroxisome which leads to cellular membrane damage. Silica nanoformulation alleviate the negative effect of H_2O_2 by increases the catalase activity, which leads to reduction of membrane damage and lipid peroxidation. It helps maintain the total chlorophyll content, soluble protein content, membrane stability index and chlorophyll stability index then relative water content may be impressed due to silica deposited in the epidermal layer of the leaf surface. Among the treatments, 400ppm of nanosilica treatments (T_4) registered higher total chlorophyll content (CO54-36.27 and CO53- 80.5%), soluble protein content (CO54- 23.9%% and CO53- 10.35%), chlorophyll stability index (CO54-20.4% and CO53-16.7%), membrane stability index (CO54- 12.4% and CO53- 8.8%), relative water content (CO54-19.1% and CO53-12.0%) and antioxidant like catalase activity decreased (CO54-039.48% and CO53-33.28%). The proline accumulation decreased (CO54- 26.1% and CO53- 26.0%) and catalase activity also decreased (CO54-40% and CO53- 33.7%) compare with drought. In the present study concluded that foliar application of nanosilica improves the drought tolerant capacity effectively in rice.

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