Role of soluble silica in alleviating oxidative stress in soybean crop

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ABSTRACT

Sudden changes in environment causes abiotic and biotic stress due to which farmers are facing problem of decreased yield. The present study was designed to improve yield by alleviating oxidative stress in crops. Silicon is gaining importance in promoting growth and yield of plants. Soluble silica in the form of Agribooster™ was sprayed on test field of soybean during cultivation. Proline content was found to be decreased significantly in soybean sprayed with soluble silica. Significantly decreased malondialdehyde content was observed in soybean which indicates beneficial role of silica in preventing lipid peroxidation. Soluble silica increased peroxidase activity by increasing water availability and total phenol content was also found to be increased significantly in soybean. So from the present study it can be concluded that silica when applied on crops in soluble form prevent oxidative stress caused due to various environmental factors which may help in better quality product and yield.

Key words: Agribooster TM, Malondialdehyde, Peroxidase, Phenol, Proline.

INTRODUCTION

One of the main determinants of agricultural production is climate. There is significant concern about the effects of climate change and its variability on agricultural production throughout the world (Chakraborty and Hazari, 2017). In today’s scenario sudden climatic changes are causing damage to crops by increasing oxidative stress. Silicon which was not believed to be essential for plant growth is becoming a boon for the development of plants. Silica in soluble form imparts many benefits for plants including enhanced growth, improvement in combating various adverse conditions like resistance to drought, salinity; heavy metal toxicity stresses etc and yield (Epstein, 1999; Ma et al., 2006). Silica have been shown to protect plant against various abiotic stress such as extremes of temperature, UV radiation etc. Water losses may be decreased through deposition of silica (Haynes, 2014). Silica imparts erectness and also improves photosynthesis in plants (Dorneles et al., 2016). Exposure of plants to different environmental stress, biotic or abiotic, produces various reactive oxygen species (Chiban et al., 2011). As shown in Fig. 1, superoxide radical (O2•−), hydrogen peroxide (H2O2), hydroxyl radical (OH) and singlet oxygen are all reactive oxygen species which trigger synthesis of each other (Marschner, 1995). Silica shows positive effect on growth of many plants and provides resistance against stress (Broadley et al., 2012). In plants subjected to multiple stresses, silica is actively involved in various metabolic and physiological activities (Sacala, 2009).

Mechanism involved in ameliorative effect of silica to different stresses is activation of antioxidative defence (Liang et al., 2003).

These ROS cause damage to cell membrane, proteins, nucleic acid and lipids (Foyer et al., 1994). However, the most susceptible target is polyunsaturated fatty acids. Removal of hydrogen atoms from polyunsaturated fatty acid initiates the pathway for lipid peroxidation and causes accumulation of malondialdehyde as shown in Fig. 2.

Within the plant cell, enzymatic as well as non-enzymatic antioxidant system is present which helps to maintain ROS balance (Yan et al., 1996). Peroxidase is one of the components of enzymatic antioxidant system which convert H2O2 to water and oxygen causing detoxification of ROS.

AH2 + H2O2 $\xrightleftharpoons{\text{Peroxidase}}^{}$ A + 2H2O

AH2 + ROOH $\xrightarrow{\text{Peroxidase}}$ A + ROH + H2O

Proline is believed to act as osmoprotectant by accumulating in high concentration in plant cells. It also helps in detoxification of ROS, thus maintain of membrane integrity in plants (Demiralaray et al., 2013). Proline is mainly involved in osmotic adjustment of cell, stabilization of membrane and detoxification of harmful ions in plants exposed to salt stress (Ashraf and Foolad, 2007).

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Figure 1: Generation of reactive oxygen species, where MPO is myeloperoxidase and SOD is superoxide dismutase. Grisham, (1992) and Mosker (1194)

Figure 2: Peroxidation of polyunsaturated fatty acid Foyer et al.,(1994)

Phenolic compounds as a part of non-enzymatic system is believed to show antioxidant action. Phenolic compounds accumulation is induced in various stress condition like presence of heavy metals which suggest their role in combating such stress. Phenol has ability to form chelates with metal ions which shows its antioxidant property. These metal ions are involved in catalysing oxygenation reactions. Phenolic compounds inhibit lipid peroxidation by trapping the lipid alkoxyl radical and thereby preventing damage caused by ROS. This activity depends on the structure of the phenolic compounds and the number and position of the hydroxyl group in the molecules (Millic et al., 1998). Phenolic compounds also inhibit activity of oxidizing enzymes. (Elavarthi and Martin, 2010)

In the present study, oxidative stress parameters were assessed in soybean obtained after cultivation in absence and presence of soluble silica to know the beneficial role of silica in alleviating stress. Foliar spray of soluble silica was done. Foliar spray helps in rapid and direct utilization of nutrients (Geetha and Velayutham, 2016).

MATERIALS AND METHODS

Soybean (*Glycine max* L.) was sown on 23rd June 2016 at five fields located in District Dewas, Madhya Pradesh, India. Geographical location of fields selected as per Latitude Longitude read by GPS 76 Garmin was N- 22° 58’32.2” – 34.4” E- 076° 10’ 32.9”– 32.2”

First flowering was observed on 28th July 2016. Agribooster™ containing soluble silica was diluted to 1%
and sprayed on soybean crop twice (22nd July and 25th August 2016) approximately at the interval of one month after sowing seeds. Unsprayed crop served as control. The soybean crop was harvested on 13th September 2016 and seeds were collected to evaluate different oxidative stress parameters.

**Proline content:** Proline was estimated according to the method of Bates *et al.*, (1973). Proline was selectively extracted from soybean using aqueous sulphosalicylic acid. The extracted proline was made to react with 2 ml glacial acetic acid and ninhydrin which is prepared by using glacial acetic acid and phosphoric acid. (pH 1.0) to form chromophore (red color) which was read at 520nm. Except proline other amino acid reacts with ninhydrin prepared in ethanol.

**MDA content:** Malondialdehyde (MDA), a decomposition product of polyunsaturated fatty acid component of membrane lipid was extracted using 5% TCA and estimated using thiobarbituric acid (TBA) as reactive material (Heath and Packer, 1968).

**Peroxidase activity:** It was determined according to method of Summer and Gjessing, (1943). The enzyme activity was assayed using ortho-dianisidine as hydrogen donor and H$_2$O$_2$ as electron acceptor. The rate of formation of yellow orange colored dianisidine dehydrogenation product was measure of peroxidase activity and was assayed spectrophotometrically at 430 nm.

**Total phenol content:** It was estimated using Folin-Ciocalteu reagent which contains oxidizing agent phosphomolybdate. Under alkaline condition phenol reacts to form blue color complex, the molybdenum blue which is measured at 650 nm colorimetrically. Catechol was used as standard. (Bray and Thorpe, 1954)

**RESULTS AND DISCUSSION**

Values are expressed as mean ± SD. p- value was calculated to assess significant difference as compared to control (Table 1).

Proline content was found to be decreased significantly (p value < 0.05) in soybean of test fields T1 and T3. In T2 and T4 fields of soybean, decrease in proline content was highly significant (p value < 0.01) as compared to control (Fig. 3). Decrease in proline content in soybean of test field T1, T2 T3, T4 was found to be 58.8%, 71.5%, 56.6% and 68.7% respectively.

**Table 1:** Oxidative stress parameters in soybean after foliar spray of Agribooster™

| Parameters                  | Control plant | Treated plant (T1) | Treated plant (T2) | Treated plant (T3) | Treated plant (T4) |
|-----------------------------|---------------|--------------------|--------------------|--------------------|--------------------|
| Proline content (µmoles/gm) | 5.03 ± 1.26   | 2.07 ± 0.73*       | 1.43 ± 0.25**      | 2.18 ± 0.39*       | 1.57 ± 0.36**      |
| Malondialdehyde content (µmoles/gm) | 2.65± 0.27 | 0.73 ± 0.16***     | 0.45± 0.05***      | 0.49±0.006***      | 0.69±0.19***       |
| Peroxidase Activity (units/min/gm) | 0.61 ± 0.32  | 18.01 ± 3.44***   | 5.2± 2.05**        | 2.68 ±1.21*       | 2.23±0.57*         |
| Total Phenol content (mg/gm) | 64.33 ±8.14 | 80 ± 3*           | 80 ± 6.08*        | 85.33 ± 5.85*      | 77.33 ± 2.51*      |

* indicates p value < 0.05 and is significant as compared to control
** indicates p value < 0.01 and is highly significant as compared to control
*** indicates p value < 0.001 and is extremely significant as compared to control
Parenthesis indicates standard error

![Figure 3: Effect of soluble silica on proline content of soybean](image)
fields as compared to control (Fig. 4). 72.4%, 83.01%, 81.50% and 73.96% decrease was observed in malondialdehyde content in soybean of test field T1, T2, T3 and T4 respectively.

Activity of peroxidase enzyme was observed to be increased significantly (p value <0.05) in the seeds of test fields T3 (3.93%) and T4 (2.65%) as compared to control (Fig. 5). In soybean of T1 (28.52%) field increase in peroxidase activity was extremely significant (p value < 0.000) and in soybean of field T2 (7.52%) increase was highly significant (p value < 0.01) as compared to control.

Total phenol content in soybean was significantly increased in all the test fields as compared to control. Increase in total phenol content was found to be 24.35%, 24.35%, 32.64% and 20.2% in soybean of T1, T2, T3 and T4 fields respectively (Fig. 6).

Proline is an amino acid involved in osmoregulation. It provides high turgor potential for maintaining growth (Ashraf and Harris, 2004). Proline is also considered as a potent antioxidant and potential inhibitor of programmed cell death in plants (Pireivatlooum et al., 2010). Accumulation of proline is indicator of immediate response to stress (Kundar et al, 2016). Decrease in proline content was observed in P. multiflorum when sprayed with silica (Rubinowska et al., 2014). Similar results were obtained by Kaya et al., (2006) and Tuna et al., (2008) while cultivating corn and wheat respectively under different stress conditions. Silica have been shown to enhance the water...
status; relieving the oxidative stress and improving osmotic adjustment in plants which may favour decrease in proline content in silica treated plants.

As shown in Fig. 2, hydroxyl radical mainly cause peroxidation of lipid. Application of silica in soluble form increases availability of water which releases more protons to neutralise harmful hydroxyl ion preventing peroxidation of lipid present in the membrane. Thus malondialdehyde which is major product of membrane lipid peroxidation had decreased in presence of silica. Liang et al., (2003) found that in silica-treated plants membrane lipid peroxidation had decreased, which indicates greater membrane stability under stress. Silica prevented membrane damage caused due to drought and UV B radiation (Shen et al., 2010). Kazemi et al., (2012) observed decrease in malondialdehyde content under influence of silica solution on Lisanthus.

Various studies have suggested that silicon protect plant from oxidative damage by promoting conversion of superoxide to H₂O and O₂ and also by increasing activities of H₂O₂ scavenging enzymes peroxidase (Dong et al., 2014). Silica activates antioxidant defence system in plants in condition of salt stress (Mahmoud et al., 2017). Peroxidase is believed to have scavenging role against stress condition. Increased availability of water in presence of silica helps in activation of peroxidase enzyme which has protective role in combating stress condition. Thus silica helps to relieve stress condition so that plants can utilize major part of their energy in synthesis of macromolecules which results in improved quality of plant product. Present results are supported by the work of Ahmad and Haddad (2011) who also found significant increase in activity of peroxidase in wheat under water deficit stress in presence of silica. Sayed and Gadallah (2014) suggested that silica induce increase in oxidative defence abilities in drought stressed maize. In the case of saline soils, silica increased the activity of antioxidant enzymes in wheat (Saqib et al., 2008). Silica provides protection from harmful effect of UV B light by enhancing activity of peroxidase enzyme (Shen et al., 2010).

Secondary metabolites such as phenol are synthesized under drought stress and act as antioxidant to protect plants (Nascimento and Fett-Neto, 2010). Phenolic compounds protect cells against the negative effects of ROS such as lipid peroxidation, protein denaturation and DNA damage (Allakhverdiev et al., 2008). Phenolic compounds can scavenge ROS (Amar-owicz and Weidner 2009). It has been shown that plants which are supplemented with silica can have better defence system by elevating production of phenolics (Ma and Yamaji, 2006). (Kidd et al., 2001) reported release of phenolic compounds against aluminium tolerance.

**CONCLUSION**

Silica when used in soluble form can enhance antioxidant capacity of crops so that they can survive and grow under adverse environmental conditions. Silica can become a boon for the farmers to increase their yield.

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