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Beneficial Role of Soluble Silica in Enhancing Chlorophyll Content in Onion Leaves

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Abstract

Silicon which is not considered essential element, improves growth and development in onion. The present study was designed to investigate beneficial role of soluble silica in increasing chlorophyll content in onion leaves. Soluble silica under tradename Agribooster™ was used to alleviate environmental stress. Eight treatments were given at the interval of 15 days after one month of sowing in randomised block design as follows: T1- without fertilizer and soluble silica (Control), T2, T3, T4- foliar spray of soluble silica viz, 7.5, 10 and 12.5 ml/ lit respectively, T5- only fertilizer, T6, T7, T8- fertilizer + foliar spray of soluble silica viz, 7.5, 10 and 12.5 ml/ lit respectively. Chlorophyll a, b and carotenoid content were determined. Malondialdehyde was estimated in leaves to determine level of stress. Malondialdehyde content was found to be significantly higher in control and only fertilizer treated leaves of onion indicating stress in plants which significantly decreased level of chlorophyll a as well as chlorophyll b. This negative effect of stress in chlorophyll content was counteracted by soluble silica. Soluble silica could be used to increase chlorophyll content which will improve photosynthesis.

Introduction

India is agriculture- based country and is the second largest producers of onion in the world. Madhya Pradesh is one of the major onions producing States. In today's scenario stress is a matter of concern for all living beings including plants. Sudden change in environment cause stress which affects photosynthesis.¹ Yield is dependent on rate of photosynthesis. Change in temperature and water availabilitycan decrease plant growth

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Keywords:

Agribooster™, Carotenoid, Chlorophyll, Malondialdehyde by affecting various physiological processes like photosynthesis, water balance and antioxidative defence mechanism.² Water stress cause decrease in chlorophyll content in *Triticum durum* seed.³

With the progression of world food demand it is necessary to increase tolerance of crops to environmental stress to maintain adequate food supplies. The predominant light absorbing pigment in photosynthesis is chlorophyll which is also required to maintain stability of light harvesting complex proteins.⁴ Decrease in chlorophyll content thus would adversely affect photosynthesis. Inhibition of chlorophyll biosynthesis leads to accumulation of tetrapyrrole intermediates which when illuminated can result in generation of reactive oxygen species (ROS).^{5,6} As chloroplast is the centre for high energetic reaction of photosynthesis along with abundant oxygen supply, it is major site for generation of reactive oxygen species.7 Rate of photosynthesis can be determined using chlorophyll content.8 Physiological character such as chlorophyll can help to assess effect of drought condition on growth and production as it is directly related to rate of photosynthesis.9 Stress lead to production of reactive oxygen species resulting in degradation of chlorophyll and peroxidation of lipid membrane.¹⁰

Silicon has been found to alleviate the negative effect of stress by restoring normal growth and physiological processes in plants. In sorghum grown in drought condition, silicon application improved the rate of photosynthesis.¹¹ In a study on capsicum silicon alleviated water deficit stress induced damage in photosynthetic mechanism and improved photosynthesis.¹² Extent of photosynthesis inhibition is inversely related to ratio of chlorophyll a/b.13 Silicon is believed to increase chlorophyll content and chlorophyll a/b ratio under stress condition.14 Plants exposed to different environmental stresses including disease infections exhibit changes in membrane permeability that lead to loss of membrane integrity.¹⁵ Membrane lipid peroxidation is measured in terms of malondialdehyde, an indicator of oxidative damage.16

Therefore the present study was aimed at evaluating effect of environmental stress on chlorophyll content of onions and the use of soluble silica for its remedy.

Materials and Methods

A field study was conducted on onion crop variety Allora from January to May 2018 at Loharpipliya village, Dewas road. Geographical location of fields selected as per Latitude Longitude read by GPS 76 Garmin was N- 22 54'40" -41.4" E- 075 59' 35.1"- 37". Seeds of onion were first used to form ropa [small plant] which were then further transferred to get onion crop. Randomized block design was used with 2.5 m² area for each block. Each block contains approx. 250-260 plants. Soluble silica as Agribooster[™] was used in liquid form in eight treatments as follows:

T1- without fertilizer and soluble silica (Control)

T2, T3 T4- foliar spray of soluble silica viz, 7.5, 10 and 12.5 ml/ lit respectively

T5- only given N, P, K fertilizer

T6, T7, T8- fertilizer + foliar spray of soluble silica viz, 7.5, 10 and 12.5 ml/ lit respectively.

After one month of sowing "ropa", three sprays with soluble silica were given at interval of 15 days. Leaf samples were collected after 15 days of each treatment and analysed for following contents in triplicates:

Determination of Chlorophyll a, b and Carotenoid Content

Chlorophyll was extracted from leaves on the basis of its solubility using 80% acetone. Chlorophyll a, b and carotenoid were calculated using absorbance of supernatant at 470, 646 and 663 in below equation¹⁷

Chlorophyll a (mg/g) = 12.21(A663) - 2.81 (A646)

Chlorophyll b (mg/g) = 20.13 (A646) - 5.03 (A663)

Carotenoid (mg/g) = [1000(A470)-3.27 (Chl a)- 104 (Chl b)]/229

Determination of Malondialdehyde(MDA) Content It was extracted from leaves using 5% trichloroacetic acid and then estimated by reacting with thiobarbituric acid prepared in 20% trichloroacetic acid. The absorbance of mixture after boiling for 30 minutes and centrifugation was taken at 532 nm.¹⁸ Concentration of MDA was calculated using extinction coefficient (ϵ = 155 mM⁻¹cm⁻¹)

Chlorophyll a/b Ratio

It was calculated by dividing chlorophyll a content by Chlorophyll b content.

Result and Discussion

Results are expressed as mean \pm SD. p value was calculated to test significant difference following the protocols of Ashfaq *et al.*, 2019; David *et al.*, 2019; Ullah *et al.*, 2019.¹⁹⁻²¹ Anova was applied to test significant difference within the treatments.

Table 1: Effect of silica on Malondialdehyde, chlorophyll and carotenoid content in
leaves of onion after 15 days of first silica spray

Treatments	MDA (µmoles/g)	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Chlorophyll a/b ratio	Cartenoid (mg/g)
T1 T2	0.022 ± 0.001 0.014 ± 0.006 $a^{ns}.b^{ns}$	8.86 ± 0.91 9.41 ± 0.88 a ^{ns} , b ^{ns}	7.34 ± 0.38 7.47 ± 0.65 a ^{ns} , b ^{ns}	1.2 ± 0.06 1.25 ± 0.02 a ^{ns} , b ^{ns}	0.022 0.002 0.018 ± 0.014 a ^{ns} , b ^{ns}
ТЗ	0.013 ± 0.003 a**,b*	10.33 ± 0.37 a ^{ns} , b ^{ns}	8.02 ± 0.73 a ^{ns} , b ^{ns}	1.28 ± 0.07 a ^{ns} , b ^{ns}	0.021 ± 0.003 a ^{ns} , b ^{ns}
T4	0.018 ± 0.001	10.28 ± 0.93	8.12 ± 0.45	1.26 ± 0.17	0.019 ± 0.016
	a*, b ^{ns}	a ^{ns} , b ^{ns}	a ^{ns} , b ^{ns}	a ^{ns} , b ^{ns}	a ^{ns} , b ^{ns}
T5	0.02 ± 0.006	9.33 ± 1.21	8.69 ± 1.14	1.09 ± 0.3	0.026 ± 0.01
	a ^{ns} , b ^{ns}	a ^{ns}	a ^{ns}	a ^{ns}	a ^{ns}
Т6	0.01 ± 0.006	12.17 ± 1.01	10.31 ± 1.32	1.18 ± 0.05	0.015 ± 0.007
	a ^{ns} ,b*	a*,b*	a*,b ^{ns}	a ^{ns} , b ^{ns}	a ^{ns} , b ^{ns}
Τ7	0.01 ± 0.003	12.21 ± 0.94	8.04 ± 2.79	1.6 ± 0.39	0.018 ± 0.004
	a*,b*	a*,b*	a ^{ns} , b ^{ns}	a ^{ns} , b ^{ns}	a ^{ns} , b ^{ns}
Т8	0.013 ± 0.0005	12.84 ± 1.36	9.98 ± 1.27	1.28 ± 0.03	0.018 ± 0.007
	a***,b**	a*,b*	a*,b ^{ns}	a ^{ns} , b ^{ns}	a ^{ns} , b ^{ns}

Table 2: Effect of silica on Malondialdehyde, chlorophyll and carotenoid content in leaves of onion after 15 days of second silica spray

Treatments	MDA (µmoles/g)	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Chlorophyll a/b ratio	Cartenoid (mg/g)		
T1	0.017 ± 0.003 a ^{ns} , b ^{ns}	7.29 ±1.12	4.11 ±2.89	1.67 ±0.61	0.015 ± 0.006		
T2	0.012 ± 0.0005	9.46 ± 0.89	6.54 ± 0.71	1.44 ± 0.04	0.017 ± 0.003		
	a ^{ns} , b ^{ns}	a*,b ^{ns}	a ^{ns} , b*	a ^{ns} , b ^{ns}	a ^{ns} , b*		
Т3	0.012 ± 0.003	11.77 ±2.27	8.45 ±3.07	1.44 ± 0.23	0.025 ± 0.009		
	a ^{ns} , b ^{ns}	a*,b*	a ^{ns} , b ^{ns}	a ^{ns} , b ^{ns}	a ^{ns} , b ^{ns}		
T4	0.011 ± 0.002	10.85 ± 0.37	7.52 ±0.46	1.44 ± 0.03	0.015 ± 0.008		
	a ^{ns} , b ^{ns}	a***,b ^{ns}	a ^{ns} , b ^{ns}	a ^{ns} , b*	a ^{ns} , b ^{ns}		
Τ5	0.015 ± 0.002	9.87 ±0.73	7.78 ± 0.22	1.26 ± 0.08	0.027 ± 0.004		
	a ^{ns}	a*	a ^{ns} , b ^{ns}	a ^{ns}	a ^{ns}		
Т6	0.006 ± 0.003	12.08 ± 1.42	9.27 ± 1.83	1.32 ± 0.22	0.020 ± 0.01		
	a*,b*	a**,b ^{ns}	a*,b ^{ns}	a ^{ns} , b ^{ns}	a ^{ns} , b ^{ns}		
Τ7	0.010 ±0.003 a*,b ^{ns}	10.98 ± 0.94 a*,b ^{ns}	9.13 ±1.6 a*b ^{ns}	1.21 ± 0.13 a ^{ns} , b ^{ns}	0.021 ± 0.008 a ^{ns} , b ^{ns}		
Т8	0.006 ± 0.003 a***,b***	12.32 ± 0.9 a**,b*	8.63 ±1.03 a*, b ^{ns}	1.44 ± 0.28 a ^{ns} , b ^{ns}	0.017 ± 0.003 a ^{ns} , b*		

Treatments	MDA (µmoles/g)	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Chlorophyll a/b ratio	Cartenoid (mg/g)		
 T1	0.042± 0.01	11.02 ± 1.66	8.52 ± 1.40	1.32 ± 0.30	0.03 ± 0.01		
T2	0.013 ± 0.003	15.72 ±0.63	10.82± 0.59	1.44± 0.13	0.01 ± 0.005		
	a*,b*	a*, b***	a ^{ns} ,b ^{ns}	a ^{ns} ,b*	a ^{ns} , b ^{ns}		
Т3	0.016 ± 0.002	14.32 ± 0.61	10.30± 0.50	1.38± 0.04	0.01 ± 0.003		
	a ^{ns} , b*	a*,b**	a ^{ns} , b ^{ns}	a ^{ns} , b**	a ^{ns} , b ^{ns}		
Τ4	0.014 ± 0.002	15.55 ± 0.56	11.44± 0.57	1.08 ± 0.06	0.007 ± 0.004		
	a ^{ns} , b***	a*,b***	a*,b*	a ^{ns} , b ^{ns}	a ^{ns} ,b*		
Т5	0.022 ± 0.001	11.46 ± 0.40	9.81 ± 0.65	1.16 ± 0.05	0.01 ± 0.003		
	a ^{ns}	a ns	a ^{ns}	a ^{ns}	a ^{ns}		
Т6	0.017 ± 0.001	14.22 ± 0.15	11.12± 0.30	1.27± 0.04	0.009 ± 0.003		
	a ^{ns} ,b*	a*,b***	a*,b*	a ^{ns} , b ^{ns}	a ^{ns} ,b*		
Τ7	0.018 ± 0.001	14.18 ± 0.61	9.63 ± 1.34	1.37 ± 0.05	0.01 ± 0.08		
	a*,b*	a*, b***	a ^{ns} , b ^{ns}	a ^{ns} , b**	a ^{ns} , b ^{ns}		
Т8	0.011 ± 0.005 a***, b***	14.11 ± 1.07 a*,b*	11.75 ± 2.10 a ^{ns} , b ^{ns}	1.27± 0.08 a ^{ns} , b ^{ns}	0.01 ± 0.01 a ^{ns} , b ^{ns}		

 Table 3: Effect of silica on Malondialdehyde, chlorophyll and carotenoid content in leaves of onion after 15 days of third silica spray

a indicates p value compared to T1 and b indicates p value compared to T5

*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

Chlorophyll and Carotenoid Content

Chlorophyll content is influenced by various environmental factors like light intensity, temperature, water content etc. Some of the important role of carotenoids is in photosynthesis and photoprotection.²² In the present study Chlorophyll a content was found to be significantly increased in all soluble silica treated leaves of onion as compared to T1 (irrigated with only water) and T5 (supplied with routine fertilizer) in all the stages. Chlorophyll content in green leaves o susceptible varieties of durum wheat decreased under environmental stress.23 Under drought stress change in chlorophyll content occurs which depends upon plant species and conditions. In some plant decrease is observed in chlorophyll content and in some chlorophyll content increase under stress.²⁴ All the three concentrations of silica viz, 7.5, 10, 12.5 ml/ lit have the same effect on chlorophyll and carotenoid content as calculated using ANOVA (Table 4).

Application of Silicon has shown to improve fresh and dry weight, chlorophyll concentration, rate of photosynthesis, conduction through mesophyll cells and water use efficiency under salinity stress.25,26 Supplementation with silica can maintain level of chlorophyll in capsicum under drought stress.12 Similar results were seen in drought stressed rice.²⁷ Turan *et al.,*²⁸ on P. *vulgris* and Taffouo et al.,29 on Vigna subterranean L observed decrease in chlorophyll content under plants exposed to salt stress. The reason for decrease in chlorophyll content under stress is attributed to both decreases in chlorophyll synthesis as well as increase in degradation of chlorophyll by chlorophyllase enzyme.³⁰ Si induces synthesis of new chlorophylls and also protects existing chlorophyll from oxidative stress induced by salinity.31 Supplementation with silica increased chlorophyll a, b and carotenoid concentration in control and salt stressed in tobacco leaves.³² Tuna et al.,³³ reported that silicon treated wheat plants have higher concentration of pigments. As shown in table 5, high positive correlation was observed between Chlorophyll a and chlorophyll b after each treatment which indicates that chlorophyll b concentration is affected by chlorophyll a in leaves.

Parameters F value between T2,T3 and T4 after first treatment		F value between T6,T7 and T8 after first treatment	F value between T2,T3 and T4 after second treatment	F value between T6,T7 and T8 after third treatment	F value between T2,T3 and T4 after third treatment	F value between T6,T7 and T8 after third treatment	
Chlorophyll a	1.83	1.33	2.19	1.42	1.99	0.083	
Chlorophyll b	1.12	1.41	1.17	0.16	3.69	2.01	
Chlorophyll a/b	0.12	3.72	0.25	0.921	1.42	3.33	
carotenoid	0.057	0.056	1.11	0.875	4	0.916	
MDA	1.25	0.63	0.94	0.44	1	3.54	

Table 4: showing F value between different treatments

Tabulated F value at 2, 7 degree of freedom is 4.74. All the calculated values are less than tabulated value therefore difference is insignificant.

Table 5: showing correlation	between	parameters

	First silica spray					Second silica spray				Third silica spray					
	Chl a	Chl b	Chl a/b	Carote noid	MDA	Chl a	Chl b	Chl a/b	Carote noid	MDA	Chl a	Chl b	Chl a/b	Carote noid	MDA
First sili	ica sp	ray													
Chl a	1	0.6	0.34	-0.18	-0.35	0.62	0.42	-0.4	0.08	-0.58	0.25	0.44	-0.13	-0.43	-0.44
Chl b		1	-0.53	-0.43	-0.1	0.37	0.178	0.02	0.35	-0.48	0.04	0.3	-0.27	-0.26	-0.25
Chl a/b			1	0.29	-0.21	0.2	0.27	-0.13	-0.27	0.03	0.18	0.08	0.15	-0.14	-0.16
Carote															
noid				1	0.18	-0.04	0.08	-0.1	-0.17	0.39	-0.28	-0.31	0.13	0.23	0.11
MDA					1	-0.43	-0.1	0.1	0.34	0.57	-0.58	-0.39	-0.28	0.31	0.4
Second	silica	spray													
Chl a						1	0.81	-0.27	0.09	-0.43	0.39	0.59	-0.11	-0.5	-0.67
Chl b							1	-0.72	0.1	-0.42	0.2	0.2	0.03	-0.22	-0.57
Chl a/b								1	-0.2	0.21	-0.04	0.17	-0.28	-0.17	0.36
Carote															
noid									1	0.03	-0.09	-0.2	0.11	0.08	0.01
MDA										1	-0.57	-0.47	-0.11	0.03	0.45
Third si	lica sp	orayt													
Chl a											1	0.59	0.21	-0.58	-0.51
Chl b												1	-0.42	-0.91	-0.5
Chl a/b													1	0.48	0.11
Carote															
noid														1	0.45
MDA															1

Oxidative Stress

In open field, environmental conditions are not constant. Environmental changes disturb the control system of plants due to accumulation of reactive oxygen species which cause damage to plants and affects its growth. ³⁴ With application of soluble silica in absence of fertilizer, MDA content decreased significantly as compared to T1 (only water) and T5 (only fertilizer). Decrease in lipid peroxidation measured by MDA content indicates increased tolerance of plant to changing environmental condition. In silica treated plants membrane lipid peroxidation is decreased indicating greater membrane stability.35 Treatment with soluble silica along with fertilizer also decreased MDA content significantly as compared to T1 and T5 indicating more tolerance in plants. MDA content was increased under saline stress as observed in alfaalfa cultivators by Babakhani et al.,36 Wang et al.,37 reported increased in MDA concentration in sensitive variety of alfalfa under salinity stress. Malondialdehyde content was decreased in presence of soluble silica.³⁸ Potassium silicate ameliorated the effect of infectious disease in onion indicated by decrease in lipid peroxidation and increasing membrane stability.³⁹ Alleviation of lipid peroxidation by supplementation of silicon was observed in *F. oxysporumf. sp. cubense, cotton- Ramularia areola, rice- P. oryzae, sorghum- C. sublineolumand wheat-P. oryzae* marked by decrease amount of MDA.^{40,41}

Pretreatment with silica before exposing plant to salt stress resulted in decrease in MDA content which shows increased tolerance of plant towards oxidative stress caused by salinity stress.⁴² Similar results were reported by Al-aghabary *et al.*,⁴³ In *Boragoofficinalis* L. MDA content was decreased in aluminium treated plant supplied with silicon.⁴⁴ MDA content was found to be negatively correlated with both Chlorophyll a and Chlorophyll b which shows that under stress condition with increase in content of MDA, there occur decrease in pigment concentration (Table 5).



Photograph 1 showing treatment of onion with soluble silica



Photograph 2 showing onion leaves after third silica spray

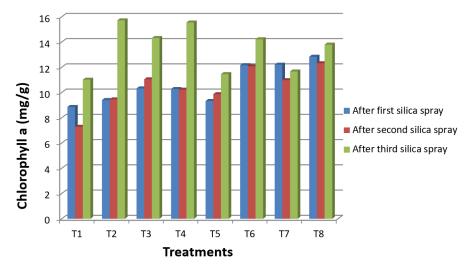


Fig.1 Effect of silica on Chlorophyll a content in leaves of onion after different treatments

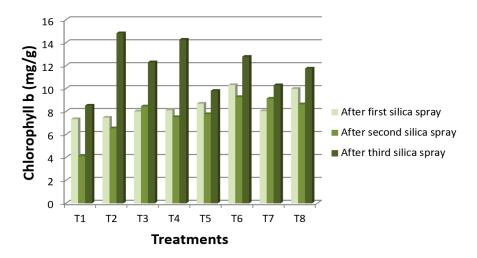


Fig. 2: Effect of silica on Chlorophyll a content in leaves of onion after different treatments

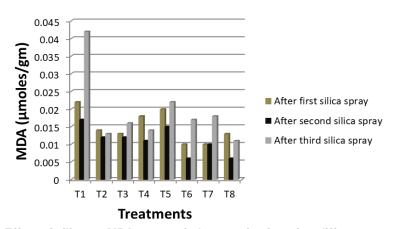


Fig.3: Effect of silica on MDA content in leaves of onion after different treatments

Conclusion

Decrease in productivity due to sudden environmental changes like adverse climatic changes, inadequate rainfall, abnormal soil properties etc. is a big challenge faced by farmers. Keeping in mind above problem the present study was designed to get a solution for it. Soluble silica according to the results proved to be helpful in reducing MDA concentration and elevating chlorophyll content. It can be concluded that application of soluble silica helps to enhances productivity by improving rate of photosynthesis. Farmers are suggested to use soluble silica to upgrade their yield and product quality.

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Conflict of Interest

Authors declare no conflict of interest.

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