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Effect of Silicon solubilize on physiological and biochemical characteristic of rice (*Oryza sativa* L.)

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Abstract
Rice is important crop for human nutrition, providing more than one fifth of the total calories consumed by human population. Proper application of silicon is important for plant growth and development. The deficiency of the silicon is one of the most important limiting factors for crop production. There are several researchers working on rice for development of high yielding varieties by using silicon. Silicon enhances the protein content, photosynthetic efficiency and other physiological and biochemical attributes. Keeping the above facts in mind a field experiment was conducted at G. B. Pant University of Agriculture and Technology, Pantnagar to elucidate the effect of Silixol (liquid source of silicon) on physiological and biochemical character of rice (*Oryza sativa* L.). Foliar application of 0.2% aqueous solution of Silixol was used in different genotypes of rice namely DRRH-3, PA-6129, PA-6201, PA-6444, PHB-71 and BPT-5204. Silicon as liquid silixol improved various attributes of rice. Si solubilizer response needs more studies in terms of soil as well as foliar application for understanding its role in essentiality.

Keywords: chlorophyll content, chlorophyll fluorescence, genotypes, rice, silicon, solubilizer, silixol

Introduction
Rice productivity and production is essential for the national point of view to provide the food security and reduce the imports (Serneels and Lambinn, 2011**)** [1]. In plants, chlorophyll content (CC) is an important indicator of nutritional status. It is well known that plant growth could be related to rate of photosynthesis which is directly proportional to chlorophyll contents in leaves (Jawahar and Vaiyapuri, 2010) [2]. The enhancements in chlorophyll (chl) are defined by the fact that silicon is absorbed by the plant and it has been accumulated in the layer of the epidermis, which promotes positive changes in plant structure and better light capture by the leaf (Barbosa *et al*., 2015) [3].
Chlorophyll fluorescence analysis has become one of the most powerful and widely used techniques available to plant physiologist and ecophysiologist. It can be used to estimate the rate of photosynthetic electron transport, photosynthetic quantum yield and capacity of thermal energy dissipation under high light stress (Demming *et al.,* 1995) [4]. Both of maximum/potential quantum efficiency of PS II (Fv/Fm) and the maximum primary yield of photochemistry of PS II (Fv/F0) are related with photosynthetic efficiency of plant leaves (Shangguan *et al.,* 2000) [5].
The storage organs of plants contain two types of starch: amylose, composed of largely linear chains of a-(1/4) linked glucose residues and amylopectin, a branched starch with a-(1/6) linkage at branch point. In addition, nutritional value is mostly determined by the synthesis of storage carbohydrates, proteins, oil and minerals during grain filling. The silicon might be play important role in the carbohydrate metabolism, translocation of sugars. Protein is macromolecule which contains all essential amino acid while rich in sulfur-containing amino acids such as cysteine and methionine, this amino acid found in more concentration. Protein synthesis is closely related to the production of new tissues and considered as a principal sink for nitrogen compounds. By applying silicon together with Al increased total protein content in roots and shoots of rice seedlings compared to the Al treatment alone (Singh *et al.,* 2011) [6].

Materials and Methods
Experimental site and Plant material
In order to study the effect of silicon solubilizer (silixol) on physiological and biochemical characters in rice, an experiment was conducted in the Norman E.

Borlaug Crop Research Centre (CRC) and Department of Plant Physiology of G. B. Pant University of Agriculture and Technology, Pantnagar (Uttarakhand), India, using six rice genotypes namely DRRH-3, PA-6129, PA-6201, PA-6444, PHB-71 and BPT-5204. The experiment was carried out using the split plot design with three replications.

Estimation of chlorophyll content

Chlorophyll content was estimated in freshly harvested leaves at active tillering and flowering by Dimethyl sulfoxide (DMSO) method which described by (Hiscox *et al*., 1979) [7]. To estimate chlorophyll content 50 mg of finely chopped leaves were taken in test tube in triplicate. Then 10 ml of DMSO was added in each tube and incubated at 65^0C for 3 hour in an oven. After incubation of 3 hour absorbance of sample were determined at 663 and 645nm using a UV spectrophotometer against pure DMSO as a blank. The chlorophyll content was then calculated by using the following formula.

$$\text{Chlorophyll a} = \frac{(12.7XA663-2.63XA645)XV}{Weight(g)X1000}$$

$$\text{Chlorophyll b} = \frac{(22.9XA645-4.48XA663)XV}{Weight(g)X1000}$$

$$\text{Total chlorophyll} = \frac{(20.2XA645-8.02XA663)XV}{Weight(g)X1000}$$

Where,
A = Absorbance of chlorophyll extract at specific indicated wavelength
V = Final volume of sample
W = Weight of tissue extracted on fresh weight basis

Chlorophyll fluorescence variable yield (F_v/F_m)

The chlorophyll fluorescence of the rice flag leaves was observed at the flowering stage with taking help of Handy PEA (Hansatech, UK). Light weight plastic leaf clips were used for the achievement the dark adaptation of the plant tissue sample. Leaf clip shutter blades were used for preventing the entry of light and left clip in that place for 10 minutes, so that provides the dark adaptation. The performance for the measurement, the sensor unit of Handy PEA was held over the clip and after that the shutter opened. After that single button-press immediately displayed automatically and calculated fluorescence parameters F_0, F_m, and F_v/F_m. The observations were recorded in the forenoon hrs (9-11 AM) to avoid photoinhibition.

F_0	=	Fluorescence level when plastoquinone electron acceptor pool (a_a) is fully oxidized.
F_m	=	Fluorescence level when plastoquinone transiently fully reduced
F_v	=	Variable fluorescence ($F_m – F_0$)

Amylose content of grains

Amylose content of grains was estimated in rice grain by using the method described by McCready and Owens, (1950) [8]. Rice grains were dehulled and grind with the help of pestle & mortar. 500 mg of powered sample was weighed, and 1 ml of distilled ethanol was added followed by 10 ml of 1N NaOH and volume was made upto 50ml and left it for overnight. 2.5 ml of the extract was taken and 20 ml distilled water was added followed by three drops of phenolphthalein. After this, 0.1 NHCl was added drop by drop until the pink colour just disappeared. Then 1 ml of iodine reagent was added and the volume was made upto 50 ml and absorbance was recorded at 590 nm using dilute iodine reagent as blank. Amylose content was calculated by using standard. For this, 100 mg amylose was dissolved in 10 ml 1N NaOH and volume was made upto 100 ml with distilled water.

Protein extraction & estimation

Protein content of grains was estimated by the method described by Bradford (1976) [9]. Rice grains were dehusked and ground in mortar and pestle. Then 500 mg of rice grains were taken in 2.5ml of protein extraction buffer. Centrifuged at 4^0C at 10, 000 rpm for 15 minutes. After centrifugation 2µl supernatant was taken out in dry test tube and 2-3 drops of PMSF (Phenyl methane sulfonyl fluoride) was added. 300µl extraction buffer was added in solution. 300ml Bradford dye was added. Absorbance was recorded at 595nm. Protein content in the grains was measured by plotting the perpendicular on the standard curve.

Statistical Analysis

The data were analysed using two-way analysis of variance (ANOVA) by using STPR statistical software followed by test at a significance level of $p<0.05$.

Results

Results of different physiological and biochemical parameters as mention below in Tables.

Chlorophyll content

Silicon solubilizer treatment affects the chlorophyll content in different rice genotypes. In 2014, maximum chlorophyll was observed by rice genotype PA-6444 (1.62) and minimum in rice genotype BPT-5204 (1.29) **(**Table-1). In 2015, maximum was observed by rice genotype PA-6444, PHB-71, PA-6201 (1.48) and minimum in rice genotype DRRH-3 (1.46) (Table-2). In 2014, maximum chlorophyll b was observed by rice genotype PA-6201 (0.48) and minimum in rice genotype PA-6444 (0.38). In 2015, maximum was observed by rice genotype PA-6201 (0.51) and minimum in rice genotype BPT-5204 (0.47). In 2014, maximum total chlorophyll was observed by rice genotype PHB-71 (2.11) and minimum in rice genotype DRRH-3 (1.79). In 2015, maximum by rice genotype PHB-71 (2.00) and minimum in rice genotype BPT-5204 (1.93). In 2015, maximum was observed by rice genotype PA-6444, PHB-71, PA-6201 (1.48) and minimum in rice genotype DRRH-3 (1.46). In 2014, maximum chlorophyll b was observed by rice genotype PA-6201 (0.48) and minimum in rice genotype PA-6444 (0.38). In 2015, maximum was observed by rice genotype PA-6201 (0.51) and minimum in rice genotype BPT-5204 (0.47). In 2014, maximum total chlorophyll was observed by rice genotype PHB-71 (2.11) and minimum in rice genotype DRRH-3 (1.79). In 2015, maximum by rice genotype PHB-71 (2.00) and minimum in rice genotype BPT-5204 (1.93).

Chlorophyll fluorescence

Silicon solubilizer treatment affects the PEA meter in different rice genotypes. In 2014, maximum photosynthetic efficiency was obtained by rice genotype PA-6444 (0.71) and minimum in rice genotype PA-6129 (0.65) (Table-3). In 2015, maximum was observed by rice genotype PA-6444 (0.71) and minimum in rice genotype PA-6129 (0.64) (Table-4).

Amylose content of grains

Amylose content decreased in different rice genotypes under the silicon solubilizer treatment. It was observed that amylose content, maximum was by rice genotype BPT-5204 (15.80) and minimum by rice genotype DRRH-3 (13.00) during 2014 (Table-5). In 2015, maximum was by rice genotype BPT-5204 (15.74) and minimum by rice genotype DRRH-3 (12.93) (Table-6).

Protein extraction & estimation

The total protein content of grains was influenced by the silicon solubilizer treatment in different rice genotypes. In 2014, maximum was observed by rice genotype PA-6129 (16.90) and minimum in rice genotype BPT-5204 (13.40) (Table-7). In 2015, maximum protein content was observed by rice genotype PA-6129 (17.00) and minimum in rice genotype BPT-5204 (13.83) (Table-8).

Table 1: Effect of foliar application of silicon solubilizer on chlorophyll content at flowering (mg g^{-1} fr. wt.) in different rice genotypes during kharif season of 2014.

Name of the rice genotypes	Chlorophyll content at flowering (mg g^{-1} fr.wt.)								
	Chlorophyll 'a'			Chlorophyll 'b'			Total Chlorophyll		
	Control	Liquid silicon solubilizer treatment	Mean	Control	Liquid silicon solubilizer treatment	Mean	Control	Liquid silicon solubilizer treatment	Mean
DRRH-3	1.50±0.04	1.58±0.07	1.54	0.41±0.08	0.41±0.07				
PA-6129	1.48±0.02	1.56±0.05	1.52	0.43±0.07	0.44±0.07				
PA-6201	1.28±0.09	1.38±0.08	1.33	0.47±0.05	0.48±0.04				
PA-6444	1.53±0.03	1.62±0.07	1.57	0.37±0.04	0.38±0.04				
PHB-71	1.46±0.09	1.59±0.01	1.52	0.42±0.03	0.44±0.03				
BPT-5204	1.18±0.04	1.29±0.04	1.23	0.41±0.03	0.42±0.03				
Mean	0.403	1.503	0.420	0.431	1.85	1.96			
S. Em. ± 0.029 0.040 0.234 0.097 0.056 CD at 5%	Genotype (G)	Treatment (T)	TxV	Genotype (G)	Treatment (T)	Tx			

Table 2: Effect of foliar application of silicon solubilizer on chlorophyll content at flowering (mg g^{-1} fr.wt.) in different rice genotypes during kharif season of 2015.

Name of the rice genotypes	Chlorophyll content at flowering (mg g^{-1} fr. wt.)								
	Chlorophyll 'a'			Chlorophyll 'b'			Total Chlorophyll		
	Control	Liquid silicon solubilizer treatment	Mean	Control	Liquid silicon solubilizer treatment	Mean	Control	Liquid silicon solubilizer treatment	Mean
DRRH-3	1.40±0.02	1.46±0.02	1.43	0.41±0.01	0.48±0.01	0.44			
PA-6129	1.40±0.03	1.46±0.02	1.43	0.42±0.00	0.50±0.00	0.46			
PA-6201	1.42±0.03	1.48±0.01	1.45	0.44±0.01	.51±0.01	0.47			
PA-6444	1.41±0.02	1.48±0.01	1.44	0.43±0.01	0.50±0.02	0.46			
PHB-71	1.43±0.01	1.48±0.01	1.45	0.43±0.02	0.50±0.02	0.46			
BPT-5204	1.40±0.02	1.46±0.00	1.43	0.43±0.02	0.47±0.00	0.45			
Mean	1.143	1.470	0.429	0.497	1.82	1.96			

Table 3: Effect of foliar application of silicon solubilizer on photosynthetic efficiency (F_v/F_m) in different rice genotypes during kharif season of 2014.

Name of the rice genotypes	Photosynthetic efficiency (F_v/F_m)		
	Control	Liquid silicon solubilizer treatment	Mean
DRRH-3	0.68±0.006	0.70±0.003	0.69
PA-6129	0.63±0.015	0.6There is some error in this table kindly make correction 5±0.012	0.64
PA-6201	0.64±0.007	0.68±0.009	0.66
PA-6444	0.68±0.003	0.71±0.003	0.69
PHB-71	0.68±0.003	0.69±0.003	0.68
BPT-5204	0.65±0.009	0.67±0.003	0.66
Mean	0.663	0.688	
	Genotype(G)	Treatment (T)	TxV
S.Em. ±	0.005	0.002	
CD at 5%	0.014	0.085	0.021

Table 4: Effect of foliar application of silicon solubilizer on photosynthetic efficiency (F_v/F_m) in different rice genotypes during kharif season of 2015.

Name of the rice genotypes	Photosynthetic efficiency (F_v/F_m)		
	Control	Liquid silicon solubilizer treatment	Mean
DRRH-3	0.68±0.003	0.69±0.003	0.68
PA-6129	0.62±0.001	0.64±0.012	0.63
PA-6201	0.63±0.009	0.68±0.009	0.65
PA-6444	0.68±0.006	0.71±0.006	0.69
PHB-71	0.67±0.007	0.69±0.003	0.68

BPT-5204	0.65±0.003	0.68±0.003	0.66
Mean	0.658	0.684	
	Genotype (G)	Treatment (T)	TxV
S.Em. ±	0.005	0.002	
CD at 5%	0.013	0.079	0.019

Table 5: Effect of foliar application of silicon solubilizer on amylose content (%) in different rice genotypes during kharif season of 2014**.**

Name of the rice genotypes	Amylose content (%)		
	Control	**Liquid silicon solubilizer treatment**	**Mean**
DRRH-3	13.36±0.18	13.00±0.11	13.18
PA-6129	16.10±0.05	15.76±0.03	15.93
PA-6201	15.26±0.08	14.76±0.12	15.01
PA-6444	15.86±0.03	15.06±0.12	15.46
PHB-71	14.03±0.03	13.60±0.11	13.81
BPT-5204	16.10±0.05	15.80±0.04	15.95
Mean	15.11	14.66	
	Genotype (G)	Treatment (T)	TxV
S.Em. ±	0.063	0.036	
CD at 5%	0.187	0.108	0.264

Table 6: Effect of foliar application of silicon solubilizer on amylose content (%) in different rice genotypes during kharif season of 2015**.**

Name of the Rice genotypes	Amylose content (%)		
	Control	**Liquid silicon solubilizer treatment**	**Mean**
DRRH-3	13.43±0.16	12.93±0.12	13.18
PA-6129	16.23±0.03	15.66±0.03	15.94
PA-6201	15.40±0.17	14.60±0.23	15.00
PA-6444	15.83±0.06	15.06±0.08	15.44
PHB-71	14.16±0.03	13.53±0.21	13.84
BPT-5204	16.30±0.11	15.74±0.07	16.02
Mean	15.22	14.57	
	Genotype (G)	Treatment (T)	TxV
S.Em. ±	0.095	0.055	
CD at 5%	0.280	0.162	0.396

Table 7: Effect of foliar application of silicon solubilizer on protein content (mg g-1 fresh weight) in different rice genotypes during kharif season of 2014**.**

Name of the rice genotypes	Protein content (mg g-1 fr. Wt.)		
	Control	**Liquid silicon solubilizer treatment**	**Mean**
DRRH-3	14.20±0.15	16.13±0.08	15.16
PA-6129	15.26±0.12	16.90±0.05	16.08
PA-6201	14.13±0.08	14.70±0.11	14.41
PA-6444	14.40±0.11	15.16±0.12	14.78
PHB-71	15.44±0.11	16.03±0.03	15.73
BPT-5204	13.50±0.05	13.40±0.11	13.45
Mean	14.48	15.38	
	Genotype (G)	Treatment (T)	TxV
S.Em. ±	0.075	0.043	
CD at 5%	0.302	0.174	0.428

Table 8: Effect of foliar application of silicon solubilizer on protein content (mg g-1 fresh weight) in different rice genotypes during kharif season of 2015**.**

Name of the rice genotypes	Protein content (mg g-1 fr. Wt.)		
	Control	**Liquid silicon solubilizer treatment**	**Mean**
DRRH-3	14.16±0.06	16.20±0.05	15.18
PA-6129	15.26±0.17	17.00±0.05	16.13
PA-6201	14.13±0.03	14.90±0.05	14.51
PA-6444	14.53±0.12	15.23±0.08	14.88
PHB-71	15.56±0.13	16.13±0.08	15.84
BPT-5204	13.66±0.06	13.83±0.06	13.74
Mean	14.55	15.54	
	Genotype (G)	Treatment (T)	TxV
S.Em. ±	0.066	0.038	
CD at 5%	0.195	0.112	0.276

Discussion

Chlorophyll (Chl) is a photosynthetic pigment and act as an essential factor of the plants photosystem. The inhibition in chlorophyll in the plants, resulting of disorders in chloroplast and their modifications in relation to proteins/lipids responsible by formation of pigment-protein complex (Parida *et al.,* 2007) [10]. Similar results were found in wheat, in which chlorophyll b was significantly increased under silicon solubilizer as compared to control. Silicon may increase cell metabolic activity and promote the amino acid biosynthesis of chlorophyll. It was reported that in soybean crops in drought stressed and UV radiated condition silicon solubilizer significantly increased the chlorophyll content in leaves (Shen *et al.*, 2010) [11]. It was reported that Chl content is positively correlated with the photosynthetic rate (Thomas *et al.,* 2013) [12].

The same observation was found in Similar in chlorophyll b, was observed in wheat (Locarno *et al.,* 2011) [13]. Silicon in concentrations of 0.25, 1.00, and 1.75 μM induced an increment in the total chlorophyll. At the time of stress, silicon treatment enhanced the levels of chlorophyll a, which indicates formation of new pigments, and maintenance of chlorophyll a previously existing (Lobato *et al.,* 2013) [14]. Silixol in concentration of 0.25, 1.00 and 1.75mM can induce progressive increased in chlorophyll a, chlorophyll b, as well as total chlorophyll in rice (Liu *et al.*, 2014) [15].

The photosynthetic efficiency is the recording of the fluorescence emitted from chlorophyll molecule located in chloroplasts of photosynthesizing organism, has allowed to understanding the photochemical and non-photochemical process occurring in thylakoid membrane of chloroplast (Fracheboud *et al.,* 2003). Similarly in maize, silicon treatment increased the photosynthetic efficiency (Fv/Fm) by 22.2% as compared with the control plants (Moussa, 2006) [17]. The decrease in F_0 could reflect damage to regulatory processes external to P_{680} (reaction center of PS II), such as impairment of the photo protective processes that facilitates the dissipation of excess energy with the leaf (Angelopoulos *et al.*, 2009) [18]. Chlorophyll fluorescence analysis suggested that Silixol alleviated water deficit-induced adverse effects by increasing no photochemical quenching, so that it improved the light use efficiency under water stress (Iqbal *et al.*, 2011) [19]. It was observed that application of Silicon had no effects on Fv/Fm and Fv/F0 under high Zn stress, while under a normal Zn level, Fv/Fm and Fv/F0 were increased by the addition of Silicon fertilizers (Song *et al.*, 2014) [20].

The component composition of rice grains is 90% starch and approximately 2% lipids, 6–8% proteins, and 1% minerals. The starch may be further divide in 2 parts (amylose content and the fine structure of amylopectin) are the most important key which determinants cooking quality of rice (Jang *et al.*, 2009) [21]. Amylose content was significantly decreases in rice grain after application of silicon solubilizer. Silicon might be improved grain quality by decreasing amylose content and stickiness of grain. Silicon play important role in carbohydrate metabolism, sugar translocation in plants. Amylose is digested more slowly, providing beneficial effects on human health. (Behall and Scholfield, 2005) [22]. It might be cause formation of complex compound made by the reaction of silicon solubilizer with sugar molecules which inhibit amylase machinery. Increasing the level of silicon in sunflower it was observed that the sugar content was increased as well as amylase content was decreased (Zahoor *et al.*, 2011) [23]. The application of silicon caused a reduction in the levels of total soluble carbohydrates. This effect was likely due to the silicon protecting the photosynthetic apparatus (Guntzer *et al.*, 2012) [24]. In case of rice silicon treatment can significantly decreased amylose content under stress condition compared with control (Emami *et al.*, 2014) [33].

The total protein content was higher, where the proper application of fertilization takes place, which is due to source activity in seed (Baxer *et al.,* 2004; Hamaker and Griffin, 1993; Lim *et al.,* 1999; Tan and Corke, 2002; Wu *et al.,* 2009) [26, 27, 28, 29, 30]. Silixol application in plants has the potential that positively affect the total protein content in different rice genotypes. After the application of silicon solubilizer in plants 0.77 to 8.32% increased total protein content was recorded. That can be due to silicon application which directly improved the synthesis of various amino acids and the activity enzymes in plants. In was also reported that in wheat silicon can reduce the decomposition of total proteins at water stress (Ahmed *et al.*, 2011) [31]. Treatment of silicon can cause increasing of amino acid content, specially Asp and total protein content in rice. It was also reported that under silica stress some new proteins can be generated, (Thomas *et al.*, 2013) [12]. In rice crop, soil application of silicon solubilizer granules can increased insoluble protein content, compared with control (no silicon treatment) under drought stress condition (Emam *et al.*, 2014) [33].

Conclusion

Thus results highlight that amongst six genotypes PA-6444 was the most efficient genotype and significantly perform better in field experiment as compared to other genotypes. On the basis of present study overall results showed that, treatment positively influenced all the attributes and ultimately helped to increasing the yield.

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