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Rakesh Sil Sarma
Department of Plant Physiology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Deepti Shankhdhar
Department of Plant Physiology, College of Basic sciences and Huminities, GBPUAT, Pantnagar, Uttarakhand, India

Pallavi Srivastava
Department of Plant Physiology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Shailesh Chandra Shankhdhar
Department of Plant Physiology, College of Basic sciences and Huminities, GBPUAT, Pantnagar, Uttarakhand, India

Correspondence
Rakesh Sil Sarma
Department of Plant Physiology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Influence of silicon solubilizers on Silicon content, chlorophyll content (mg g^{-1}) and photosynthetic efficiency in leaves at three different growth stages in rice genotypes

Rakesh Sil Sarma, Deepti Shankhdhar, Pallavi Srivastava and Shailesh Chandra Shankhdhar

Abstract
A field experiment was conducted in kharif season of 2015 to investigate the influence of silicon solublizer on different rice genotypes namely PA-6129, PA-6201, PA-6444, PHB-71, US-312, and BPT-5204. Soil application of silicon solubilizer was given at the time of maximum tillering, panicle initiation and 50% flowering stage. Different biochemical observation like Silicon content (mg g-1) and chlorophyll content (mg g-1) was taken at severel growth stages like maximum tillering, panicle initiation and 50% flowering. It was found that application of silicon solublizers can increased silicon content in rice leaves in many folds which enhanced the defence and protective mechanism from several biotic and abiotic stresses in rice genotypes. Four fold increase in silicon content was found also in genotype BPT-5204 leaves as compared to control at all the three growth stages. Silicon solublizers treated plants shows significance increased in chlorophyll a, chlorophyll b, and total chlorophyll content in rice leaves. The chlorophyll a content was increased by 1.48% in PA-6444 and chlorophyll b was increased by 21.92% in PA-6129, total chlorophyll content was maximum increase (13.81%) in PA-6201 in treated plants when compared with control. Among all the genotypes and treatments the chlorophyll fluorescence (F_v/F_m) of PA-6201 was significantly increased from rest of the genotypes at control and as well as silicon solublizers treatment.

Keywords: silicon, chlorophyll, photosynthetic, genotypes

Introduction
Rice contributes 42% of total food grains production and 45% of the cereal production. It is the most important crop with respect to human nutrition and calorie intake, providing more than one fifth of the total calories consumed by human population (Li *et al.*, 2014) [14]. Rice was consider as a great silicon accumulator and its shoot silicon content ranges above 10% of culm dry matter, glyceroporins lsi1 and lsi2 helps in assimilation of silicon from silicic acid which are present in soil solution by xylem transport system (Ma *et al.*, 2008) [7]. Due to the application of silicon rice plants become more resistant to fungal disease, and raises the percentage of the filled spikelets and seed yield by increase of cell wall thickness below the cuticle, imparting mechanical resistance to the penetration of fungi, and improvement of the leaf angle, making leaves more erect. In the absence of silicon the rice leaves become soft and droopy which causes mutual shading and reduce the photosynthetic efficiency. It is also reported in many studies that drought stress is mitigated by silicon. In case of rice it has been observed that silicon by regulating the transcription factors *OsNAC5* and *OsDREB2A* modulate the expression of drought stress genes and enhance the rice seedling stress tolerance to drought stress (Khattab *et al.*, 2014) [6]. Silicon application resulted in an increase of chlorophyll and an improvement in the antioxidant system in *Lycopersicon esculentum* plants when exposed to salt stress, but the regulatory mechanism responsible for this effect was not explained (Al-aghabary *et al.*, 2004) [1]. It was investigated that interaction between silicon and nitrogen is reported an increase in the levels of chlorophyll content in *Oryza sativa* plants, silicon proportionately increased the levels of chlorophyll a in the water-deficient cultivars, indicating the synthesis of new pigments and the maintenance photosynthetic machinary (Avila *et al.*, 2010) [2]. Study conducted on *Sorghum bicolor* plants under Si treatment showed increase in chlorophyll a, chlorophyll b and total chlorophyll as compared with non-treated plants, this might be due to improvement in light interception and better performance of the photosynthetic parameters (Tari *et al*., 2013) [12]. Addition of Si to the root growing medium of salt-stressed tomato plants enhanced Fv/Fm as well as improved the photochemical efficiency

of PSII, this might be due to the increase in the value of F_0/F_m in Si-treated plants under water-deficit conditions (Al-aghabary *et al*., 2004) [1]. It has been also observed that the Si application to *Magnoporthe oryzae* inoculated rice plants significantly increased the ratio of F_v/F_m compared with non-Si-treated infected plants (Gao *et al.*, 2006) [4]. In rice crop silicon are uptake in the form of monosilicic acid, and orthosilicic acid and normally plants uptake 60-250 kg silicon fertilizer/hectare (He *et al.*, 2012) [5]. It was observed that monocots acumulates more silicon in leaves compared with dicots approximately 2% less in root dry weights (Ma *et al*., 2011) [8]. Silicic acid (0.1-0.6 mM) occurs as one of the main constituents of soil solution and it can be regarded as a plant nutrient (Epstein *et al*., 1999) [3]. Monocots such as rice, wheat, maize and barley are categorized as Si accumulators due their very high silica contents (10–15%).

Materials and Methods

The field experiment was carried out at Norman E. Borolaug Crop Research Centre & Department of Plant Physiology, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Udham Singh Nagar (Uttarakhand) during kharif season of 2015 for the purpose of studying the influence of soil application of silicon solublizers on growth, biochemical and photosynthetic efficiency in different genotypes of rice (*Oryza sativa* L.).

Chlorophyll content

Chlorophyll content was determined in fresh flag leaves at flowering by using method described by Hiscox *et al*. (1979) [13]. Chlorophyll content was estimated in freshly harvested leaves at active tillering and flowering by DMSO method. 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^{0}C 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

Photosynthetic efficiency (F_v/F_m)

The influence of silicon solubilizer treatment on chlorophyll fluorescence (F_v/F_m) at the time of 50% flowering in flag leaves was recorded.

Estimation of silicon content:

Silicon content of rice leaves was estimated by the method described by Weimin *et al*. (2005) [11].

Setting up standard curve

Sodium silicate was used for preparing standard solution.1 ml sodium silicate solution was used for 0.1 mg silicon. Take 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 ml silicon standard solution to a 50 ml volumetric flask respectively. Add 30 ml acetic acid (20%) and 10 ml ammonium molybdate solution (54 g/litre, pH 7.0).Kept for 5 min and then add 5 ml 20% tartaric acid and 1ml reducing solution. Adjust to 50 ml, with 20% acetic acid. Stored in a tightly stopered plastic bottle in the dark. 30 minutes later, measure the absorbance at 650nm

Sample preparation

Leaves were collected from each replicate and dried in oven at 70 ^{0}C for 7 days. Each sample was grind and sifted through a 60-mesh sieve. Collected leaves powder (100mg) for silicon estimation

Sample pretreatment for analysis

Put 100mg sample into a 100ml polyethylene tube. Add 3ml 50% NaOH and cover it with a loose-fitting plastic cap. Gently vortex, then autoclave at 1210c for 20 min. Transfer to volumetric flask and adjust to 50ml with ddH2O

Sample determination

Transfer 1ml sample solution to a 50ml volumetric flask. Add 30 ml 20% acetic acid and 10 ml ammonium molybdate solution (54g/litre, pH 7.0), shake up to mix thoroughly. Keep for 5 min after that immediately add 5ml 20% tartaric acid and 1ml reducing solution. Adjust to 50ml with 20% acetic acid. After 30 minutes later, measure the absorbance at 650 nm.

Result and Discussion

Chlorophyll a content (mg g^{-1} fresh weight): The influence of silicon solubilizer on chlorophyll content in flag leaf of all genotypes at the time of 50% flowering was evaluated. PA-6444 (1.51 mg g^{-1}fr.wt.) showed maximum chlorophyll a content and the minimum in PA-6201 (1.42 mg g^{-1}fr.wt.) under silicon solubilizer. However silicon solubilizer showed maximum increase (7.82%) in BPT-5204 and minimum (0.68%) in US-312 where compared to control. Among all the genotypes and treatments the chlorophyll a content of PA-6129, PA-6444, BPT-5204, PHB-71 was found statistically significant with respect to all the genotypes at control and as well as solid treatment.

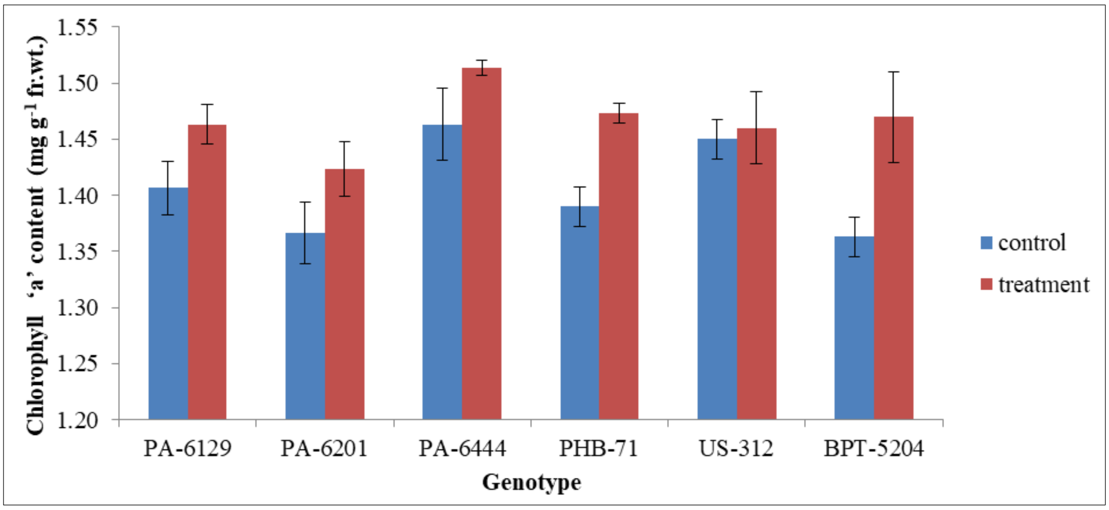

Fig 1: Effect of silicon solubilizer on chlorophyll 'a' at 50% flowering stage in different rice genotypes and vertical bars indicate ± standard error of mean

Chlorophyll b content (mg g^{-1} fresh weight): silicon solubilizer US-312 (0.54 mg g^{-1}) showed maximum chlorophyll b content and the minimum in PA-6129 (0.46 mg g^{-1}). The interaction between Treatment and genotypes was found statistically non-significant with respect to almost all the genotypes. Among all the genotypes and treatments the chlorophyll b content of, PA-6444,, PHB-71, US-312 was found statistically significant with respect to all the genotypes at control and as well as solid treatment.

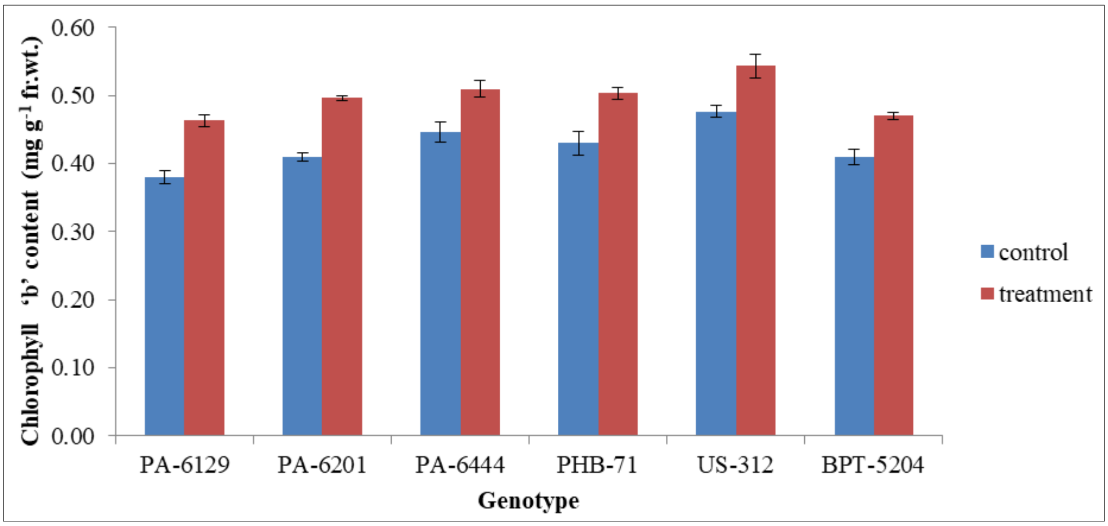

Fig 2: Effect of silicon solubilizer on chlorophyll 'b' at 50% flowering stage in different rice genotypes and vertical bars indicate ± standard error of mean

Table 1: Effect of silicon application on chlorophyll 'a' and 'b' content (mg g^{-1} F.W) in the flag leaf at 50% flowering in different rice genotypes

Name of the rice genotypes	Chlorophyll 'a' content (mg g^{-1} fr.wt.) at 50% flowering				Chlorophyll 'b' content (mg g^{-1} fr.wt.) at 50% flowering			
	Control	Silicon solubilizer treatment	Mean	% increase	Control	Silicon solubilizer treatment	Mean	% increase
PA-6129	1.40±0.02	1.46±0.01	1.43	4.02	0.38±0.01	0.46±0.00	0.42	21.92
PA-6201	1.36±0.02	1.42±0.02	1.39	4.14	0.41±0.00	0.49±0.00	0.45	21.13
PA-6444	1.46±0.03	1.51±0.00	1.48	3.41	0.44±0.01	0.51±0.01	0.47	14.17
PHB-71	1.39±0.01	1.47±0.00	1.43	5.99	0.43±0.01	0.50±0.00	0.46	17.05
US-312	1.45±0.01	1.46±0.03	1.45	0.68	0.47±0.00	0.54±0.01	0.51	13.98
BPT-5204	1.36±0.01	1.47±0.04	1.41	7.82	0.41±0.01	0.47±0.00	0.44	14.63
Mean	1.40	1.46			0.42	0.49		
	Genotype (G)	Treatment(T)		TxV	Genotype (G)	Treatment (T)		TxV
S.Em. ±	0.009	0.01		0.022	0.004	0.007		0.01
CD at 5%	0.026	0.046		0.066	0.013	0.022		0.032

Total chlorophyll content (mg g^{-1} fresh weight): PA-6444 (2.15 mg g^{-1}) showed maximum total chlorophyll content and the minimum in PA-6201 (1.98 mg g^{-1}) under silicon solubilizer treatment. However silicon solubilizer treatment showed maximum increase (13.81%) in PA-6201 and minimum (0.59%) in BPT-5204 where compared to control

Among all the genotypes and treatments the total chlorophyll content of PA-6201 was significantly increased from rest of the genotypes at control and as well as solid treatment. The silicon solubilizer treated plants led to increase the chlorophyll content might be due to silicon deposition on cuticles epidermal cells. Silicon have beneficial effects on plant photosynthesis and it was positively correlated with leaf chlorophyll content. After application of silicon solubilizer, silica content in cuticle was increased then less water is exits from the stomata, water retention capacity increased in plants, leaf surface area is significantly increased for better absorption of solar radiation in leaves which increased active chlorophyll content as well as photosynthesis. Silixol in concentration of 0.25, 1.00 and 1.75mM can induced progressive increased in chlorophyll a, chlorophyll b, as well as total chlorophyll in rice (Liu *et al.*, 2014) [14].

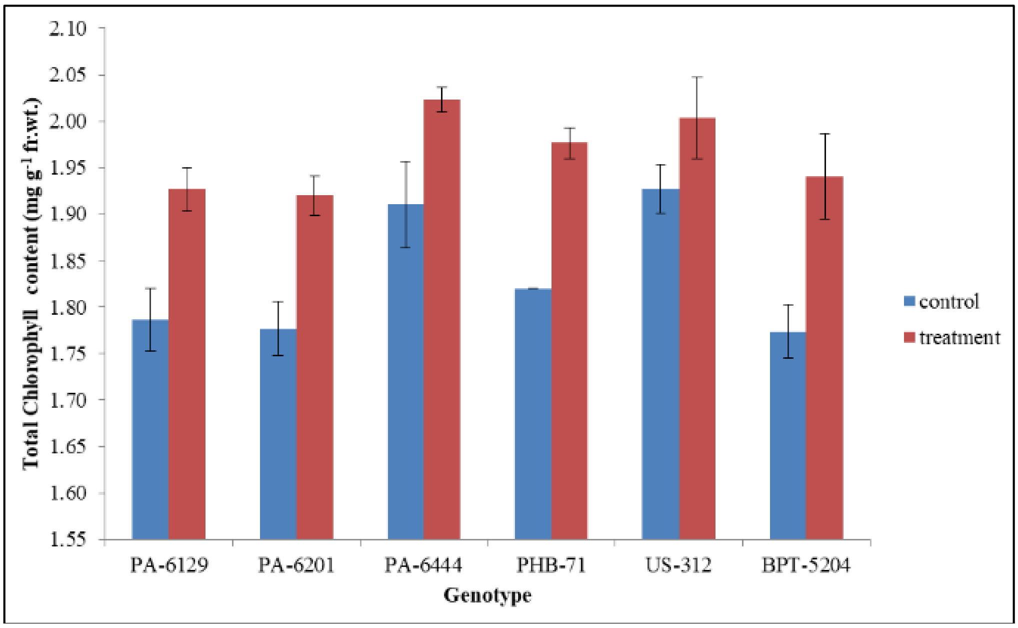

Fig 3: Effect of silicon solubilizer treatment on total chlorophyll content at 50% flowering stage in different rice genotypes and vertical bars indicate ± standard error of mean

Photosynthetic efficiency (F_v/F_m)

The influence of silicon solubilizer treatment on chlorophyll fluorescence (F_v/F_m) at the time of 50% flowering in flag leaves was recorded. PHB-71 (0.767) showed maximum chlorophyll fluorescence (F_v/F_m) and the minimum in BPT-5204 (0.737) under silicon solubilizer treatment. However silicon solubilizer treatment showed maximum increase (13.81%) in PA-6201 and minimum (0.59%) in BPT-5204 where compared to control. In silicon solubilizer treatment 0.59 to13.81% increased in chlorophyll fluorescence (F_v/F_m) as compared with control. This might be due to silicon increases leaf surface area of plants, thus enhance photosynthetic activity. In maize application of silicon fertilizers can improved chlorophyll fluorescence (F_v/F_m) by 22.2% as compared to control (no silicon treatment) (Mitani *et al.*, 2009) [10]

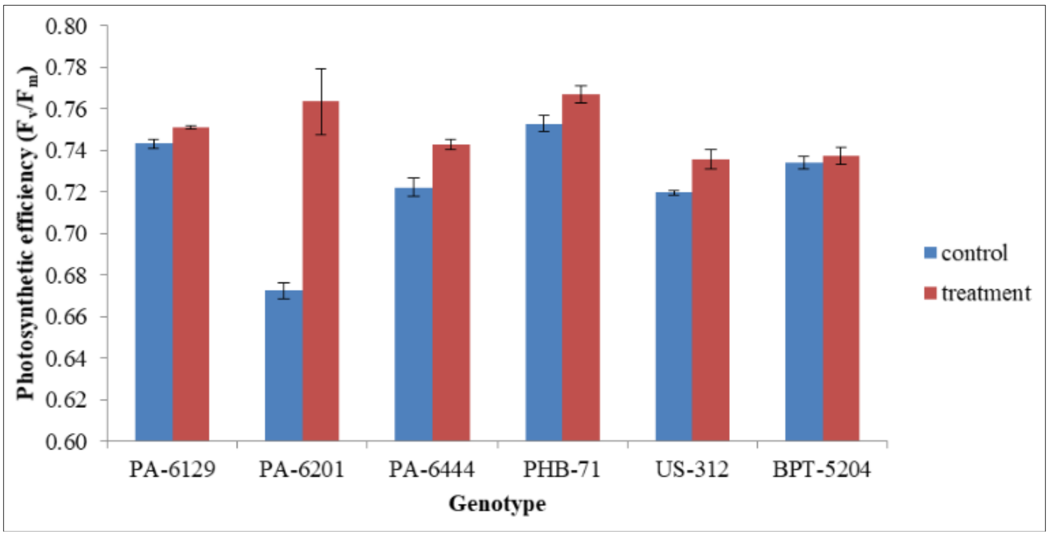

Table 2: Effect of foliar spray of silicon solubilizers on total chlorophyll content (mg g^{-1} F.W) in the flag leaf and photosynthetic efficiency (F_v/F_m) at flowering in different rice genotypes.

Name of the rice genotypes	Total Chlorophyll content (mg g^{-1} fr.wt.) at flowering				Photosynthetic efficiency (F_v/F_m)at flowering			
	Control	Solid silicon solubilizer treatment	Mean	% increase	Control	Solid silicon solubilizer treatment	Mean	% increase
PA-6129	1.83±0.002	1.98±0.000	1.85	00.71	0.743±0.002	0.751±0.000	0.747	00.71
PA-6201	1.85±0.003	1.98±0.015	1.84	13.81	0.672±0.003	0.763±0.015	0.718	13.81
PA-6444	1.97±0.004	2.15±0.002	1.96	01.64	0.722±0.004	0.743±0.002	0.732	01.64
PHB-71	1.92±0.003	2.08±0.004	1.89	01.72	0.753±0.003	0.767±0.004	0.760	01.72
US-312	1.99±0.000	2.10±0.004	1.96	02.46	0.719±0.000	0.735±0.004	0.727	02.46
BPT-5204	1.83±0.002	2.12±0.004	1.85	00.59	0.734±0.002	0.737±0.004	0.735	00.59
Mean	1.89	2.06			0.724	0.749		
	Genotype (G)	**Treatment (T)**		**TxV**	**Genotype (G)**	**Treatment (T)**		**TxV**
S.Em. ±	0.011	0.019		0.028	0.002	0.003		0.005
CD at 5%	0.033	0.058		0.082	0.006	0.011		0.015

Silicon content (mg g^{-1}) in leaves at maximum tillering, panicle initiation, 50% flowering: In maximum tillering, BPT-5204 (30.20 mgg^{-1}) showed maximum silicon content and the minimum in US-312 (17.19) under solid treatment. However silicon solubilizer showed maximum increase (287%) in BPT-5204 and minimum (21.73%) in PHB-71 where compared to control. Among all the rice genotypes and silicon treatment the silicon content of BPT-5204 (4 fold), PA-6444 (3 fold), PA-6201 (2 fold), had a significantly increased from all other genotypes at maximum tillering. In panicle initiation, The silicon content of leaves was recorded maximum in rice genotypes PA-6201 (34.96) and minimum in US-312 (22.56) at silicon solubilizer treatment. However silicon solubilizer showed maximum increase (324.16%) in BPT-5204 and minimum (6.51%) in PHB-71 where compared to control. Among all the rice genotypes and silicon treatment the silicon content of BPT-5204 (4 fold), PA-6444 (3 fold), US-312 (3fold), had significantly increased from all other genotypes at panicle initiation.At 50% flowering, genotype BPT-5204 (41.10) was recorded maximum silicon content in leaves and minimum in PHB-71 (25.29) at silicon solubilizer treatment. However silicon solubilizer showed maximum increase (347.04%) in BPT-5204 and minimum (2.27%) in PHB-71 where compared to control. In silicon solubilizer silicon content in leaves was highly increased which ranges from 21.73 to 287.74% at maximum tillering, 6.51 to 324.16% at panicle initiation and 2.27 to 347.04% at 50% flowering stages as compare to control was observed.). In rice it was found that crop accumulate silica behave the range 0.1–15% of their dry weight. The degree of accumulation depends on uptake and transport mechanisms in plant leaves (Ma *et al.*, 2015) [9]. The uptake and distribution of silicic acid by plants is increased many folds by the presence of different type of influx and efflux transporters in leaves (Ma and Yamaji, 2011) [8].

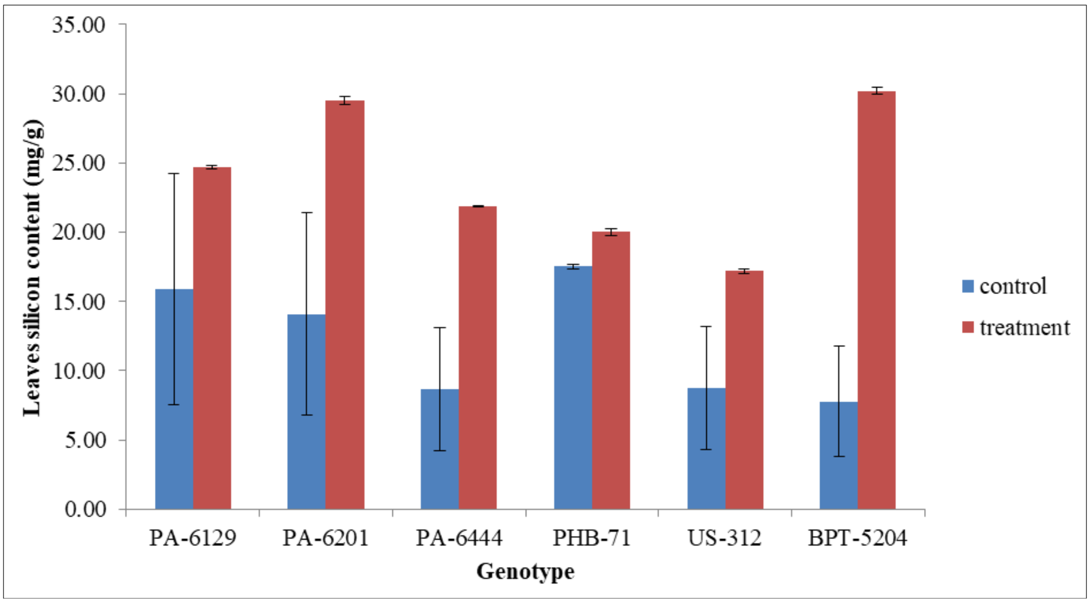

Fig 5: silicon content at maximum tillering stage in different rice genotypes and vertical bars indicate ± standard error of mean

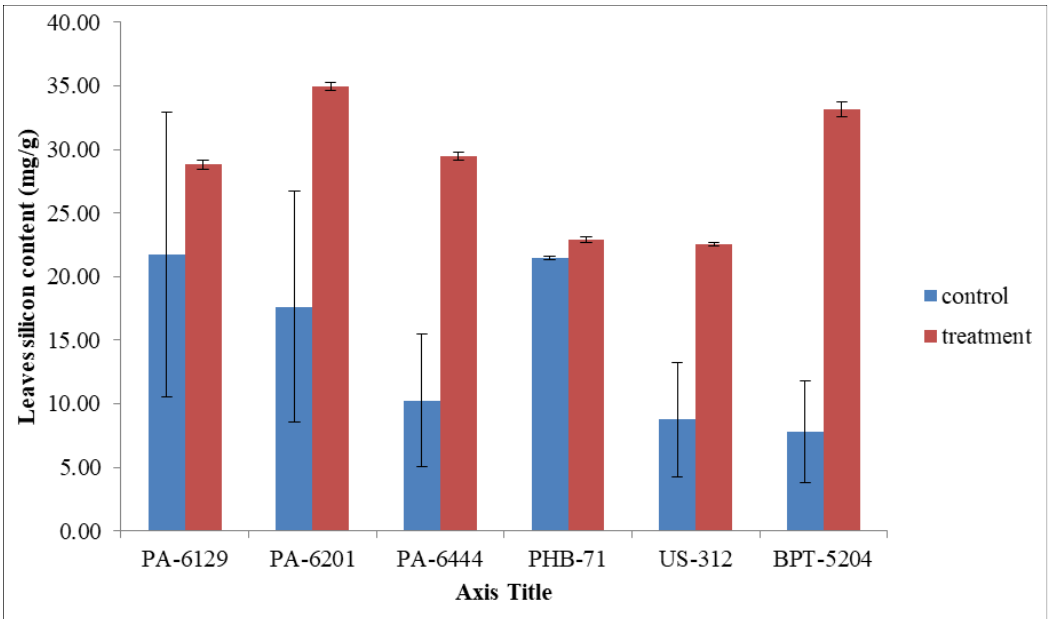

Fig 6: silicon content at panicle initiation stage in different rice genotypes and vertical bars indicate ± standard error of mean

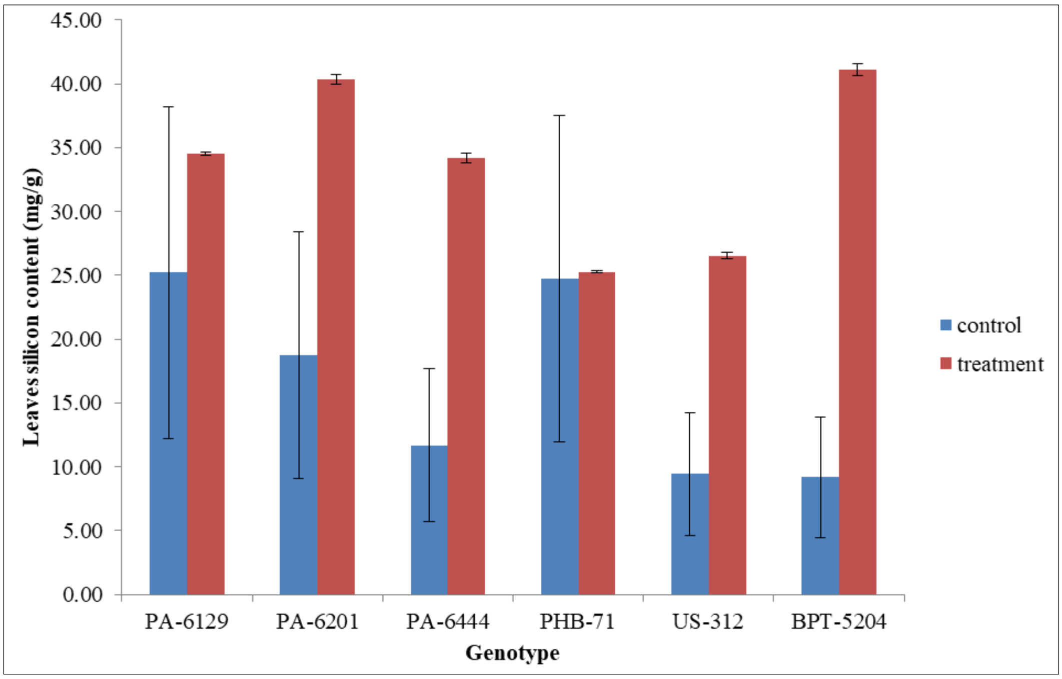

Fig 7: Effect of silicon solubilizer on leaves silicon content at 50% flowering stage in different rice genotypes and vertical bars indicate ± standard error of mean

Table 3: Effect of silicon application on silicon content in leaves at maximum tillering, panicle initiation and at 50% flowering in different rice genotypes.

Name of the rice genotypes	Silicon content in leaves at tillering(mg/g)				Silicon content in leaves at panicle initiation(mg/g)				Silicon content in leaves at 50% flowering(mg/g)			
	Control	Silicon solubilizer treatment	Mean	% increase	Control	Silicon solubilizer treatment	Mean	% increase	Control	Silicon solubilizer treatment	Mean	% increase
PA-6129	15.89±0.17	24.73±0.15	20.31	55.59	21.70±0.16	28.80±0.31	25.25	32.70	25.22±0.28	34.55±0.13	29.88	36.97
PA-6201	14.10±0.10	29.45±0.31	21.77	108.89	17.64±0.21	34.96±0.32	26.30	98.19	18.76±0.18	40.33±0.36	29.55	114.97
PA-6444	8.63±0.36	21.83±0.05	15.23	152.92	10.24±0.08	29.49±0.29	19.86	187.93	11.68±0.08	34.21±0.40	22.94	192.78
PHB-71	17.52±0.24	20.01±0.16	18.77	21.73	21.47±0.21	22.92.±0.11	22.20	6.51	24.73±0.18	25.29±0.10	25.01	2.27
US-312	8.74±0.08	17.19±0.17	12.96	96.57	8.75±0.09	22.56±0.14	15.65	158.03	9.43±0.05	26.55±0.28	17.99	181.34
BPT-5204	7.79±0.05	30.20±0.24	18.99	287.74	7.82±0.13	33.20±0.58	20.51	324.16	9.19±0.11	41.10±0.47	25.14	347.04
Mean	12.53	23.49			14.84	28.41			16.50	36.67		
	Genotype (G)	Treatment (T)	TxV		Genotype (G)	Treatment (T)	TxV		Genotype (G)	Treatment (T)	TxV	
S.Em. ±	0.07	0.13	0.18		0.10	0.18	0.26		0.10	0.17	0.25	
CD at 5%	0.22	0.38	0.54		0.32	0.55	0.78		0.30	0.52	0.73	

Conclusion

The silicon content in leaves was significantly affected by soli application of silicon solublizers at three different growth stages in plants. In solid treatment the silicon content in leaves was significantly 4 fold increase in rice genotype BPT-5204 as compared to control at three stages. The overall result was showed that the overall mean of treatment showed the maximum total silicon content for most of the genotypes at solid treatment. Increased silicon content in plants is directly related to improvement of lodging, biotic and abiotic stress resistance in severe adverse condition in climate. Chlorophyll content also significantly increased in different rice genotypes under silicon solublizer treatment, photosynthetic efficiency, chlorophyll fluorescence (F_v/F_m) shows positive response under soil application of silicon solublizers. Hence by using the silicon solublizers one would expect to raise the yield many fold, decreased insect, disease infestation of rice and fulfilling the demand of overgrowing population in the up coming years.

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