



# Assessing the Impact of Co-fertilization of Silicon with Macronutrient Fertilizers on Yield, Nutrient Uptake, use Efficiency and Grain Quality of Rice in Sandy Clay Loam Soil

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## Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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## ABSTRACT

This study wanted to clarify the impact of silicon (Si) fertilization on nutrient uptake, silicon use efficiency, yield and quality of rice and to detect the rate and mode of application of Si fertilizer in silicon deficient soil. The field experiment was conducted with ten treatment combinations of 100% NPK, 100% NPK + potassium silicate (PS) @ 0.25%, 0.50% and 1.0% Foliar spray (FS) at tillering stage, 100% NPK + PS @ 0.25%, 0.50% and 1.0% FS at tillering and panicle initiation stages, 100% NPK + PS @ 50, 100 and 150 kg Si ha<sup>-1</sup> soil application (SA). Application of 50 kg Si ha<sup>-1</sup> through

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PS recorded the highest grain (6183 kg ha<sup>-1</sup>) and straw (6740 kg ha<sup>-1</sup>) yield, and higher uptake of macronutrients (NP&K) in rice and it was on par with foliar spray of 1% Si sprayed at tillering stage. Silicon uptake increased linearly with Si levels and the maximum uptake was recorded with 150 kg Si ha<sup>-1</sup> and foliar spray at 1% Si. Foliar spray recorded higher Si use efficiency compared to soil application. Rice grain quality viz., protein, amylose and carbohydrate were significantly influenced by silicon addition. The results proved that both foliar and soil application of Si through potassium silicate in sandy clay loam soil could enhance the rice yield, nutrient uptake and grain quality by means of improving nutrient use efficiency.

*Keywords: Amylase; potassium silicate; silicon use efficiency; sandy clay loam; true protein content; yield.*

## 1. INTRODUCTION

Soil is definitely the most important resources among the natural resources as it performs incredibly vital functions such as environmental, social and economic in nature [1,2]. As it regulates water, recycling raw materials, habitat for soil organisms, and so on which directly and or indirectly connect with crop production i.e. soil quality and it may consider as the ability of a soil to produce safe and nutritious crops in sustained manner over the long run, without impairing the resource base [3]. But the modern farming grown with high yielding varieties has led to rise of multiple nutrient deficiencies owing to heavy withdrawal of nutrients by crops without replenishing [4] and ignoring the soil resilience [5] leads to low productivity, reduced nutritional quality of agricultural produces and malnutrition in animal /human [6]. As soils are major sources of nutrients for plant productivity. Plants absorb the desired nutrients from soil in various levels as per requirements. Due to absorption and utilization of macro and micro nutrients by crops when monocropping system is followed especially rice soils. Apart from multinutrient deficiency single nutrient deficiency have been reported in different soils of the world, including India [7]. Silicon increases resistance to lodging and drought in rice and also Si positively affect the activity of certain photosynthetic enzymes, increases crop growth and development, and improves availability of applied nutrients [8] whereas Si deficiency makes rice plants susceptible to pest and diseases. In general, tropical and sub-tropical soils are associated with low plant available silicon [9]. Rice crop demand more silicon for its growth, and Ma and Takahashi [10] reported that for every 100 kg brown rice, 20 kg SiO<sub>2</sub> ha<sup>-1</sup> is removed. Soils of tropical and sub-tropical regions are considered low in plant available silicon and crops grown in these soils respond to silicon fertilization significantly [9]. Silicon is present in different

forms either alone or in complex with organic and inorganic constituents, however the plant available Si used by crops have primary influence on growth. Mitani and Ma [11] have observed that rice is greater accumulator of silicon owing to prolific nature of rice roots to extract silicon from soil and hence silicon is beneficial element for cereal crops more so for rice [12]. Ma [13] and Ahmad et al. [14] reported that "besides N, P and K, silicon plays an important role in balancing the macronutrients in rice cultivation to improve the quality and yield. Silicon plays a significant role in not only promoting growth and yield of crops, but also improves nutrient availability and helps to mitigate biotic and abiotic stresses in several crops". "In the soil solution, Si is present as silicic acid (mono and or poly) as well as complexes with organic and inorganic compounds. The plant available silicon (PAS) taken up by the plants and has a direct influence on crop growth. Beneficial nutrients (BNs) are equally significant as macronutrients [14], and balancing these beneficial nutrients in rice cultivation can enhance the yield and quality" [13]. "Silicon plays an important role in the nutrient availability in soil and uptake by plants. Further, silicon is the only one non-essential element that is included in the guidelines for rice fertilization, besides all plant-essential nutrients already have established fertilization programs for rice crop" [15]. "Besides rice yield increase, Si has many fold advantages of increasing nutrient availability (N, P, K, Ca, Mg, S, Zn), decreases nutrient toxicity (Fe, Mn, P, Al), minimizing stress (biotic and abiotic) in plants, and improving fertilizer use efficiency" [16]. "Si fertilizer has been used in many countries for improving rice productivity" [17]. Considering the role of Si to rice grain yield and quality especially "reduces the chalkiness" [18], Si is now considered as an important determinant in rice production [19-21]. Recent studies shown that effect of applying Si fertilizers on rice productivity without clarifying the soil

silicon supply in different soil types especially different textural classes. Thus, it is necessary to understand the response of rice to Si fertilizers and establish an optimized silicon fertilizer and application mode at critical stages. Till now, scanty information available on soil Si supply level and suitable Si fertilizer application rate for different soils. Hence, this study was conducted to identify the influence of co-fertilization silicon with macronutrient fertilizers in sandy clay loam textured soil on rice yield, nutrient uptake, and quality and silicon use efficiency (SUE) especially to find out the potassium silicate fertilizer rate and suitable mode application for enhancing nutrient use efficiency.

## 2. MATERIALS AND METHODS

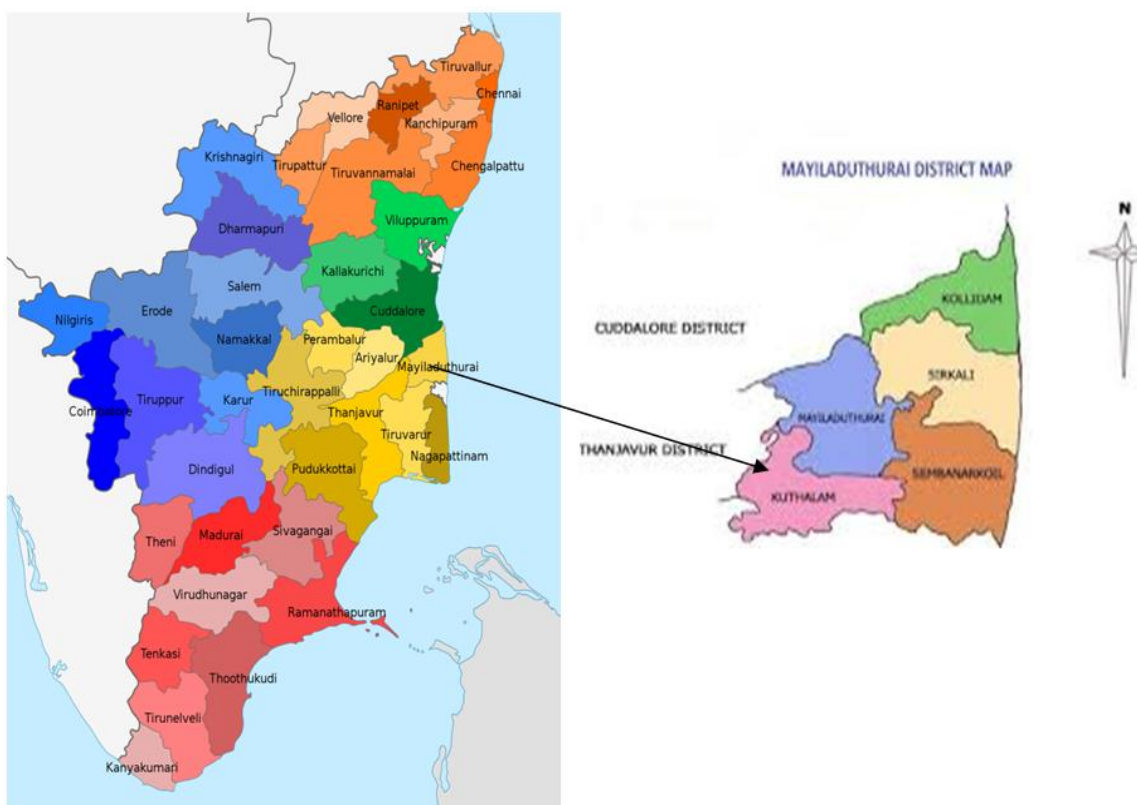
### 2.1 Experimental Site

The field experiment was conducted in the farmer's holding located at Rajagopalapuram village under Kuttalam taluk, Mayiladuthurai district, Tamilnadu, India. The experimental field is geographically situated 11.10°N and 79.67°E at an altitude of 16 m above mean sea level. The

experimental soil identified as sandy clay loam soil belonging to Padugai series (Typic Ustifluvent). When initial soil was analysed and found that the soil having pH-7.25, EC-0.15 dSm<sup>-1</sup>, soil organic carbon-3.5 g kg<sup>-1</sup>, KMnO<sub>4</sub>-N-265 kg ha<sup>-1</sup>, Olsen P- 18.4 kg ha<sup>-1</sup>, NH<sub>4</sub>OAc-K-228 kg ha<sup>-1</sup> and available silicon- 25 mg kg<sup>-1</sup>.

### 2.2 Treatment Structure and Design

The treatment structure includes T<sub>1</sub> - NPK (RDF), T<sub>2</sub> - NPK + Potassium silicate (FS) - 0.25%, T<sub>3</sub>- NPK + Potassium silicate (FS) - 0.50%, T<sub>4</sub> - NPK + Potassium silicate (FS) - 1.00%, T<sub>5</sub>-NPK + Potassium silicate (FS) - 0.25%, T<sub>6</sub> -NPK + Potassium silicate (FS) - 0.50%, T<sub>7</sub>- NPK + Potassium silicate (FS) - 1.00%, T<sub>8</sub> - NPK + Potassium silicate (SA) - 50 kg ha<sup>-1</sup>, T<sub>9</sub> - NPK + Potassium silicate (SA) - 100 kg ha<sup>-1</sup> and T<sub>10</sub>- NPK + Potassium silicate (SA) – 150 kg ha<sup>-1</sup>. From T<sub>2</sub> to T<sub>4</sub> foliar spray was done at tillering stage and from T<sub>5</sub> to T<sub>7</sub>, foliar spray was done at tillering and panicle initiation stage. The field experiment was conducted in randomized block design (RBD) design with three replications.



Map 1. Location of the study area (11.10°N and 79.67°E at an altitude of 16 m amsl)

## 2.3 Crop Husbandry

The main field was puddled rice to bring the soil to a satisfactory colloidal condition. After levelling, the experimental plots were laid out as per the specification of plot size (5 m x 4m), treatments and replications. The bunds of the plots were raised and strengthened in between the replications to avoid seepage of water nutrients from one plot to another. Rice crop var. ADT 43 of twenty-five days old seedlings transplanted in the main field. Transplanting was done at the rate of two seedlings hill<sup>-1</sup>. The spacing adopted was 15 x 10 cm. Gap filling was done at 7(days after transplanting (DAT) with seedlings of same age. The recommended dose of 150:50:50 N, P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O ha<sup>-1</sup> through urea, superphosphate and muriate of potash was added uniformly to all the plots. The potassium silicate contains 22 per cent Si and 11 per cent K<sub>2</sub>O. Foliar and soil application of silicon was applied as per the treatment schedule and amount of potassium silicate based on treatment was calculated. The amount of potassium supplied through potassium silicate was taken out while applying in recommended dose of potassium to all the plots.

## 2.4 Data Collection and Analysis

Grain and straw yield was recorded separately from each plot and expressed as kg ha<sup>-1</sup> at harvest. Grain and straw samples were analysed for nitrogen by wet digestion of 0.5 g plant material with 12 ml of diacid mixture (H<sub>2</sub>SO<sub>4</sub>:HClO<sub>4</sub>-5:2) [22], phosphorus by Vanadomolybdate phosphoric yellow colour method [23], potassium by flame photometric method [23], and silicon [24] content and respective nutrient uptake was computed. Grain samples were analysed for its quality viz., Carbohydrate [25], Amylose [26] and true protein [27]. The data were subjected to anova analysis using SPSS version 28.0.0.0 (190) and wherever the treatment differences were found significant (F test), the critical differences were worked out at five per cent probability level (p=0.05) and the values are furnished. Treatment differences which were not significant are denoted as "NS".

## 3. RESULTS AND DISCUSSION

### 3.1 Rice Yield

Perusal of data furnished in Fig. 1 showed significant impact of graded dose of foliar and soil application of silicon through potassium

silicate on grain and straw yield of rice variety ADT-43 over control. The grain and straw yield ranged from 5283 to 6183 kg ha<sup>-1</sup> and 5653 to 6740 kg ha<sup>-1</sup>, respectively. Soil application of 50 kg Si ha<sup>-1</sup> recorded the highest grain and straw yield of 6183 and 6740 kg ha<sup>-1</sup>, respectively. Foliar spray of silicon increased the grain yield from 5530 to 6133 kg ha<sup>-1</sup> and straw yield from 6028 to 6747 kg ha<sup>-1</sup> over control (5283 and 5653 kg ha<sup>-1</sup>). Twice foliar spray of silicon recorded higher rice yield compared to single spray except foliar spray @ 1%. Among the foliar spray treatments, application @ 0.5 and 0.25% twice recorded higher rice yield compared to their single spray significantly. However, when 1.0 % Si foliar spray was applied twice, it reduced the yield. Soil application of 50 kg Si ha<sup>-1</sup> was comparable with foliar spray of Si @ 1% applied at tillering stage. Per cent increase in grain yield ranged from 4.7 to 17 and straw yield ranged from 6.6 to 19.4 due to various treatment and the highest per cent increase in grain yield was noticed with soil application of 50 kg Si ha<sup>-1</sup> (17.0) followed by single spray @ 1% Si applied at tillering stage (16.1). The lowest response was noticed with foliar spray of Si @ 0.25 per cent applied at tillering stage. Nitrogen fertilization can influence rice to response to silicon fertilizer, Ke et al. [28] reported that ample supply of nitrogen can augment rice yield by responding to silicon fertilization. In the present study, silicon application either through soil or foliar was accompanied by recommended dose of N fertilizer. This has improved N status of the soil and nitrogen uptake in rice. The practical increase in grain yield with silicon application to plants would have the potential to enhance the roots adsorptive power and uptake of nutrients [29]. Fertilizer application methods used in different crops has very large effect on Si uptake. When silicon in soil solutions is more (soluble Si), the plant content of the element is generally higher [29]. Drymatter production (DMP) is linearly related to plant Si uptake. In the current study, addition of Si either through soil or foliar enhanced both in leaf and soil Si concentration which would have contributed to higher grain yield. This was supported by significant positive correlation between grain yield with Si content ( $r = 0.621^{**}$ ), Si uptake ( $r = 0.687^{**}$ ) and soil available Si ( $r = 0.78^{**}$ ). This was further confirmed by regression equation (Fig. 2) where grain silicon content accounted 69.2 % variation in grain yield and Si uptake accounted for 38.3 %, 45.6 % and 49.5 % variation in grain yield at tillering, panicle and harvest stage, respectively. The improvement in

rice yield (grain and straw) due to silicon might be attributed to enhancing the pollen viability and photosynthetic activity [30] and improving nutrient uptake [31-33]. In the present study, higher uptake of nutrients by grain and straw was witnessed due to application of silicon. Prakash et al. [34] reported increase in rice yield on additions of calcium silicate (CS) @ 3 - 4 t ha<sup>-1</sup> in coastal and hilly soils of Karnataka. Yogendra et al. [35], Jawahar et al. [36], Nagula et al. [37], Malav et al. [38] and Ali et al. [39] opined that application of silicon alone produced the highest yield in rice. Foliar spray at different concentration increased the grain yield. It is likely to play active role in biochemical processes of the plant. Okamoto [40] and Hooda and Srivastava [41] reported that spraying soluble Si @ 0.1 to 0.2 mg lit<sup>-1</sup> solution as sodium silicate or 1% soluble as sodium silicate to rice plant increased plant growth and yield, and the effect was attributed to a condensed rate of transpiration. Mobasser et al. [42] and Ghanbari Malidarreh et al. [43] reported increase in rice yield on foliar applied silicon. The reproductive stage in the most affected by absence of silicon with reduction of up to 40 per cent in the number of grain bearing spikelet and 10 per cent on the total number of spikelet panicle<sup>-1</sup> [44]. In the present study, foliar spray of 0.25 and 0.5% potassium silicate sprayed at tillering and panicle initiation stage recorded higher grain yield compared to application of same concentration at tillering stage only. Ahmad et al. [14] reported higher rice yield with 1% Si foliar spray. Si induced modifications in crop growth and yield largely depends on the composition and availability of Si | Si based fertilizers [45] which was proved in the present study that application Si fertilizer both via soil and foliar methods enhanced the growth and yield of rice significantly in sandy clay loam soil.

### 3.2 Macro Nutrient Uptake

Accretion macro nutrients (N, P and K) by rice was appreciably improved on addition of soil and foliar application of silicon over control (Table 1). In all cases, grain and straw accumulated lower N, P, K and Si in RDF alone compared to silicon supplemented treatments. The highest N, P and K uptake was noticed with basal application 50 kg Si ha<sup>-1</sup> and foliar spray at 1% applied at tillering stage. But silicon uptake increased with silicon levels and foliar concentrations. The percent improvement in uptake of nutrients due to silicon fertilization ranged from (10.2 to 49.5,

13.1 to 27.8- nitrogen), (12.1 to 48.6, 42.5 to 99.4 – phosphorus), (3.9 to 37.0, 11.9 to 29.2-potassium) and (17.9 to 61.7, 44.2 to 75.3-silicon) in grain and straw, respectively. Si application potentially enhanced the availability of N in soil, resulting in an enhanced N uptake due to ample N availability [46]. Silicon application enhanced more optimum use of nitrogen applied to rice [47]. Meena et al. [9] reported that with adequate Si, the uptake of N was improved. Silicon increased P content in grain and straw, which caused higher P uptake, which is attributed to enhanced translocation of P from root to shoot. Patra and Neue [48] opined that silicon content of soil solution influenced P concentration in the plant at different stages of crop growth and uptake of P. Singh et al. [46] also reported increase in K concentration and uptake in rice grain and straw on addition of 180 kg Si ha<sup>-1</sup>. The improved uptake of N, P and K in above-ground rice biomass in the present study (Fig. 3) is consistent with earlier findings of Pati et al. [31], Crooks and Prentice [32] and Cuong et al. [33]. The amplified Si uptake with the addition of Si fertilizer might be due to the better Si availability in soil and enhanced root system, which in turn stimulated the plant to uptake more Si [31]. Throughout the crop growth, there was close agreement between the probable uptake of Si and available silicon in soil were recorded. Regression analysis as shown (Fig. 4) showed a great relationship between available silicon with silicon uptake at all stages rice crop growth. It indicated that 78.0, 82.0 and 66.0 per cent variation in silicon uptake in grains at tillering, panicle initiation and harvest stage, respectively was accounted by available silicon in soil.

### 3.3 Silicon Use Efficiency

Nutrient use efficiency has been extensively used as a “measure of capacity of a plant to acquire and utilize nutrients for biological and grain yield” [49,14]. Si has the potential of enhancing the translocation of nutrients within the plants [50]. From the study results we observed that Si fertilization (soil / foliar application) significantly enhanced silicon use efficiency (SUE) (response ratio, physiological efficiency and apparent Si recovery). Foliar applications of silicon recorded higher silicon use efficiency compared to soil application. Response ratio, physiological efficiency (PE) and apparent Si recovery (AR) decreased with Si levels and maximum value was noticed with basal application of PS @ 50 kg Si ha<sup>-1</sup>. Similarly

foliar spray of 0.5% Si at tillering stage recorded higher SUE compared to 0.25 and 1% Si. Further SUE was higher when foliar spray was done at once compared to twice, irrespective of Si concentration (Table 2). Higher SUE in rice could

be due to elevated uptake of silicon in rice grain. Greater nutrient use efficiency at lower level is common because of efficient utilization of nutrients at lower level as reported by Fageria [51].

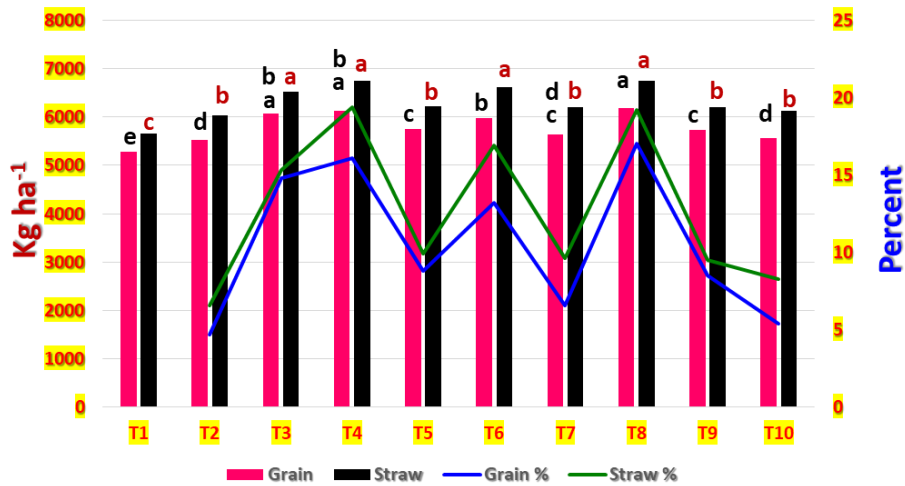


Fig.1 Effect of potassium silicate on rice yield and percent increase over control

Different letters in each treatment and each column show significant difference at P 0.05 by least significant difference (LSD)

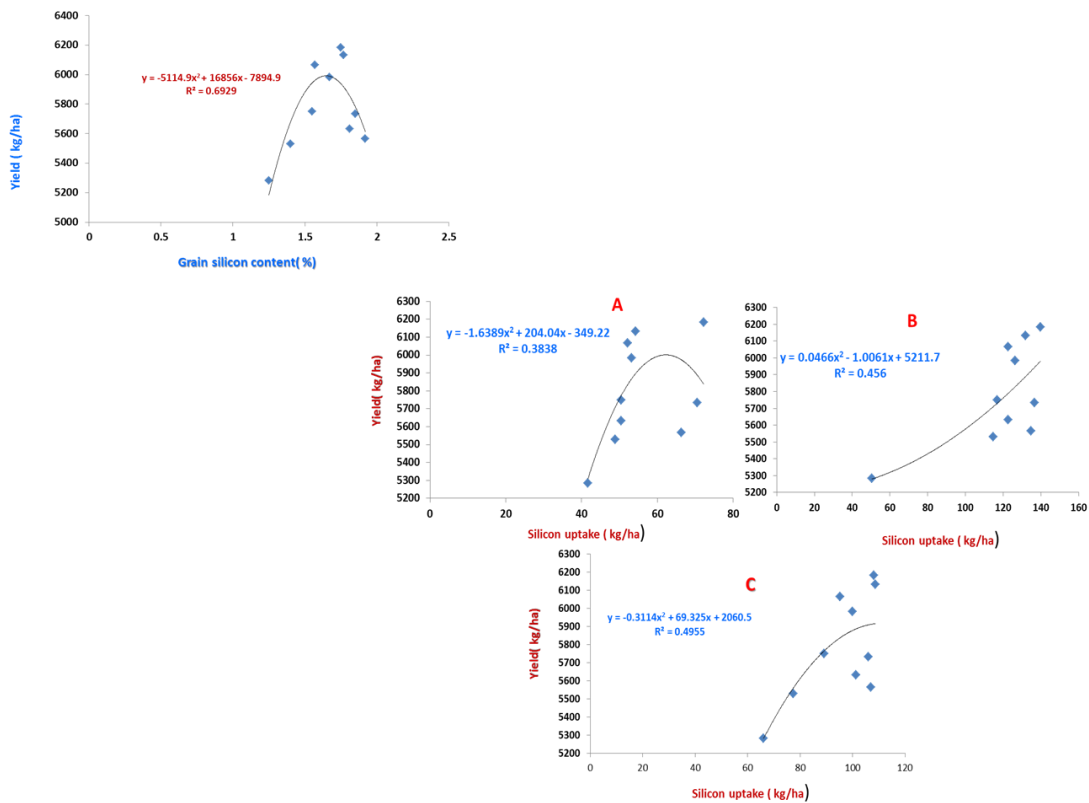


Fig.2. Quadratic relationship between grain yield with grain Si content and with Si uptake at a) Tillering stage b) Panicle initiation c) Harvest

**Table 1. Effect of potassium silicate application on nutrient uptake (kg ha<sup>-1</sup>) by grain and straw**

Treatments	Nitrogen		Phosphorus		Potassium		Silicon	
	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw
T <sub>1</sub> – RDF (Control)	20.6	37.3	7.40	3.39	18.1	39.4	66.1	96.1
T <sub>2</sub> – RDF + PS (FS) - 0.25% @ TS	22.7	42.2	8.29	4.83	18.8	44.1	77.4	138.6
T <sub>3</sub> – RDF + PS (FS) - 0.50% @ TS	26.1	46.9	10.3	5.87	22.0	48.3	95.2	157.2
T <sub>4</sub> – RDF + PS (FS) - 1.0% @ TS	27.6	50.6	11.1	6.74	22.7	50.6	108.6	171.3
T <sub>5</sub> – RDF + PS (FS) - 0.25% @ TS & PI	24.1	43.2	9.08	4.82	20.2	45.3	89.1	144.7
T <sub>6</sub> – RDF + PS (FS) - 0.5% @ TS & PI	26.5	49.2	10.5	6.62	22.1	51.1	99.9	163.3
T <sub>7</sub> – RDF + PS (FS) - 1.0% @ TS & PI	24.2	44.5	8.83	5.57	20.1	45.3	101.2	163.5
T <sub>8</sub> – RDF + PS (SA) - 50 kg ha <sup>-1</sup>	30.8	47.7	11.0	6.76	24.8	50.9	108.2	157.9
T <sub>9</sub> – RDF + PS (SA) - 100 kg ha <sup>-1</sup>	26.9	44.5	9.14	5.47	21.2	47.2	106.1	158.6
T <sub>10</sub> – RDF + PS (SA) - 150 kg ha <sup>-1</sup>	24.5	44.1	8.33	4.90	19.8	47.4	106.9	168.5
SE <sub>d</sub>	2.55	2.19	0.42	0.47	2.14	2.80	1.21	1.38
CD @ 5%	3.36	4.61	0.89	0.98	4.51	5.89	3.58	4.10

[RDF- Recommended dose of fertilizer; PS- Potassium silicate; FS- Foliar spray; SA-Soil application; TS- tillering stage; PI-Panicle initiation; SEd- Standard error deviation ; CD- critical difference]

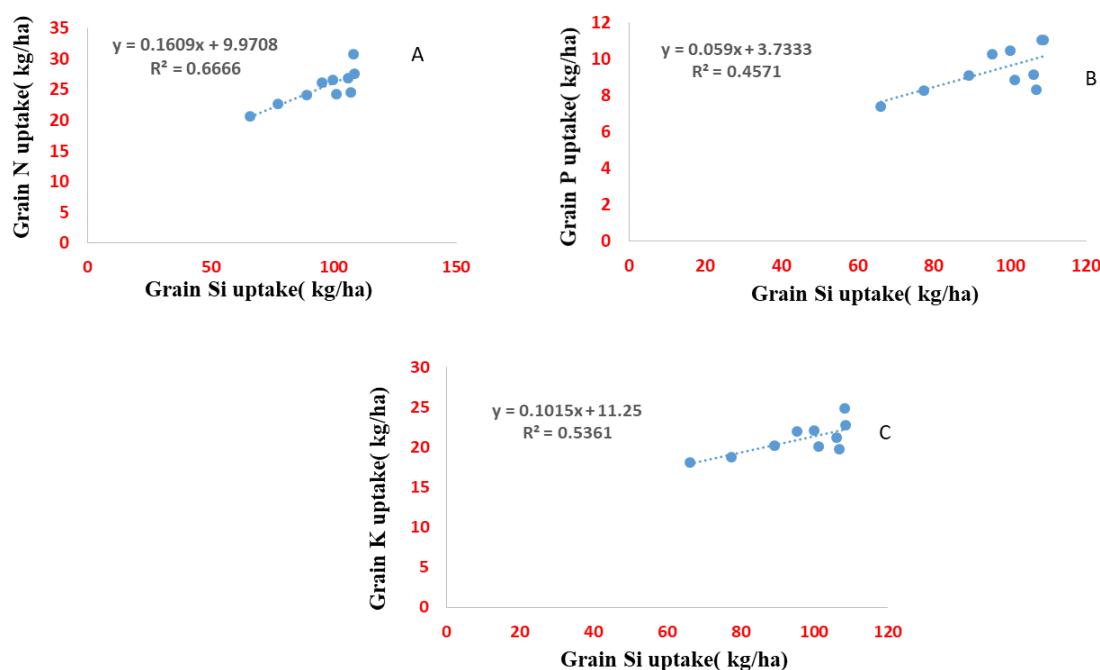


Fig.3. Linear relationship between Si uptake with a) N uptake b) P uptake c) K uptake

**Table 2. Effect of potassium silicate application on silicon use efficiency**

Treatments	Response Ratio (kg kg <sup>-1</sup> )	Apparent Si Recovery (%)	Physiological Efficiency (kg kg <sup>-1</sup> )
T <sub>1</sub> – RDF (Control)	-	-	-
T <sub>2</sub> – RDF + PS (FS) - 0.25% @ TS	98.7	452.0	21.8
T <sub>3</sub> – RDF + PS (FS) - 0.50% @ TS	156.7	582.0	26.91
T <sub>4</sub> – RDF + PS (FS) - 1.0% @ TS	88.30	425.0	20.0
T <sub>5</sub> – RDF + PS (FS) - 0.25% @ TS & PI	110.0	460.0	20.3
T <sub>6</sub> – RDF + PS (FS) - 0.5% @ TS & PI	70.0	338.0	20.7
T <sub>7</sub> – RDF + PS (FS) - 1.0% @ TS & PI	17.5	176.0	9.97
T <sub>8</sub> – RDF + PS (SA) - 50 kg ha <sup>-1</sup>	18.0	84.2	21.4
T <sub>9</sub> – RDF + PS (SA) - 100 kg ha <sup>-1</sup>	4.50	40.0	11.3
T <sub>10</sub> – RDF + PS (SA) - 150 kg ha <sup>-1</sup>	2.17	27.2	6.95
SE <sub>d</sub>	15.5	5.49	0.33
CD @ 5%	32.8	11.63	0.70

[RDF- Recommended dose of fertilizer; PS- Potassium silicate; FS- Foliar spray; SA-Soil application; TS- tillering stage; PI-Panicle initiation; SEd- Standard error deviation ; CD- critical difference]

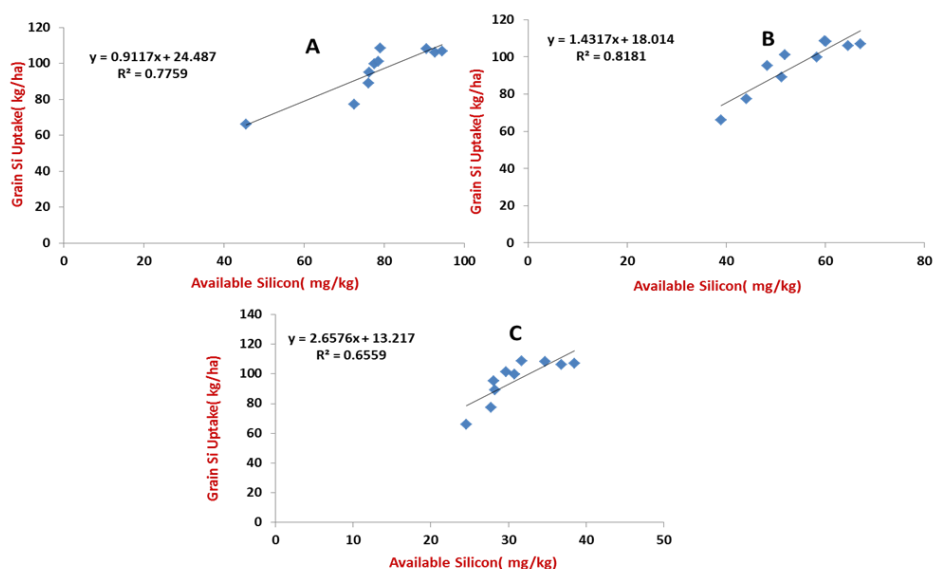


Fig.4. Linear relationship between grain Si uptake with available silicon at a) Tillering b) Panicle initiation c) Harvest

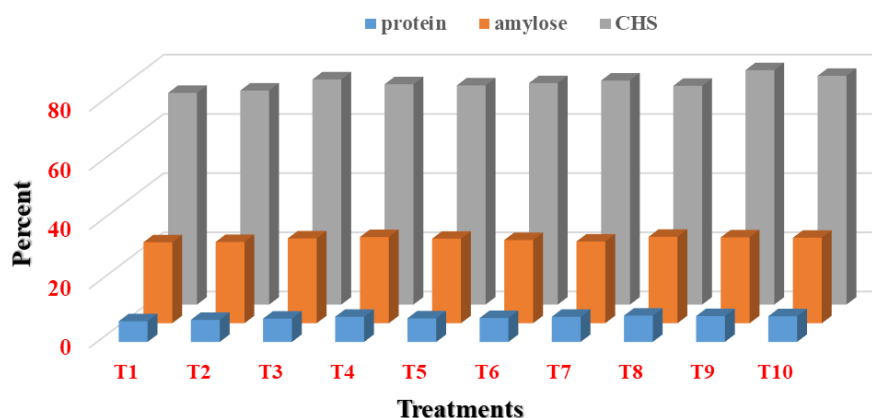


Fig.5. Effect of silicon on rice grain quality

### 3.4 Rice Grain Quality

Silicon supply via potassium silicate (PS) influenced carbohydrates (CHS), amylose and true protein content of rice grain compared to control (Fig. 5). Foliar application of silicon at varied concentration and time interval did not improve carbohydrate content over control. However, soil application of silicon at 100 and 150 kg Si/ha did significantly improved carbohydrate content. With regards to amylose content, increasing rate of soil application of silicon reduced amylose content. Foliar spray of silicon from 0.25 to 1% applied once increased amylose content compared to double spray. Soil

application of silicon netted higher true protein content than foliar spray. However, different levels of silicon had equal effect on true protein. Foliar spray of silicon from 0.25 to 1% increased true protein. The notional improvement in carbohydrate due to silicon and it might be attributed to the increments of photosynthetic pigments thus results in enhancement of carbohydrate synthesis. Manal et al. [52] also reported increase in carbohydrate content of rice grain due to rice seeds pre-treated with silicon. As reported by Miflim and Habash [53] that glutamic-pyruvic transaminase (GPT) is an important enzyme being involved in amino acids and protein synthesis in rice grain. Some reports



confirmed that the nitrogen content in rice grains was increased after applying Si, due to the promotion of transportation efficiency (TE) of N from leaves to grains induced by enhanced activity of GPT in rice leaves [54,31].

Talebi et al. [55] established that exogenous application of potassium silicate had a positive significant effect by increasing soluble protein and carbohydrate contents in the leaves of potato plants. Liu et al. [56] also reported increase in protein concentration in rice grain due to silicon. Ahmad et al. [14] and Jawahar et al. [57] reported increase in amylose content in rice with silicon. Present study results coherence with reports of previous studies that silicon application improved the rice grain quality significantly.

#### 4. CONCLUSIONS

The study shows that rice responded to silicon addition and soil application of 50 kg Si/ha recorded the maximum rice yield and was on par with foliar spray of Si@1% at critical growth stages of rice crop. Also the application of 50 kg Si/ha showed maximum recovery of N, P and K by rice plant. However silicon uptake was higher with 150 kg Si ha<sup>-1</sup> and 1% Si foliar sprays. Silicon use efficiency by rice crop was also benefitted by silicon application. Foliar spray registered higher silicon use efficiency than soil application. Further, silicon application found to be improved the grain quality compared to rice plants not received silicon. However, the optimum Si fertilizer rate both via soil and foliar application at critical stages of rice crop growth in different textured soils should be carried out according to the soil available Si levels for wider adoption of Si co-fertilization with recommended inorganic fertilizers.

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#### DATA AVAILABILITY STATEMENT

The data that support this work are available within the article itself.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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