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Original Article

Silicon improves strawberry plants nutrient uptake and epicuticular wax formation in a rhizosphere cooling system

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Abstract

Bioavailable silicon (silicic acid) is considered to be beneficial for plant growth and development. This study therefore aimed to determine the effects of silicic acid [$Si(OH)_4$] and potassium bicarbonate ($KHCO_3$) application on strawberry (*Fragaria* x *ananassa* Duch) plants' nutrient uptake and leaf wax formation. The strawberry plants (cvs Festival and Fortuna) were grown in a rhizosphere cooling system (RCS) at a mean rhizosphere temperature of 18°C ±2. Accumulation of plant nutrients in the plant parts was analysed by using inductively coupled plasma – optical emission spectrometry (ICP-OES), whilst the formation of wax on the leaves was observed with scanning electron microscopy (SEM). The results showed that plants given 0.25% (v/v) $Si(OH)_4$ via the roots had the highest amount of silicon accumulated in the leaves, roots, and crowns of both cultivars. The greatest amount of Ca and Mg was also found in the leaves of cv. Festival plants treated with 0.25% (v/v) $Si(OH)_4$ through the roots; whereas in cv. Fortuna, Ca, Mg and P had their highest amount accumulated in the leaves of plants sprayed with 0.25% (v/v) $Si(OH)_4$ mixed with 0.5% (s/v) $KHCO_3$. Root application of the $KHCO_3$ treatments on the other hand, limited Ca, Mg, Mn and Fe uptake into the plant parts. The leaves of the $Si(OH)_4$ treated plants were covered with a denser mass of wax when observed under SEM. Thus, $Si(OH)_4$ application was found to enhance strawberry plants' uptake of essential nutrients and also improved the formation wax on leaves, that may delay plant diseases invasion.

Keywords: Cooling system, Silicic acid, Silicon, Plant nutrient, Strawberry

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Introduction

Plants can be categorized as high, intermediate or non-accumulators of silicon (Si) based on the mechanism of Si absorption, transport, and depositions in plant tissues (Epstein, 1999). Plants that rely primarily on active transport of Si, are Si accumulators, while those that transport Si passively, are intermediate

accumulators. Silicon rejective plants are classified as non-accumulators (Ma and Takahashi, 2002). The high Si accumulating plants have shoot Si content ranging from 1.0-10% of dry mass and are mostly monocotyledonous plants, whereas plants with less than 0.5% shoot Si dry mass belong to the non-accumulators group (Ma and Takahashi, 2002; Liang et al., 2007). The low Si accumulation in the Si non-accumulating plants may be due to the lack of specific gene transporters to facilitate the radial transport and xylem loading of Si (Mitani and Ma, 2005).

Strawberries are traditionally classified as Si non-accumulators (Epstein, 1999). However, a recent study (Ouellette et al., 2017) concluded that the genes for Si transport (Lsi1 and Lsi2) are also present in strawberries. The study also concluded that strawberries can accumulate Si up to 3% of plant dry matter. Other studies have likewise reported relatively higher Si accumulation in strawberry (Soppelsa et al., 2019), peas (Jan et al., 2018) lettuce, carrot (Greger et al., 2018) and melon (do Nascimento et al., 2020) plant parts, even though these plants are traditionally not classified as Si accumulators.

When bioavailable Si [silicic acid: $Si(OH)_4$] gets absorbed by plants, it is transported through the xylem and is deposited in the leaf abaxial epidermis. Essential nutrients are reported to be carried along in the absorption process (Soppelsa et al., 2019). The deposited Si has the ability to improve the formation of the protective outer layer (cuticle) that is composed of silica, on plant leaf surfaces (Ali et al., 2013). The thicker cuticle is known to reduce dehydration and aids in crop protection (Avestan et al., 2019). This known advantage of Si application is consequently advantageous to strawberry production in the rhizosphere cooling system (RCS). The RCS is a plant growing system capable of supporting strawberry growth at tropical lowland conditions with the shoots exposed to ambient temperature. Therefore, reducing the impact of the harsh ambient conditions on the strawberry plants will likely improve plant performance. However, little is known of the effects of direct application of $Si(OH)_4$, and its combination with potassium bicarbonate ($KHCO_3$), on strawberry plants' nutrient uptake and epicuticular wax density; even though it has been reported (Dodgson et al., 2008) that strawberry farmers apply the two together.

The reported enhancement of strawberry plants after Si application, and the account of Si nutrients being combined with $KHCO_3$ and applied on strawberries, necessitated a study on how $Si(OH)_4$ application and its combination with $KHCO_3$ affects the strawberry plants' nutrient uptake and leaf wax formation. This study was therefore designed to determine the amount of Si and other plant macro- and micro nutrients accumulated in the leaves, crowns and roots of strawberry cultivars 'Festival' and 'Fortuna' after root and foliar application of $Si(OH)_4$, $KHCO_3$, and their equal volume combination. This study also sought to observe the epicuticular wax formed on the leaves, and visually rank the treatments based on the wax density.

Material and Methods

Planting material

Strawberry runner plants of cultivars 'Festival' and 'Fortuna' were acquired from Taman Agro Al-Mashoor, Cameron Highlands, Malaysia. Only healthy runner plants of similar size were selected. A rhizosphere cooling system (RCS) was then constructed and kept under a polythene tunnel at the plant nursery site of Universiti Malaysia Terengganu (UMT).

Rhizosphere cooling system (RCS) construction

The RCS constructed was a modification of the root cooling system described by Ilahi et al. (2017). The modification included building the RCS with aluminium and wood to a height of 70 cm, width 30 cm, depth 20 cm and length 3 m with linoleum lining the groove. Pipes of thermal conductivity 0.12 $W.M^{-1}.K^{-1}$ were used for cooling the growing media and were set in two turns. This resulted in the coir and perlite mixture used as growing media recording an average temperature of 18°C ±2.

The chiller system used to pump chilled water through the pipes in the troughs was made up of a 350 L water tank, one split air conditioner with an input power of 799 W, a digital temperature sensor (Dixell XR30CX-5N0C0), submersible water pumps (ASTRO AT-106), and a copper coil as condenser. The copper coil condenser cooled the water in the tank to temperatures of 7-13°C, whereas the pipes in the troughs were connected to the submersible water pumps in the water tank. The chilled water in the tank was then pumped to recycle through the pipes in the troughs and return into the tank. The troughs were covered with white plastic material and holes created for each strawberry plant to be planted.

Experimental design

The strawberry runner plants were grown and

observed for 70 days. This was to ensure that the assessments were carried out before the plants were matured enough to enter the reproductive phase. The runner plants were first transplanted into the RCS and observed for 28 days for the plants to establish and acclimatise to the growing conditions before treatment applications began. Five plants from each of the two strawberry cultivars in three replications were selected as control (no treatments applied) and other sets of five plants, in three replications of each cultivar, were given 45 mL root application or foliar spray of: 0.25% (v/v) silicic acid [$Si(OH)_4$] (72% silicic acid, Si-ben Co., Taiwan); 0.5% (s/v) potassium bicarbonate ($KHCO_3$) (R&M Chemicals, UK); and equal volumes of 0.25% (v/v) silicic acid + 0.5% (s/v) potassium bicarbonate as recommended by Jin et al. (2014), in a completely randomized design (CRD). Root applications were done by pouring the treatments directly into the root zone of the plants, whilst foliar spraying was done with a hand-held spray gun until the leaves were completely wet. The treatments were applied every week for five weeks (35 days).

The temperature of the growing media was monitored daily with a digital thermometer (ALLA, France) at a depth of 10 cm, and was found to be between 16-20°C. A drip irrigation system was also constructed with the strawberry plants irrigated once a day. Fertilizer application was once every three days with 45 mL (2.5 mS/cm) standard strawberry fertilizer obtained from the Malaysian Agricultural Research and Development Institute (MARDI).

Silicon and other plant nutrients analysis

Three plants from each treatment replication were selected and had their roots, crowns, and leaves, separated, dried, and ground into fine powder seven days after treatment applications. The ground samples of each plant part were then composited for each treatment replication for onwards analysis.

A modification of the dry ash method described by Pereira et al. (2016) was used to extract the samples for the determination of Si, P, K, Mg, Ca, Mn, B, Fe, Zn Cu, and Pb content in the various plant parts. One gram of each composited sample was weighed into a crucible and ash in a muffle furnace at 500°C for eight hours. The crucibles were then removed and the samples were heated to dryness after adding 2 mL HCl (36%). The samples were then incubated in a water bath at 70°C for 1 h after adding 10 mL HNO_3 (20%). The extracts were then filtered through a Whatman No. 1 filter paper into a 100 mL volumetric flask and washed several times with distilled water to the 100 mL mark. Samples of the extracts were then used for the nutrient analysis.

Inductively Coupled Plasma Mass Spectrometry

An inductively coupled plasma optical mass spectrometer (ICP-MS) (ELAN 9000, PerkinElmer, USA) was used for the Si nutrient determination in the plant parts. For plasma generation, nebulization and auxiliary gas, argon gas with purity of 99.995% was used. The operation conditions for the element detection is shown as Table 1.

Table-1. Operational conditions for determination of silicon by ICP-MS.

Internal Parameter	ICP-MS Value
Nebulizer gas flow	0.8 L/min
RF power	1100 W
Analog voltage	-1700 V
Pulse stage voltage	900.00 V
Lens voltage	6 V
Speed of peristaltic pump	26 rmp
Argon Gas Pressure	60 psi
Discriminator threshold	70.00 V
Detector	Dual
Analytical mass	^{28}Si
Sample flow rate	1 mL/min
Reading	1
Number of replicates	3
Rinse Delay Time	35 Sec
Flush Delay Time	35 Sec
Read Delay Time	15 Sec

Table-2. Operational conditions for determination of elements by ICP-OES.

Internal Parameter	ICP-OES Value
Sample uptake rate	1 mL/min
Spray chamber	Cyclonic
Injector	1.6 mm sapphire
Nebulizer gas flow rate	0.7 L/min
Auxiliary gas flow rate	0.2 L/min
Plasma flow rate	8 L/min
Viewing distance	15 mm
View	Axial - Radial
Source equilibrium time	15 sec
Read delay	60 sec
Replicates	3
Gas	Argon
RF Power	1500W

Inductively Coupled Plasma Optical Emission Spectroscopy

An inductively coupled plasma - optical emission spectrometer (ICP-OES) (Optima 8300, PerkinElmer, USA) was used for the other macro- and micro nutrient content determination. The operation conditions for the elements detection by the Optima 8300 is shown as Table 2.

Epicuticular wax formation

Scanning electron microscopy (SEM) was used to observe the epicuticular wax formed on the leaf adaxial surfaces of both strawberry cultivars. Three leaves from the outer whorl were sampled from three plants with each treatment replication, seven days after treatment application. The sampled leaves were cut into squares close to the midrib and fixed with 100% methanol for five minutes as described by Neinhuis and Edelmann (1996). The fixed leaves were dehydrated with different ethanol concentrations for 10 minutes and then cut into tiny strips. The strips were mounted on studs, air dried, and then placed in an auto fine coater (JFC-1600, JEOL, Japan) for gold coating.

An analytical scanning electron microscope (JSM - 6360LA) was used to observe and micrograph the wax formed on the adaxial surface of leaves of each treatment, of the two strawberry cultivars. One micrograph which was representative of the treatment was then selected and presented.

Statistical analysis

Data in this study are means and was computed with Genstat software (19th edition) with significance set at $p < 0.05$. Tukey's HSD test was used for all multiple comparisons in ANOVA.

Results

Silicon content in the plant parts

The amount of silicon (Si) in the leaves, roots and crowns of the two strawberry cultivars varied with significant differences ($p < 0.05$) based on the treatments applied (Figure 1). A comparison of the Si contents in the plant parts showed higher amounts in the leaves compared to the crowns, and roots. Moreover, the amount of Si accumulated in the plant parts was cultivar dependent. The application of the $Si(OH)_4$ treatments especially through the roots, resulted in higher amounts of Si in the various plant parts, whereas root application of $KHCO_3$ singly, generally limited the uptake and accumulation of Si. Notwithstanding the reduced Si uptake after the root application of $KHCO_3$, its application with $Si(OH)_4$ through the roots led to improved Si uptake and accumulation in the plant parts (Figure 1).

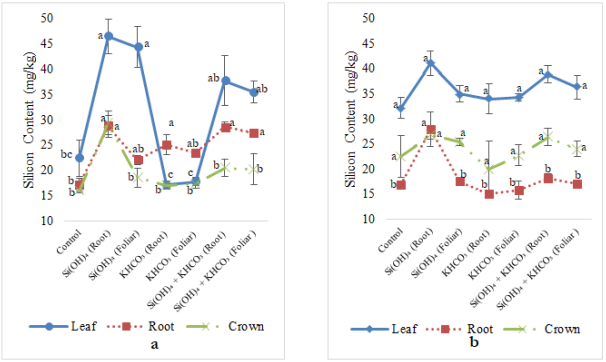

Figure-1. Silicon Content in Leaves, Roots and Crowns of Strawberry Cultivars Festival (a) and Fortuna (b) after Treatment Application. Error bars are standard error of means and different alphabet labels indicate significant difference at $p < 0.05$.

Plant essential nutrients and lead (Pb) content of the strawberry plant parts

The macronutrient (K, Ca, Mg, P) content of the leaves, crowns, and roots of the two strawberry cultivars varied with significant differences ($p < 0.05$) depending on the treatments applied (Tables 3, 4 and 5). The K content of the leaves and crowns of plants of both strawberry cultivars supplemented with $KHCO_3$ were relatively higher. However, no significant differences ($p > 0.05$) were observed except in cv. Fortuna (Tables 4 and 5). More so, plants treated with $Si(OH)_4$ often recorded the highest amount of Ca, Mg, and P in the leaves, crowns and roots. Meanwhile, the application of $KHCO_3$ only via the roots limited the uptake and accumulation of Fe, Mn, Ca, and Mg into the various plant parts even to deficiency levels in the case of Fe, though combining it with $Si(OH)_4$ often reduced this inhibitive effect. The application of $Si(OH)_4$ treatments also inhibited the uptake and accumulation of Pb in the plant parts. In general, when the micronutrient contents in the leaves, crowns and roots of both cultivars exceeded or were approaching toxicity levels, the values recorded in plants of both strawberry cultivars given treatments containing $Si(OH)_4$ were typically lower. However, when the amounts were below or within the optimal range, the values determined in plants given $Si(OH)_4$ treatments were mostly higher with the exception of the Zn and Mn contents (Tables 3, 4 and 5).

Table-3: Nutrient composition of the leaves of strawberry cultivars Festival and Fortuna after silicic acid and potassium bicarbonate application.

Cultivar	Treatments	Nutrient Content (micro = mg/kg, macro = g/kg)									
		K	Ca	Mg	P	Zn	Mn	Fe	Pb	Cu	B
Festival	Control	18.45 ±3.93^{a}	7.96 ±1.88^{a}	2.55 ±0.45^{a}	5.65 ±0.69^{a}	18.86 ±1.02^{a}	100.98 ±20.99^{a}	46.44 ±7.17^{ab}	2.32 ±0.56^{a}	2.86 ±0.51^{a}	74.03 ±2.95^{a}
	$Si(OH)_4$ (root)	25.62 ±3.18^{a}	8.75 ±1.87^{a}	2.86 ±0.54^{a}	5.05 ±0.70^{a}	15.03 ±2.50^{a}	107.41 ±27.88^{a}	58.08 ±11.03^{a}	0.73 ±0.06^{b}	1.66 ±0.75^{a}	60.19 ±12.40^{a}
	$Si(OH)_4$ (foliar)	25.41 ±4.09^{a}	5.73 ±0.73^{a}	1.89 ±0.08^{a}	4.85 ±0.70^{a}	13.32 ±2.04^{a}	59.44 ±8.00^{a}	40.32 ±10.84^{ab}	0.97 ±0.04^{b}	1.17 ±0.54^{a}	49.20 ±4.32^{a}
	$KHCO_3$ (root)	29.60 ±1.23^{a}	4.23 ±0.21^{a}	1.71 ±0.11^{a}	4.64 ±0.25^{a}	14.42 ±3.95^{a}	39.12 ±2.77^{a}	19.17 ±3.42^{b}	0.74 ±0.23^{b}	1.35 ±0.16^{a}	45.60 ±7.78^{a}
	$KHCO_3$ (foliar)	24.92 ±2.67^{a}	6.01 ±0.48^{a}	1.83 ±0.08^{a}	4.29 ±0.16^{a}	12.87 ±2.12^{a}	71.91 ±3.58^{a}	40.27 ±2.21^{ab}	0.92 ±0.06^{b}	1.43 ±0.24^{a}	47.82 ±3.97^{a}
	$Si(OH)_4$ + $KHCO_3$ (root)	25.34 ±6.40^{a}	6.73 ±0.39^{a}	2.14 ±0.25^{a}	5.50 ±0.49^{a}	12.37 ±2.20^{a}	60.46 ±3.54^{a}	24.78 ±4.39^{ab}	0.62 ±0.09^{b}	1.50 ±0.26^{a}	64.36 ±12.70^{a}
	$Si(OH)_4$ + $KHCO_3$ (foliar)	24.98 ±3.45^{a}	7.66 ±0.87^{a}	2.56 ±0.42^{a}	6.13 ±1.03^{a}	16.29 ±3.82^{a}	85.87 ±10.44^{a}	47.87 ±10.37^{ab}	0.87 ±0.08^{b}	2.81 ±1.51^{a}	75.23 ±20.62^{a}
Fortuna	Control	17.45 ±0.49^{a}	7.85 ±0.62^{ab}	2.24 ±0.09^{a}	5.50 ±0.36^{a}	17.62 ±1.78^{a}	60.80 ±4.82^{ab}	40.72 ±1.50^{a}	0.72 ±0.22^{a}	2.07 ±0.10^{a}	50.50 ±2.25^{a}
	$Si(OH)_4$ (root)	19.62 ±0.78^{a}	6.72 ±0.39^{ab}	2.71 ±0.21^{a}	6.63 ±0.76^{a}	16.67 ±1.54^{a}	51.26 ±3.89^{abc}	42.27 ±3.94^{a}	0.74 ±0.27^{a}	2.51 ±0.77^{a}	52.12 ±3.69^{a}
	$Si(OH)_4$ (foliar)	18.28 ±0.22^{a}	7.69 ±0.31^{ab}	2.49 ±0.06^{a}	6.34 ±0.29^{a}	17.05 ±1.09^{a}	62.68 ±6.51^{a}	43.11 ±1.25^{a}	0.47 ±0.04^{a}	2.70 ±0.77^{a}	58.14 ±1.70^{a}
	$KHCO_3$ (root)	26.73 ±3.02^{a}	3.89 ±1.36^{b}	1.98 ±0.39^{a}	6.27 ±1.16^{a}	17.05 ±4.32^{a}	27.63 ±7.44^{bc}	38.85 ±4.05^{a}	0.80 ±0.30^{a}	1.99 ±0.30^{a}	53.59 ±2.38^{a}
	$KHCO_3$ (foliar)	21.11 ±0.66^{a}	7.33 ±0.15^{ab}	2.44 ±0.03^{a}	7.03 ±0.13^{a}	20.17 ±0.83^{a}	52.68 ±5.66^{abc}	44.15 ±0.83^{a}	0.64 ±0.18^{a}	2.58 ±0.34^{a}	67.47 ±5.33^{a}
	$Si(OH)_4$ + $KHCO_3$ (root)	27.15 ±2.33^{a}	4.32 ±0.22^{b}	2.33 ±0.05^{a}	6.06 ±0.50^{a}	18.05 ±2.47^{a}	21.82 ±7.51^{c}	44.43 ±3.68^{a}	0.46 ±0.26^{a}	3.18 ±1.13^{a}	55.77 ±1.31^{a}
	$Si(OH)_4$ + $KHCO_3$ (foliar)	22.37 ±3.99^{a}	8.74 ±1.73^{a}	2.88 ±0.40^{a}	7.45 ±0.87^{a}	19.02 ±4.56^{a}	63.75 ±11.04^{a}	48.35 ±12.41^{a}	0.20 ±0.14^{a}	2.27 ±0.36^{a}	71.42 ±17.59^{a}

Different letters superscript within each column for a cultivar indicates significant differences ($p < 0.05$).

Table-4. Nutrient composition of the roots of strawberry cultivars Festival and Fortuna after silicic acid and potassium bicarbonate application.

Cultivar	Treatments	Nutrient Content (micro = mg/kg, macro = g/kg)									
		K	Ca	Mg	P	Zn	Mn	Fe	Pb	Cu	B
Festival	Control	12.10 ±0.48^{a}	3.27 ±0.54^{ab}	1.50 ±0.20^{a}	2.98 ±0.31^{a}	74.71 ±11.85^{a}	33.66 ±9.35^{ab}	37.10 ±2.31^{b}	1.27 ±0.29^{a}	4.03 ±0.73^{a}	21.30 ±4.39^{a}
	$Si(OH)_4$ (root)	20.83 ±2.78^{a}	4.21 ±0.68^{a}	1.62 ±0.02^{a}	2.61 ±0.16^{a}	61.81 ±1.90^{ab}	30.14 ±3.48^{ab}	74.65 ±12.48^{a}	1.03 ±0.18^{a}	5.36 ±0.97^{a}	20.38 ±3.42^{a}
	$Si(OH)_4$ (foliar)	18.43 ±2.40^{a}	2.70 ±0.12^{ab}	1.36 ±0.06^{a}	3.04 ±0.38^{a}	49.49 ±3.99^{ab}	23.79 ±0.42^{b}	56.96 ±9.43^{ab}	1.02 ±0.06^{a}	2.97 ±0.07^{a}	17.03 ±1.00^{a}
	$KHCO_3$ (root)	20.90 ±1.16^{a}	1.79 ±0.29^{b}	1.37 ±0.08^{a}	2.04 ±0.39^{a}	41.08 ±4.42^{b}	38.45 ±3.43^{ab}	24.49 ±4.32^{b}	1.16 ±0.09^{a}	6.95 ±1.91^{a}	13.61 ±2.13^{a}
	$KHCO_3$ (foliar)	19.98 ±1.86^{a}	3.35 ±0.64^{ab}	1.52 ±0.18^{a}	2.96 ±0.20^{a}	35.71 ±7.43^{b}	51.68 ±7.79^{a}	36.94 ±4.58^{ab}	1.10 ±0.11^{a}	4.29 ±0.53^{a}	19.78 ±2.14^{a}
	$Si(OH)_4$ + $KHCO_3$ (root)	19.76 ±0.60^{a}	2.86 ±0.27^{ab}	1.14 ±0.06^{a}	2.26 ±0.34^{a}	36.47 ±0.39^{b}	22.06 ±2.46^{b}	20.80 ±7.16^{b}	1.00 ±0.11^{a}	2.96 ±1.02^{a}	13.59 ±0.70^{a}
	$Si(OH)_4$ + $KHCO_3$ (foliar)	13.39 ±6.48^{a}	3.64 ±0.46^{ab}	1.65 ±0.25^{a}	2.66 ±0.42^{a}	73.26 ±7.34^{a}	27.35 ±4.22^{ab}	43.47 ±10.64^{ab}	1.20 ±0.02^{a}	4.24 ±0.18^{a}	17.52 ±2.43^{a}
Fortuna	Control	11.65 ±3.87^{a}	3.14 ±0.52^{a}	1.60 ±0.17^{a}	4.16 ±0.64^{a}	81.94 ±11.79^{a}	24.07 ±5.06^{a}	53.39 ±21.34^{a}	1.20 ±0.17^{a}	4.47 ±1.89^{a}	21.69 ±5.63^{a}
	$Si(OH)_4$ (root)	11.40 ±1.36^{a}	3.24 ±0.16^{a}	1.53 ±0.12^{a}	5.41 ±0.56^{a}	74.60 ±8.87^{a}	21.20 ±0.70^{a}	164.97 ±72.77^{a}	1.07 ±0.04^{a}	7.07 ±1.29^{a}	17.46 ±2.24^{a}
	$Si(OH)_4$ (foliar)	9.21 ±1.84^{a}	2.98 ±0.33^{a}	1.69 ±0.35^{a}	5.25 ±0.71^{a}	65.72 ±11.34^{a}	21.66 ±2.66^{a}	83.69 ±23.30^{a}	0.88 ±0.10^{a}	8.17 ±0.62^{a}	17.55 ±2.37^{a}
	$KHCO_3$ (root)	10.24 ±1.84^{a}	2.67 ±0.25^{a}	1.17 ±0.51^{a}	3.87 ±1.26^{a}	67.89 ±11.41^{a}	19.17 ±3.96^{a}	32.54 ±10.11^{a}	1.19 ±0.08^{a}	5.84 ±0.91^{a}	15.45 ±2.61^{a}
	$KHCO_3$ (foliar)	9.90 ±3.34^{a}	2.43 ±0.61^{a}	1.30 ±0.44^{a}	4.26 ±1.54^{a}	51.58 ±17.03^{a}	16.76 ±5.19^{a}	70.94 ±16.58^{a}	0.96 ±0.40^{a}	5.29 ±1.42^{a}	14.08 ±4.40^{a}
	$Si(OH)_4$ + $KHCO_3$ (root)	6.34 ±1.54^{a}	2.06 ±0.47^{a}	0.86 ±0.14^{a}	2.86 ±0.50^{a}	40.08 ±6.39^{a}	11.53 ±2.00^{a}	37.40 ±7.26^{a}	1.23 ±0.30^{a}	5.53 ±1.47^{a}	11.11 ±2.78^{a}
	$Si(OH)_4$ + $KHCO_3$ (foliar)	9.43 ±1.54^{a}	3.11 ±0.17^{a}	1.81 ±0.08^{a}	5.09 ±0.36^{a}	74.69 ±4.17^{a}	22.89 ±0.94^{a}	73.94 ±3.31^{a}	1.26 ±0.13^{a}	6.85 ±0.81^{a}	18.26 ±0.95^{a}

Different letters superscript within each column for a cultivar indicates significant differences ($p < 0.05$)

Table-5. Nutrient composition of the crowns of strawberry cultivars Festival and Fortuna after silicic acid and potassium bicarbonate application

Cultivar	Treatment	Nutrient Content (micro = mg/kg, macro = g/kg)									
		K	Ca	Mg	P	Zn	Mn	Fe	Pb	Cu	B
Festival	Control	16.85 ±2.77^{a}	5.77 ±0.05^{a}	1.81 ±0.28^{a}	5.52 ±0.52^{ab}	89.02 ±7.01^{a}	32.45 ±0.84^{a}	14.82 ±2.22^{bc}	0.63 ±0.17^{a}	6.73 ±0.23^{a}	22.80 ±1.25^{a}
	$Si(OH)_4$ (root)	18.94 ±0.82^{a}	5.65 ±0.19^{a}	2.39 ±0.30^{a}	7.95 ±0.47^{a}	77.51 ±18.80^{a}	32.02 ±2.01^{a}	23.49 ±2.66^{abc}	0.65 ±0.13^{a}	10.28 ±1.46^{a}	25.00 ±1.60^{a}
	$Si(OH)_4$ (foliar)	17.88 ±2.69^{a}	4.66 ±0.48^{ab}	2.04 ±0.25^{a}	7.80 ±0.90^{a}	76.50 ±14.06^{a}	26.78 ±3.01^{a}	24.67 ±8.32^{abc}	0.91 ±0.12^{a}	10.76 ±1.93^{a}	24.87 ±1.33^{a}
	$KHCO_3$ (root)	21.71 ±0.81^{a}	3.48 ±0.41^{ab}	1.54 ±0.23^{a}	3.84 ±0.79^{b}	41.93 ±1.78^{a}	23.35 ±4.25^{a}	7.86 ±2.57^{c}	1.25 ±0.13^{a}	7.64 ±1.07^{a}	15.59 ±2.30^{a}
	$KHCO_3$ (foliar)	21.11 ±1.29^{a}	2.99 ±0.16^{b}	1.41 ±0.25^{a}	4.86 ±0.61^{ab}	55.66 ±8.41^{a}	38.40 ±4.50^{a}	36.30 ±0.60^{a}	0.89 ±0.08^{a}	9.04 ±0.47^{a}	18.95 ±1.21^{a}
	$Si(OH)_4 + KHCO_3$ (root)	20.60 ±1.28^{a}	4.71 ±0.86^{ab}	1.98 ±0.43^{a}	6.12 ±1.29^{ab}	48.74 ±5.32^{a}	23.44 ±4.97^{a}	11.61 ±3.21^{c}	0.95 ±0.01^{a}	5.83 ±1.00^{a}	20.47 ±4.57^{a}
	$Si(OH)_4 + KHCO_3$ (foliar)	18.07 ±2.75^{a}	5.42 ±0.66^{a}	2.08 ±0.37^{a}	6.59 ±0.66^{ab}	68.04 ±15.64^{a}	39.50 ±4.38^{a}	33.34 ±3.19^{ab}	1.03 ±0.31^{a}	10.88 ±1.18^{a}	23.34 ±2.18^{a}
Fortuna	Control	16.32 ±0.76^{ab}	6.11 ±0.50^{a}	2.52 ±0.12^{a}	8.89 ±0.48^{ab}	159.23 ±9.67^{a}	73.99 ±4.34^{a}	64.85 ±3.63^{a}	0.89 ±0.14^{a}	9.57 ±0.30^{ab}	23.68 ±1.87^{a}
	$Si(OH)_4$ (root)	14.20 ±0.81^{bcd}	5.27 ±0.81^{ab}	2.30 ±0.08^{ab}	8.96 ±0.13^{ab}	110.33 ±1.94^{bc}	33.96 ±1.83^{cd}	38.13 ±0.95^{a}	0.80 ±0.16^{a}	9.04 ±0.35^{ab}	21.73 ±1.09^{a}
	$Si(OH)_4$ (foliar)	13.25 ±0.08^{cd}	5.91 ±0.28^{a}	2.63 ±0.14^{a}	9.28 ±0.28^{ab}	149.21 ±11.25^{ab}	62.33 ±5.21^{ab}	43.17 ±6.87^{a}	0.95 ±0.31^{a}	10.07 ±0.47^{a}	21.01 ±0.63^{a}
	$KHCO_3$ (root)	18.56 ±0.44^{a}	5.71 ±0.42^{a}	2.43 ±0.20^{a}	9.01 ±0.60^{ab}	145.68 ±13.13^{ab}	46.31 ±8.66^{bcd}	34.01 ±2.48^{a}	0.51 ±0.19^{a}	9.99 ±0.34^{a}	24.37 ±1.82^{a}
	$KHCO_3$ (foliar)	15.40 ±0.33^{bc}	5.17 ±0.15^{ab}	2.46 ±0.05^{a}	10.14 ±0.10^{a}	134.84 ±9.45^{ab}	67.31 ±4.70^{ab}	43.15 ±3.73^{a}	0.86 ±0.02^{a}	8.86 ±0.20^{ab}	24.29 ±0.45^{a}
	$Si(OH)_4 + KHCO_3$ (root)	15.25 ±0.35^{bcd}	3.32 ±0.14^{b}	1.83 ±0.06^{b}	8.15 ±0.10^{b}	72.11 ±3.67^{c}	25.95 ±4.93^{d}	19.72 ±3.38^{a}	0.84 ±0.08^{a}	3.77 ±0.32^{c}	21.33 ±0.61^{a}
	$Si(OH)_4 + KHCO_3$ (foliar)	12.55 ±0.81^{d}	5.01 ±0.21^{ab}	2.37 ±0.07^{ab}	8.51 ±0.32^{ab}	135.58 ±4.86^{ab}	55.21 ±1.85^{abc}	82.62 ±33.46^{a}	0.77 ±0.14^{a}	8.06 ±0.16^{b}	22.75 ±0.96^{a}

Different letters superscript within each column for a cultivar indicates significant differences ($p < 0.05$)

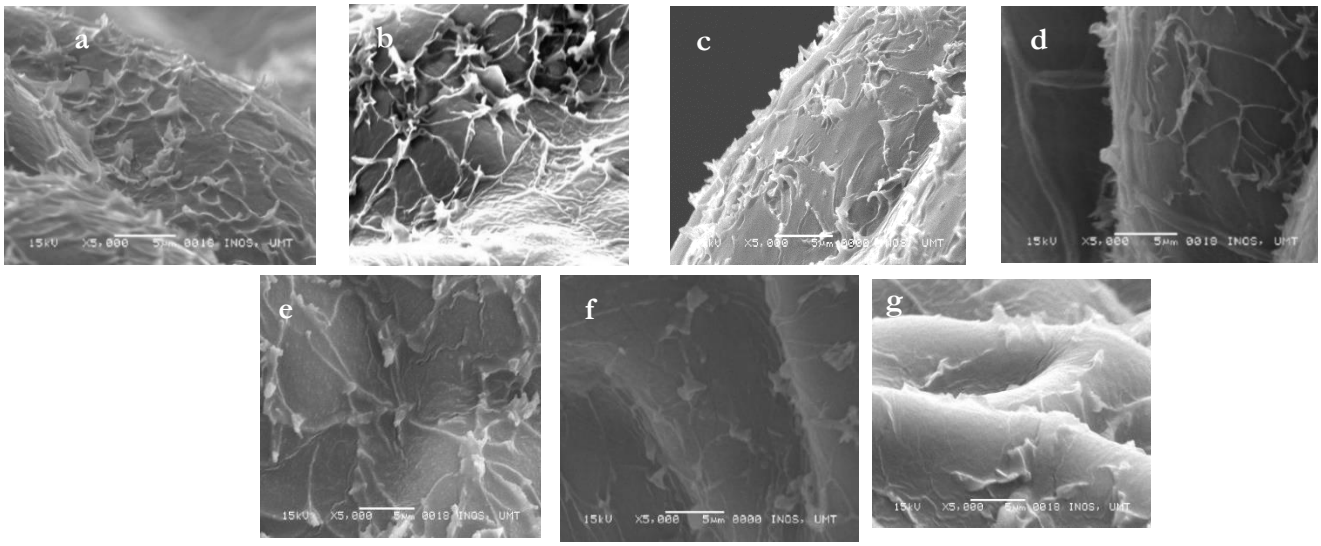

Figure-2. (a) $Si(OH)_4$ (root), (b) $Si(OH)_4$ (foliar), (c) $Si(OH)_4$ + $KHCO_3$ (foliar), (d) $KHCO_3$ (foliar) compared to (e) control, (f) $Si(OH)_4$ + $KHCO_3$ (root) and (g) $KHCO_3$ (root).

Epicuticular wax formation

The application of the various treatments had visible effects on the formation and density of wax on the leaf surface of both strawberry cultivars after treatment application. The micrographs taken showed that wax densities on the leaf adaxial surface of both cv. Festival and cv. Fortuna after treatment applications ranked as: $Si(OH)_4$ (root) > $Si(OH)_4$ (foliar) > $Si(OH)_4$ + $KHCO_3$ (foliar) > $KHCO_3$ (foliar) > Control > $Si(OH)_4$ + $KHCO_3$ (root) > $KHCO_3$ (root) (Figure 2)

Discussion

Silicic acid application has been shown to increase the Si content of the strawberry plant parts in this study (Figure 1). Similarly, the application of SiO_2 on strawberry cv. Hokowase was reported to have increased the Si content of the crowns, resulting in improved flower production and fruit yield (Miyake and Takahashi, 1986). The increased Si content in the roots of both strawberry cultivars in this study after $Si(OH)_4$ application via the roots, is also in line with the findings of Dehghanipoodeh et al. (2018) who reported that the highest concentration of Si in the roots of strawberry cultivar Camarosa, was found in plants given root application of 10 mM potassium silicate. Contrarily, the application of $KHCO_3$ only through the roots in this study, generally reduced the accumulation of Si in the leaves, crowns and roots of plants of both strawberry cultivars (Figure 1). This is in line with a study which suggested that root application of bicarbonates increased root zone pH of peas, which then reduced its nutrient absorption capacity (Jelali et al., 2011). The advantages of high Si content in strawberry plant parts include: increased essential plant nutrient uptake (Hajiboland, et al., 2018), increased leaf chlorophyll content (Muneer et al., 2017), reduced transpiration (Avestan et al., 2019), reduced heavy metals in leaves (Treder and Cieslinski, 2005) and improved resistance to strawberry foliar diseases (Jin et al., 2014; Ouellette et al., 2017). Thus, the application of the $Si(OH)_4$ treatments increasing the Si content in the plant parts of both strawberry cultivars will confer the advantages of increased silicon in plant tissues.

This study's results also showed improved macronutrients uptake and accumulation in the plant parts of both strawberry cultivars after they were given treatments containing $Si(OH)_4$ (Tables 3, 4 and 5). The application of other Si nutrients has likewise been described in other studies to increase the Ca, Mg, and P contents in the above ground tissues of strawberries (Park et al., 2018; Soppelsa et al., 2019). Moreover, the high K content in the roots of cv. Festival given treatments of $Si(OH)_4$ (Table 4), is in line with the findings of Yaghubi et al. (2019), who concluded that the highest K content in the roots of strawberry cultivars Kurdistan and Paros was found in plants given silicon treatments. These earlier studies therefore support the assertion that the application of the 0.25% (v/v) $Si(OH)_4$ may have been responsible for the improved macronutrients uptake in the organs of the two strawberry cultivars (Tables 3, 4 and 5). The high K concentrations recorded when treatments containing $KHCO_3$ was applied is also similar to the values determined when the above ground tissues of Seolhyang strawberries grown in Hoagland solution with 90 mg/L bicarbonate were assessed after 126 days (Lee et al., 2014).

Silicon fertilization improving the Mn, Fe, and Cu contents of strawberry plant parts has also been documented (Hajiboland et al., 2018; Moradtalab et al., 2019). Excess K and Si in plant tissues have however, been shown to be antagonistic to Zn uptake (Huang and Ma, 2020). In a study on Elsanta strawberries, one of the lowest Zn content in the roots was thus observed when Si nutrient (siliforce) was applied (Soppelsa et al., 2019). Therefore, the improved micronutrients uptake and the relatively lower Zn content detected in the plant parts of cv. Festival and cv. Fortuna in this study after being given treatments of $Si(OH)_4$ (Tables 3, 4 and 5), may be due to the ability of $Si(OH)_4$ in regulating plant micronutrient uptake.

The applications of bicarbonates through the roots have been shown to cause Fe deficiencies and reduce essential plant nutrient uptake in several crops including strawberries (Sánchez-Rodríguez et al., 2013; Lee et al., 2014; Ding et al., 2020). The low Fe contents and the reduced amount of the other essential nutrients in the plant parts of both cultivars in this study after being given root applications of $KHCO_3$ (Tables 3, 4 and 5), are thus supported by these earlier findings. Bicarbonates are known to increase the pH of growing media; whilst high pH increases Pb uptake (Lee et al., 1998). Therefore, the relatively higher Pb content observed in the control plants and plants given treatments of $KHCO_3$ in this study is most likely due to the same reason. Silicon fertilizers on the other hand, have been proven to reduce Pb uptake in several plants (Tripathi et al., 2016; Chen et al., 2019). Thus, the reduced Pb accumulation in the strawberry plant parts after $Si(OH)_4$ application and the increased Pb uptake in the control plants, and plants given $KHCO_3$ alone via the roots (Tables 3, 4 and 5), are in line with previous findings.

This study's results also showed that the application of the $Si(OH)_4$ treatments increased epicuticular wax formation compared to root application of treatments of $KHCO_3$ (Figure 2). The epicuticular wax functions mainly to reduce surface wetting and moisture loss. It is composed of lipophilic compounds, which makes it plant nutrient-dependent (Pollard et al., 2008). Nano-

silicon application was therefore reported to have increased the plant nutrient uptake and wax density of Camarosa strawberry under NaCl stress (Avestan et al., 2019). Other studies (Kanto et al., 2007; Liu et al., 2021) also reported improved strawberry wax densities after silicon application. The improved leaf wax density in the $Si(OH)_4$ treated plants may thus have been as a result of the improved plant essential nutrients uptake, whereas the lower wax densities observed after root application of $KHCO_3$ may be due to the reduction in the uptake of some plant essential nutrients.

Conclusion

This study showed that the application of silicic acid [$Si(OH)_4$] increased the amount of silicon accumulated in the leaves, crowns and root of strawberry cultivars 'Festival' and 'Fortuna'. Furthermore, it was proven that applying 0.25% (v/v) $Si(OH)_4$ alone, or a combination of equal volumes of 0.25% (v/v) $Si(OH)_4$ and 0.5% (s/v) $KHCO_3$, increased the Mg, Ca, P, Fe Cu, and Mn in the various strawberry plant parts. However, the application of 0.5% (s/v) $KHCO_3$ alone through the roots, reduced Fe uptake significantly and also the uptake of several other plant essential elements. Meanwhile, the $Si(OH)_4$ treatments application caused a reduced Pb uptake. Epicuticular wax formation also improved when 0.25% (v/v) $Si(OH)_4$ was applied, especially through the roots. Therefore, the application of 0.25% (v/v) $Si(OH)_4$ especially through the roots, is recommended to improve strawberry plant nutrient uptake. Additionally, 0.25% (v/v) $Si(OH)_4$ application was found to have the potential to improve leaf wax formation that may reduce biotic and abiotic stresses.

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Osei AF: Research planning, data collection, data analysis and manuscript write up.
Jin X & Abdullah WZBW: Research planning and manuscript editing.
Sidique SNM: Research planning and discussion of results, manuscript editing and final approval of the manuscript.