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Effect of silicon nutrition on physiological and biochemical parameters of maize at different dates of sowing

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Abstract
A field experiment was conducted to study 'effect of silicon nutrition on physiological and biochemical parameters of maize at different dates of sowing' during *Kharif* 2018 at Main Agricultural Research Station (MARS), University of Agricultural Sciences Dharwad, Karnataka, India. The main plot treatments consist of three dates of sowing (DS_1: Second fortnight of June, DS_2: First fortnight of July, and DS_3: Second fortnight of July) and the sub plot treatments consist of three rates of soil application of Potassium silicate (S_1, S_2 and S_3) and two rates of Foliar application of silicic acid (S_4 and S_5) spray at 45 DAS along with recommended dose of fertilizers and absolute control. Sowing of maize at second fortnight of June recorded significantly higher relative leaf water content (62.0 and 55.7% at 60 and 90 DAS, respectively) over other dates of sowing and soil application of Potassium silicate @ 60 kg ha^{-1} recorded significantly higher relative leaf water content (61.4 and 55.0% at 60 and 90 DAS, respectively) as compared to other treatments. The proline content (0.390 µg g^{-1}) of maize recorded lower at sowing of maize at second fortnight of June with foliar application of silicic acid at 0.50 per cent than all other treatment combinations, RDF and absolute control. Soil application of potassium silicate @ 60 kg ha^{-1} and sowing of maize at second fortnight of July recorded significantly lower transpiration rate (4.01 m mole of H_2O m^{-2} s^{-1}at 60 DAS) and higher stomotal conductance (0.588 µ mole m^{-2} s^{-1}) than all other treatment combinations, RDF and absolute control. Significantly higher total soluble sugars content (23.9 mg g^{-1}) was recorded with sowing of maize at second fortnight of June with soil application of potassium silicate @ 60 kg ha^{-1} over all other treatment combinations, RDF and absolute control. However, these parameters were on par with sowing at second fortnight of June with foliar application of silicic acid at 0.5 per cent. Sowing of maize at second fortnight of June recorded significantly higher starch (45.0 mg g^{-1}) over other dates of sowing and soil application of potassium silicate @ 60 kg ha^{-1} recorded significantly higher starch content in maize grain (43.5 mg g^{-1}) than other treatments. However it was on par with soil application of potassium silicate @ 40 kg ha^{-1} (42.5 mg g^{-1}) and with foliar application of silicic acid at 0.50 per cent (43.0 mg g^{-1}).

Keywords: Potassium silicate, Silicic acid, Silicon, Proline, RLWC, transpiration rate, stomotal conductance, starch, TSS

Introduction
Maize (*Zea mays* L.) is one of the important cereal crops which stands first with respect to production in the world. Maize is considered as a staple food in many parts of the world with the total production of maize surpassing that of wheat or rice. It is an important source of carbohydrate, protein, iron, vitamin B and minerals. The demand for maize as an animal feed will continue to grow faster than the demand for its use as a human food, particularly in Asia. Due to its higher production potential and wider adaptability, maize is called as "Queen of cereals".

Maize is an exhaustive crop, the nutrient requirement cannot be supplied only through soil nutrient reserves, the additional nutrients can be met by fertilizer application. In Karnataka, maize yield is low due to imbalanced application of nutrients. The recommendations of a fertilizer dose is a challenge to scientists as it should meet both nutrient demand of crop and sustain the production system (Shankar and Umesh, 2008) [14].

Optimum time of sowing is one of the important factors which provide scope for better utilization of natural resources by the crop during its growing season. Suitable time of sowing enables the crop to take full advantage of favourable weather conditions during growing seasons. Studies have shown that, delay in sowing of maize beyond July results in yield reduction. In the event of late onset of monsoon rains and erratic rainfall, farmers are forced to take-up sowing late (Leelarani *et al.*, 2012) [10].

Silicon is the most abundant element (in the earth's crust) after oxygen with soils containing approximately 28 per cent silicon by weight (Lindsay, 1979) [11]. Because of its abundance in the biosphere, the essentiality of silicon as a micronutrient for higher plants is very difficult to prove. Agricultural activity tends to remove large quantities of silicon from soil. Even highly purified water contains about 20 nM Si (Werner and Roth, 1983) [15] and correspondingly, the leaves of silicon accumulator plants that were subjected to no silicon treatment usually contain between 0.5-1.9 mg Si g^{-1} leaf dry weight.

Silicon has been regarded as an essential element in a number of species of the Poaceae and Cyperaceae but it has not been possible to demonstrate that it is essential to all higher plants because direct evidence is still lacking that, it is part of the molecule of an essential plant constituent or metabolite (Epstein, 1999) [6].

Due to climate change scenario, maize crop is affecting from moisture stress under rainfed conditions. Aberrant weather especially late onset of monsoon, early onset of monsoon followed by gap of rainfall for 30- 40 days leads to moisture stress at critical crop growth stages leading to reduction in yield. One of the options to mitigate moisture stress is application of silicon either in the form of soil or foliar nutrition. Silicon plays important role in inducing drought tolerance and lodging resistance to plants. Application of silicon has been considered beneficial for improving crop tolerance to both biotic and abiotic stresses (Haynes, 2017) [9]. As water scarcity demands the maximum use of every drop of water, there is a need to use silicon for sustainable productivity of crops. Further analysis on silicon application and water uptake is required to understand silicon enhanced crop tolerance to water stress. However, still information regarding drought tolerance and water uptake ability in conjunction with silicon is lacking (Ahmed *et al.*, 2011) [2].

Material Methods

The experiment was conducted during *kharif* season of 2018 at MARS, UAS, Dharwad. The geographical co-ordinates of experimental site is 15° 26′ N latitude and 75° 07′ East longitude at an altitude of 678 m above mean sea level. UAS, Dharwad comes under Northern Transitional Zone (Zone -8) of Karnataka. The soil of the experimental site was deep black soil (*Vertic Inceptisol*).

The treatments were laid out in a split plot design with three dates of sowing as second fortnight of June, First fortnight of July and Second fortnight of July (DS_1, DS_2, and DS_3 respectively) with three treatments of soil application of potassium silicate @ 20, 40 and 60 kg ha^{-1} (S_1, S_2 and S_3) and two treatments of foliar application of silicic acid @ 0.25 and 0.50% (S_4 and S_5) along with recommended dose of fertilizers and absolute control. Date of sowing (DS) were included as a main plot and silicon nutrient levels as sub plot. The soil of the experimental site was deep black soil (*Vertic Inceptisol*) with neutral in pH (7.2), low in organic carbon (0.38%), medium in available nitrogen (285 kg N ha^{-1}) and available P_2O_5 (32 kg ha^{-1}) and high in available potassium (410.4 kg K ha^{-1}).

Maize hybrid NK-6240 was used for sowing. Sowings were under taken on 30th June (Second fortnight of June), 13th July (First fortnight of July) and 28th July (Second fortnight of July), respectively. Two control treatments (Recommended dose of fertilizer and absolute control) were also sown on 30th June with same hybrid and following same method of sowing as that of main and subplot treatments. Well decomposed FYM @ 7.5 t ha^{-1} was incorporated into soil two weeks prior to sowing. The nutrients *viz.*, nitrogen, phosphorus and potassium were applied in the form of urea, di-ammonium phosphate (DAP) and muriate of potash (MOP), respectively. Recommended dose of fertilizers (RDF) @ 100: 50: 25 N: P_2O_5: K_2O kg ha^{-1} was used and were calculated as per treatments based on gross plot size. The silicon nutrients were applied in the form of potassium silicate and silicic acid. Entire dose of (100 per cent) potassium silicate was applied as basal fertilizer at the time of sowing (S_1: 20, S_2: 40 and S_3: 60 kg ha^{-1}, respectively) and silicic acid was applied as foliar spray at 45 DAS (S_4: 0.25 and S_5: 0.50%, respectively).

Results and Discussion

Effect of dates of sowing on physiological and biochemical parameters: Sowing of maize at second fortnight of June recorded significantly higher relative leaf water content (62.0 and 55.75% at 60 and 90 DAS, respectively) over first fortnight (59.0 and 53.9% at 60 and 90 DAS respectively) and second fortnight of July (58.6 and 51.4% at 60 and 90 DAS, respectively). Higher relative leaf water content at second fortnight of June was mainly due to sufficient rainfall received at critical growth stages and there was no dearth of soil moisture as compared to other dates of sowing where moisture stress was observed (Table 1).

Sowing of maize at second fortnight of July recorded significantly higher proline (1.34 µg g^{-1}) content at anthesis silking stage as compared to second fortnight of June (0.51 µg g^{-1}) and first fortnight of July (0.69 µg g^{-1}). It was mainly because of absence of favourable soil moisture at anthesis silking stage and mainly under stress condition proline accumulation was more due to moisture stress and presence of more proline content in plant adversely affects physiological aspects of crop like relative leaf water content and stomatal conductance. Significantly lower proline content was recorded at second fortnight of June and first fortnight of July over second fortnight of July. Hence, crop suffered due to moisture stress at critical stage was more in second fortnight of July sown crop over other dates of sowing (Table 2).

Significantly higher transpiration rate (4.38 m mole of H_2O m^{-2} s^{-1}) was recorded in second fortnight of June over other dates of sowing. Under delayed sowing crop undergone stress and moisture availability is less hence, transpiration was lower. Second fortnight of June recorded higher transpiration due to higher moisture availability (Table 3).

Stomatal conductance (0.575 µ mole m^{-2} s^{-1} at 60 days) was more during second fortnight of June as compared to other dates of sowing. Gaseous exchange and carbon dioxide absorption in plant is influenced by stomatal conductance. Due to delay in sowing because of imbibition and low osmosis, stomotal conductance was affected. Due to delayed sowing the plants suffered by moisture stress which affected the stomatal movement activity, gaseous exchange and carbon dioxide absorption. Stomotal conductance was more in second fortnight of June over other dates of sowing.

Sowing of maize at second fortnight of June recorded higher starch (45.0 mg g^{-1}) and total soluble sugars (22.5 mg g^{-1}) over other dates of sowing (Table 4). Early sowing of maize resulted higher starch and total soluble sugars than delayed sowing. This was in line with the findings of Buriro *et al.*, 2015 [5].

Influence of silicon nutrition on physiological and biochemical parameters

Soil application of potassium silicate @ 60 kg ha^{-1} recorded significantly higher relative leaf water content (61.4 and 55.0% at 60 and 90 DAS, respectively) as compared to soil application of potassium silicate @ 20 kg ha^{-1} and foliar application of silicic acid at 0.25 per cent (Table 1). However, it was on par with soil application of potassium silicate @ 40 kg ha^{-1} and foliar application of silicic acid at 0.50 per cent. Silicon nutrition maintained higher water potential, lowered osmotic potential and improved relative leaf water content that showed improved drought tolerance in silicon-treated maize compared to plants that were grown without silicon under limited moisture supply. Improved performance of drought-stressed plants may be contributed to silicon nutrition that causes osmotic adjustment by maintaining the turgor pressure at low water potential. Exogenous silicon application considerably improved the relative leaf water content under both well watered and water deficit condition (Amin *et al.*, 2016) [4].

The improvement in photosynthesis might be related to ameliorative effect of silicon on plants under water stress that deposited as colloidal silica gel (SiO_2) in the xylem vessels and cell walls of leaves. So, decreases the bypass flow of transpired water that crosses the root cells towards the xylem vessels and provides a barrier to cuticular transpiration (Savvas *et al.*, 2009) [13]. Such effects of silicon increases the relative leaf water content of plant tissues to hold leaves erect and strengthening the stem to prevent lodging that results in improved accommodation of light in plant community thus improving photosynthesis (Abdalla, 2009) [1].

Among different levels of silicon, foliar application of silicic acid at 0.5 per cent (0.694 µg g^{-1}) was recorded significantly lower proline content over soil application of potassium silicate @ 20 kg ha^{-1} (0.997 µg g^{-1}) and foliar application of silicic acid at 0.5 per cent (0.915 µg g^{-1}) (Table 2). However, there was no significant difference between soil application of potassium silicate @ 60 kg ha^{-1} (0.733 µg g^{-1}) with foliar application silicic acid at 0.5 per cent (0.694 µg g^{-1}). Proline accumulation in response to stress has been reported widely and may play a vital role in stress adaptation within the cell (Gilbert *et al.*, 1998) [8].

Soil application of potassium silicate @ 60 kg ha^{-1} was recorded significantly lower transpiration rate (4.16 m mole of H_2O m^{-2} s^{-1}) over soil application of potassium silicate at 20 kg ha^{-1} (4.37 m mole of H_2O m^{-2} s^{-1}) and foliar application of silicic acid at 0.25 per cent (4.33 m mole of H_2O m^{-2} s^{-1}). Soil application of potassium silicate @ 60 kg ha^{-1} was on par with potassium silicate @ 40 kg ha^{-1} and foliar application of silicic acid at 0.50 per cent (Table 3). Reduced transpiration rates under water stress and silicon fertilization were observed for maize. Reduction of transpiration rates might be a consequence of silicon leaf depositions, that is, mainly the silicification of the extracellular matrix. Silicon depositions differ between abaxial (lower) and adaxial (upper) leaf surfaces. Application of silicon decreased transpiration from stomatal pores instead of from cuticular layers. It is well known that transpiration from leaves of maize plants is reduced considerably by silicon (silicic acid) application. This effect has been explained by a well-thickened layer of silica gel associated with the cellulose in the epidermal cell walls (Gao *et al.*, 2004) [7]. Similar results recorded by Ahmed *et al.*, 2013 [3] and Amin *et al.*, 2016 [4].

Significantly higher stomatal conductance recorded at soil application of potassium silicate @ 60 kg ha^{-1} (0.543 µ mole m^{-2} s^{-1}) over other silicon nutrition. However, it was on par with soil application of potassium silicate @ 40 kg ha^{-1} and foliar application of silicic acid at 0.50 per cent. Silicon application in sorghum increased stomotal conductance and alleviated the photosynthetic reduction by water stress. Similarly, antioxidants processes in crops were activated by silicon under water stress. Hence, silicon application may affect physiological traits to enhance crop tolerance under deficit irrigation (Ahmed *et al.*, 2011) [2].

Soil application of potassium silicate @ 60 kg ha^{-1} recorded significantly higher total soluble sugars (23.9 mg g^{-1}) and starch (43.5 mg g^{-1}) over other treatments. Soil application of silicon can enhance and maintain the starch and total soluble sugars in maize. Similar results quoted by Xie *et al.*, 2016 [16].

Interaction effect of dates of sowing and silicon nutrition

Overall interaction data with respect to relative leaf water content was found non significant. Among treatment combinations sowing of maize at second fortnight of June with soil application of potassium silicate @ 60 kg ha^{-1} resulted significantly higher relative leaf water content (63.3 and 57.5% at 60and 90 DAS respectively) as compared to other treatments, recommended dose of fertilizer and absolute control (Table 1). These results were in conformity with findings of Amin *et al.*, 2016 [4].

Foliar application of silicic acid at 0.50 per cent with sowing of maize at second fortnight of June recorded significantly lower proline content (0.390 µg g^{-1}) over other treatment combinations, recommended dose of fertilizer (0.648 µg g^{-1}) and absolute control (0.587 µg g^{-1}). Under moisture stress condition accumulation of proline was more. Second fortnight of June received 27.5 per cent less rainfall than normal rainfall and silicic acid spray at 0.50 per cent mitigate moisture stress and recorded lower proline. But under first and second fortnight of July the crop undergone more stress (36 and 46%) than second fortnight of June and hence accumulation was still more. But application of silicic acid spray at 0.50 per cent significantly reduced the proline content over other treatments, recommended dose of fertilizer and absolute control (Table 2).

Transpiration rate of maize at second fortnight of July with soil application of potassium silicate @ 60 kg ha^{-1} recorded significantly lower (4.01 m mole of H_2O m^{-2} s^{-1} at 60 DAS) than all other treatment combinations, recommended dose of fertilizer and absolute control. Similar results were reported by Maghsoudi *et al.*, 2016 [12]. Silicon mediated increase in growth of moisture stressed plants may be due to the important role of silicon in the promotion of water status of stressed plants that might be the reason of lowered transpiration. However, increase in the photosynthetic rate in silicon-fertilized drought-stressed plants may improve the growth (Ahmed *et al.*, 2013) [3].

The overall interaction data on total soluble sugars (TSS) and starch revealed that sowing of maize at second fortnight of June with soil application of potassium silicate @ 60 kg ha^{-1} recorded significantly higher total soluble sugars and starch (23.9 and 46.2 mg g^{-1} respectively) of maize as compared to all other treatment combinations, recommended dose of fertilizer (13.9 and 15.6 mg g^{-1} respectively) and absolute control (9.1 and 14.8 mg g^{-1} respectively). Under delayed sowing (first and second fortnight of July) starch and TSS was reduced due to moisture stress (Table 4).

Table 1: Relative leaf water content of maize as influenced by silicon nutrition at different dates of sowing

Treatments	Relative leaf water content (%)		
	30 DAS	60 DAS	90 DAS
Main plot (Dates of sowing)			
D_1	80.7	62.0	55.7
D_2	79.0	59.0	53.9
D_3	79.1	58.6	51.4
S.Em±	0.4	0.2	0.4
C.D. at 5%	NS	1.1	1.7
Sub plot (Silicon nutrition)			
S_1	79.0	58.0	51.6
S_2	79.7	60.1	54.2
S_3	80.0	61.4	55.0
S_4	79.4	58.8	52.9
S_5	79.9	61.1	54.4
S.Em±	0.3	0.3	0.3
C.D.at 5%	NS	0.9	1.02
Interaction (Dates of sowing × Silicon nutrition)			
D_1S_1	79.9	60.9	53.9
D_1S_2	80.9	62.0	56.2
D_1S_3	81.3	63.3	57.5
D_1S_4	80.2	61.0	54.3
D_1S_5	81.2	63.0	56.4
D_2S_1	78.7	57.7	51.7
D_2S_2	79.0	59.1	54.3
D_2S_3	79.3	60.2	54.8
D_2S_4	79.0	58.1	53.8
D_2S_5	79.2	60.1	54.6
D_3S_1	78.6	55.3	49.2
D_3S_2	79.0	59.3	52.0
D_3S_3	79.5	60.7	52.7
D_3S_4	79.1	57.4	50.7
D_3S_5	79.3	60.3	52.1
S.Em±	0.5	0.5	0.6
C.D. at 5%	NS	NS	NS
Control			
RDF	73.6	59.2	50.1
Absolute control	72.0	55.7	46.2
S.Em±	1.7	0.6	0.6
C.D. at 5%	4.9	1.61	1.8

Table 2: Proline content of maize as influenced by silicon nutrition at different dates of sowing

Treatments	Proline (µg g^{-1})	
	45 DAS	Anthesis-silking stage
Main plot (Dates of sowing)		
D_1	0.281	0.512
D_2	0.304	0.688
D_3	0.329	1.326
S.Em±	0.007	0.010
C.D. at 5%	0.029	0.038
Sub plot (Silicon nutrition)		
S_1	0.313	0.997
S_2	0.309	0.872
S_3	0.301	0.733
S_4	0.295	0.915
S_5	0.305	0.694
S.Em±	0.007	0.014
C.D. at 5%	NS	0.041
Interaction (Dates of sowing × Silicon nutrition)		
D_1S_1	0.290	0.614
D_1S_2	0.278	0.512
D_1S_3	0.272	0.391
D_1S_4	0.269	0.652
D_1S_5	0.295	0.390
D_2S_1	0.303	0.716
D_2S_2	0.311	0.478
D_2S_3	0.296	0.393
D_2S_4	0.300	1.057

D_2S_5	0.310	0.798
D_3S_1	0.347	1.660
D_3S_2	0.337	1.626
D_3S_3	0.335	1.415
D_3S_4	0.317	1.035
D_3S_5	0.310	0.895
S.Em±	0.013	0.024
C.D. at 5%	NS	0.071
Control		
RDF	0.283	0.648
Absolute control	0.290	0.587
S.Em±	0.013	0.023
C.D. at 5%	NS	0.067

Table 3: Transpiration rate and stomotal conductance of maize as influenced by silicon nutrition at different dates of sowing

	Transpiration rate (m mole of H_2O m^{-2} s^{-1})		Stomotal conductance (μ mole m^{-2} s^{-1})	
Treatments	**30 DAS**	**60 DAS**	**30 DAS**	**60 DAS**
Main plot (Dates of sowing)				
D_1	9.05	4.38	0.932	0.575
D_2	8.98	4.24	0.931	0.531
D_3	8.88	4.13	0.928	0.476
S.Em±	0.05	0.02	0.00	0.001
C.D. at 5%	NS	0.09	NS	0.005
Sub plot (Silicon nutrition)				
S_1	9.06	4.37	0.924	0.508
S_2	9.03	4.21	0.939	0.537
S_3	8.85	4.16	0.948	0.543
S_4	8.97	4.33	0.922	0.510
S_5	8.96	4.18	0.920	0.539
S.Em±	0.07	0.02	0.008	0.005
C.D.at 5%	NS	0.06	NS	0.013
Interaction (Dates of sowing × Silicon nutrition)				
D_1S_1	9.10	4.41	0.922	0.562
D_1S_2	9.07	4.36	0.941	0.584
D_1S_3	9.00	4.36	0.946	0.588
D_1S_4	9.10	4.41	0.928	0.555
D_1S_5	8.97	4.37	0.924	0.586
D_2S_1	9.00	4.40	0.931	0.521
D_2S_2	8.98	4.20	0.942	0.534
D_2S_3	8.90	4.13	0.954	0.538
D_2S_4	9.00	4.33	0.918	0.529
D_2S_5	9.03	4.14	0.911	0.535
D_3S_1	9.07	4.30	0.919	0.440
D_3S_2	9.03	4.06	0.934	0.493
D_3S_3	8.65	4.01	0.943	0.503
D_3S_4	8.80	4.24	0.921	0.447
D_3S_5	8.87	4.04	0.924	0.497
S.Em±	0.12	0.04	0.013	0.008
C.D. at 5%	NS	0.11	NS	0.023
Control				
RDF	9.10	4.62	0.850	0.556
Absolute control	9.30	4.63	0.813	0.553
S.Em±	0.12	0.04	0.012	0.008
C.D. at 5%	NS	0.114	0.035	0.023

Table 4: Grain quality parameters of maize as influenced by silicon nutrition at different dates of sowing

Treatments	TSS (Total soluble sugars) (mg g^{-1})	Starch (mg g^{-1})
Main plot (Dates of sowing)		
D_1	22.5	45.0
D_2	21.1	42.2
D_3	17.6	38.9
S.Em±	0.1	0.1
C.D. at 5%	0.4	0.5
Sub plot (Silicon nutrition)		
S_1	18.5	39.6
S_2	20.6	42.5
S_3	21.9	43.5
S_4	19.5	41.3

S_5	21.5	43.0
S.Em±	0.1	0.3
C.D.at 5%	0.3	0.8
Interaction (Dates of sowing × silicon nutrition)		
D_1S_1	20.1	42.1
D_1S_2	23.0	45.7
D_1S_3	23.9	46.2
D_1S_4	21.7	44.9
D_1S_5	23.6	45.7
D_2S_1	20.0	40.2
D_2S_2	20.7	43.0
D_2S_3	22.1	43.9
D_2S_4	20.5	40.4
D_2S_5	22.2	43.5
D_3S_1	15.4	36.5
D_3S_2	18.2	39.0
D_3S_3	19.6	40.3
D_3S_4	16.4	38.7
D_3S_5	18.6	40.0
S.Em±	0.2	0.5
C.D. at 5%	0.6	NS
Control		
RDF	14.0	27.5
Absolute control	9.2	20.3
S.Em±	0.2	0.4
C.D. at 5%	0.6	1.4

Conclusion

Foliar application of silicic acid at 0.50 per cent with sowing of maize at second fortnight of July recorded significantly lower proline content (0.895 µg g^{-1}) than other treatment combination, recommended dose of fertilizer (0.648 µg g^{-1}) and absolute control (0.587 µg g^{-1}).Soil application of potassium silicate @ 60 kg ha^{-1} with sowing of maize at second fortnight of July recorded significantly lower transpiration rate (4.01m mole of H_2O m^{-2} s^{-1}) over other treatment combinations, recommended dose of fertilizers and absolute control. Second fortnight of June with soil application of potassium silicate @ 60 kg ha^{-1} recorded significantly higher stomotal conductance (0.588 µ mole m^{-2} s^{-1} at 60 DAS) over recommended dose of fertilizers and absolute control. Sowing of maize at second fortnight of June recorded significantly higher starch (45mg g^{-1}) and total soluble sugars (22.5 mg g^{-1}) at harvest as compared to other dates of sowing. Soil application of potassium silicate @ 60 kg ha^{-1} recorded significantly higher starch content (43.5mg g^{-1}) and total soluble sugars (21.9 mg g^{-1}) as compared to soil application of potassium silicate @ 20 kg ha^{-1} (39.6 and 18.5 mg g^{-1}, respectively).

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