

ORIGINAL ARTICLE

Growth and Nitrogen Fixation in Silicon and/or Potassium Fed Chickpeas Grown under Drought and Well Watered Conditions

Fawaz Kurdali, Mohammad Al-Chammaa and Ahmad Mouasess

Atomic Energy Commission, Agriculture Department P.O. Box 6091, Damascus, Syria

*E-Mail: ascientific@aec.org.sy

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A pot experiment was conducted to study the effects of silicon (Si) and/or potassium (K) on plant growth, nitrogen uptake and N₂-fixation in water stressed (FC1) and well watered (FC2) chickpea plants using ¹⁵N and ¹³C isotopes. Three fertilizer rates of Si (Si₅₀, Si₁₀₀ and Si₂₀₀) and one fertilizer rate of K were used. For most of the growth parameters, it was found that Si either alone or in combination with K was more effective to alleviate water stress than K alone. Increasing soil water level from FC1 to FC2 often had a positive impact on values of almost all studied parameters. The Si₁₀₀K⁺ (FC1) and Si₅₀K⁺ (FC2) treatments gave high enough amounts of N₂-fixation, higher dry matter production and greater nitrogen yield. The percent increments of total N₂-fixed in the above mentioned treatments were 51 and 47% over their controls, respectively. On the other hand, increasing leaf's dry matter in response to the solely added Si (Si₅₀K⁺ and Si₁₀₀K⁺) is associated with lower Δ¹³C under both watering regimes. This may indicate that Si fertilization had a beneficial effect on water use efficiency (WUE). Hence, Δ¹³C could be an adequate indicator of WUE in response to the exogenous supply of silicon to chickpea plants. Our results highlight that Si is not only involved in amelioration of growth and in maintaining of water status but it can be also considered an important element for the symbiotic performance of chickpea plants. It can be concluded that the synergistic effect of silicon and potassium fertilization with adequate irrigation improves growth and nitrogen fixation in chickpea plants.

Key words: Silicon, Potassium, 15N, 13C, Nitrogen fixation, Chickpea

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Key words: Silicon, Potassium, 15N, $\Delta^{13}\text{C}$, Nitrogen fixation, Chickpea

Water stress is one of the major limitations to the agricultural productivity worldwide. Chickpea (*Cicer arietinum*.) is one of the oldest grain legumes, and is grown in a wide geographical range with varied agroclimatic conditions. It is a cool season annual crop which grows well in limited rainfall areas of the world. In the Mediterranean basin, this

grain legume is cultivated on a large scale under rainfed conditions, where water stress is considered a major limiting abiotic factor, reducing growth and N₂-fixation (Kurdali, 1996).

Increasing biological nitrogen fixation (BNF) in cropping systems is one way to increase productivity and to improve farming systems of

rain-fed areas in the Mediterranean region. It has been reported that soil water content in rain fed grown grain legumes decreased severely after flowering (Kurdali, 1996; Kurdali et al., 1997). This causes a decline in N₂-fixation and affects the distribution of nitrogen (N) in the plants. Since water is infrequently available for irrigation in semi-arid areas, another approach can be adopted to reduce the problem of drought stress, which frequently occurs during the reproductive stages of these grain legumes.

The management of plant nutrients is very useful to develop plant tolerance to drought. Better plant nutrition can effectively alleviate the adverse effects of drought by a number of mechanisms (Farooq et al., 2009; Waraich et al., 2011). Potassium (K) is reported to improve plant's resistance against drought stress (Marschner 1995) and can alleviate water shortages in many legume crops (Sangakkara et al., 1996, Kurdali et al., 2002, Kurdali and Al-Shammaa, 2010). This is attributed to its role in cell turgor control and metabolic activity (Beringer et al., 1983). Moreover, numerous reports indicate that silicon (Si) improve growth parameters of plants growing under water stress. Although silicon is not considered to be an essential nutrient for most terrestrial plants, it plays an important role in protecting plants from abiotic and biotic stresses (Ma 2004, Epstein 2009; Liang et al., 2007). Sacala (2009) reported that the beneficial effects of Si may result from better and more efficient osmoregulation, improved plant water status, reduction in water loss by transpiration, maintenance of adequate supply of essential nutrients, restriction in toxic ions uptake and efficient functioning of antioxidative mechanisms. Furthermore, the role of Si in plants is not restricted to formation of physical or mechanical barrier in

cell wall, lumens and intercellular voids, it can modulate plants' metabolism and alter physiological activity.

Carbon isotope discrimination ($\Delta^{13}\text{C}$) of C3 plant leaves is related to photosynthetic gas exchange, because $\Delta^{13}\text{C}$ is in part determined by C_i/C_a , the ratio of CO₂ concentration in the leaf intercellular spaces (C_i) to that of the atmosphere (C_a), (Farquhar and Rishards, 1984). Foliar $\Delta^{13}\text{C}$ values have been used as an integrated measure of the response of photosynthetic gas exchange to environmental variables such as water availability (Gondon et al., 2002). Low $\Delta^{13}\text{C}$ has been proposed as an indicator of high water use efficiency (WUE) in C3 plants (Farquhar and Richards, 1984). Although the relationship between water uptake, transpiration rate and Si deposition or K states in the plant is well established, there seems to be a distinct lack of work on the effects of these two nutrients on $\Delta^{13}\text{C}$, and consequently on water use efficiency.

Applied Si seems to interact favorably with other nutrients (N, P, and K) and offers the potential to improve efficiency in terms of yield response (Singh et al., 2005). Ashraf et al., 2009 reported that potassium and silicon improve yield and juice quality in sugarcane under salt stress. Since potassium and silicon have been noted for their particular role in enhancing drought tolerance of crops, very little is known about their beneficial effects on N₂-fixation by legumes in response to drought tolerance. Although the role of Si on nodulation and nitrogen fixation in legumes remains ambiguous, Nelwamondo and Dakora (1999) and Mali and Aery, (2008a) showed that silicon nutrition promotes nodule formation in cowpea plants. Hence, a better understanding of the interactions between silicon and other nutrient applications and plant responses will contribute to

more efficient fertilizer practices, particularly under water stress conditions. The present pot experiment aimed at investigating the effects of potassium and/or silicon fertilizer on dry matter production, N uptake, N₂-fixation and carbon isotope discrimination in chickpea plants subjected to different soil moisture levels at the beginning of flower bud initiation stage, using ¹³C and ¹⁵N isotopes.

MATERIALS AND METHODS

Soil Properties and Plant Materials

The experiment was conducted in pots, each one containing 10 kg of thoroughly mixed soil. The main physical and chemical soil properties are: pH 7.7; E_c 0.83 dS /m; organic matter 0.91%; cations mmol (e) /L (Ca²⁺ 1.1, Mg²⁺ 0.47, K⁺ 0.14, Na⁺ 1.27); anions mmol (e) /L (SO₄²⁻ 1.27, HCO₃⁻ 0.97, Cl⁻ 0.74); available P (Olsen) 6.8 µg/ g; total N 0.07%; NO₃⁻ 42µg/g; NH₄⁺ 26.1µg/g; clay 30.85%; Loam 17.99% and sand 51.26%.

Seeds of chickpea (cv. Gab 3 a local cultivar) and barley (a non-fixing reference crop) were used. After germination, plants were thinned to 2 plants per pot. The pots were set outdoors under natural climatic conditions. Since abundant nodules have already been observed on the roots of such legume species grown in the area, from which the soil was collected and used for this experiment, the seeds were therefore not inoculated.

Experimental Design and Treatments

The design was a split plot factorial, with irrigation regimes being the two main plots and the factors potassium and silicon fertilizers randomized within each irrigation regime as a 3 factorial (2 x 2 x 4) design. The irrigation regimes constitute two watering levels: water stress (FC1, 45-50% of field capacity) and well watered (FC2, 75-80% of field

capacity). Within each irrigation regime the first treatment was potassium fertilizer (K) applied in the form of K₂SO₄, at two levels: K⁻ and K⁺ (equivalent to 150 K₂O/ha). Within each K treatment, silicon fertilizer (Si) was applied in the form of sodium metasilicate (Na₂SiO₃·9H₂O) at four levels: no fertilizer (Si⁻) versus application of 50, 100 and 200 µg Si/g air-dried soil (abbreviated as Si₅₀, Si₁₀₀ and Si₂₀₀, respectively). All treatments were replicated four times. Total number of pots was 128 (i.e. 2 crops x 2 water regimes x 2 K levels x 4 Si levels x 4 replicates). Soil water content in all pots was maintained at around 75% of field capacity from planting up to bud flower initiations. Thereafter, plants were subjected to the two above-mentioned soil moisture regimes, starting from bud flower initiation to physiological maturity stage. Pots were weighed every 3 days and water was added to maintain the soil moisture levels previously described.

¹⁵Nitrogen Fertilizer

For estimating the fractional contribution of fixed N₂ derived from air (Ndfa), soil (Ndfs) and from fertilizer (Ndff) using ¹⁵N isotope dilution method (Fried and Middelboe 1977), an equivalent rate of 20 kg N/ha of ¹⁵N labeled urea (10%¹⁵N atom excess) was applied at planting of both plant species. Barley was used as a non-fixing reference crop for estimating %Ndfa by chickpea plants.

Plant Sampling and Isotopic Composition Analyses

Plants were harvested 12 weeks after planting. The various plant parts of both species were oven dried at 70 °C, weighed and ground to a fine powder. Concentration of total N and C, and isotopic composition of ¹⁵N and ¹³C were determined on sub-samples (7 and 2 mg dry weight for N and C determination, respectively) of different

plant parts using the continuous-flow isotope ratio mass spectrometry (Integra-CN, PDZ Europea Scientific Instrument, UK). Isotopic compositions are expressed using delta notation (δ) in parts per thousand (‰):

$$\delta (\text{‰}) = [(R_{\text{sample}}/R_{\text{standard}}) - 1] 1000$$

where R is the ratio of $^{15}\text{N}/^{14}\text{N}$ or $^{13}\text{C}/^{12}\text{C}$.

Carbon isotope discrimination ($\Delta^{13}\text{C}$) was calculated according to Farquhar et al. (1982):

$$\Delta^{13}\text{C} = (\delta^{13}\text{C}_{\text{air}} - \delta^{13}\text{C}_{\text{sample}}) / (1 - \delta^{13}\text{C}_{\text{sample}}/1000)$$

where $\delta^{13}\text{C}_{\text{air}}$ is the $\delta^{13}\text{C}$ value in air (-8‰) and $\delta^{13}\text{C}_{\text{sample}}$ is the measured value in the plant.

Statistical Analysis

The data were subjected to analysis of variance (ANOVA) test, and means were compared using the Least Significant Difference (Fisher's PLSD) test at the 0.05 level of confidence.

RESULTS

Dry Matter Yield

Both soil moisture levels and fertilizers had significant effects on dry matter yield in different plant parts of chickpea plants (Table 1). For shoots, the lowest dry matter yield (8.6 g/pot) was observed in plants grown without fertilization (S'K) under water stress treatment (FC1). Sole application of silicon (K'Si₅₀, K'Si₁₀₀ and K'Si₂₀₀) or in combination with potassium (Si₅₀K⁺, Si₁₀₀K⁺ and Si₂₀₀K⁺) significantly increased dry matter yield of shoots by 19, 17, 19, 18, 40 and 21%, respectively. However, no significant effect was observed between S'K⁺ and S'K. Increasing soil water level from FC1 to FC2 often had a positive impact on shoot dry matter yield. The highest value was in the Si₁₀₀K⁺ where the percent increment rate was 28% as compared with the control (S'K). Moreover, Si₅₀K⁺ and Si₅₀K⁺ in FC2 showed significant increments by

15 and 24% compare with S'K, respectively. The pattern of pod's dry matter yield was relatively similar to that of shoots. In water stressed plants, sole application of Si or in combination with K significantly increased dry matter yield of pods. The highest increment rates (75%) were in K⁺Si₁₀₀ and Si₂₀₀K⁺ treatments. Increasing soil water level from FC1 to FC2 also showed positive impacts on pod's dry matter yield regardless of fertilization. The addition of Si along with K fertilizers (Si₅₀K⁺ and Si₁₀₀K⁺) in FC2 surpassed the other treatments in terms of pod's dry matter yield which was improved by 40 and 22%, respectively, as compared with the control (S'K). In roots, however, chickpea showed no significant response to fertilization under both watering regimes excepting in the FC1-Si₁₀₀K⁺ treatment where the percent increment rate was 64% over the control. Moreover, root's dry matter yield generally improved in the FC2 as compared with FC1.

Nodule dry matter yield in well-watered plants was significantly higher than that in water stressed plants in all fertilization treatments. The lowest value (0.67 g/pot) was observed in plants grown under water stress treatment (FC1) without fertilization (S'K). However, the addition of Si along with K fertilizer (particularly K⁺Si₁₀₀) increased nodule dry weight by 69% over the control.

The effect of soil moisture and fertilizer amendment on the whole plant dry matter yield is shown in Fig. 1. Under water stress conditions (FC1), the whole plant dry matter yield ranged between 13.2 g/pot (S'K) and 19.8 g/pot (Si₁₀₀K⁺). Sole application of silicon (Si₅₀K⁺, Si₁₀₀K⁺ and Si₂₀₀K⁺) or in combination with potassium (Si₅₀K⁺, Si₁₀₀K⁺ and Si₂₀₀K⁺) significantly increased dry matter yield of the whole plant by 19, 22, 25, 21, 50 and 30%, respectively as compared with the control (S'K).

However, K alone did not significantly affect dry matter yield of chickpea under both watering regimes. The whole plant dry matter yield in well-watered plants was significantly higher than that in water stressed plants in almost all of the fertilized treatments. Under well watered conditions (FC2), whole plant dry matter yield ranged between 17.6 g/pot (S'K) and 22 g/pot (Si₅₀K⁺). Sole application of silicon (Si₅₀K) or in combination with potassium (Si₅₀K⁺, Si₁₀₀K⁺ and Si₂₀₀K⁺) significantly increased dry matter yield of the whole plant by 14, 25, 23, and 14%, respectively.

Nitrogen Yield

The effect of soil moisture and fertilizer amendment on total N (TN) yield in different parts of chickpea plants is shown in Table 2. Under water stress conditions (FC1), sole applications of Si or K did not significantly increase N yield of chickpea shoots. However, Si in combination with K, particularly the Si₁₀₀K⁺ treatment, significantly improved shoot N yield by 27% as compared with the control (S'K). Shoot N yield in well-watered plants were generally higher than that in water stressed plants regardless of fertilization treatments. In comparison with the control, sole application of silicon (KSi₅₀) or in combination with potassium (Si₅₀K⁺, Si₁₀₀K⁺ and Si₂₀₀K⁺) significantly increased shoot N yield of the well watered plants by 31, 33, 39, and 32%, respectively. The effect of fertilizers on pod's N yield in water stressed plants was more pronounced than that on shoots. Sole application of silicon (Si₅₀K, Si₁₀₀K and Si₂₀₀K) or in combination with potassium (Si₅₀K⁺, Si₁₀₀K⁺ and Si₂₀₀K⁺) significantly increased pod's N yield by 39, 70, 61, 59, 83 and 70%, respectively as compared with the control (S'K). However, K alone did not significantly affect pod N yield of chickpea under both watering regimes. Under well watered

conditions, Si plus K significantly increased pod's N yield by 28, 16 and 20% for Si₅₀K⁺, Si₁₀₀K⁺ and Si₂₀₀K⁺, respectively. The pattern of root N yield was relatively similar to that of dry matter yield. Chickpea showed no significant response to fertilization under water stress conditions excepting in the Si₁₀₀K⁺ treatment where the percent increment rate of root N yield was 62% over the control. Under well watering regime, however, a positive impact on root N yield in response to Si and/or K was generally observed.

Nodule N yield in well-watered plants was significantly higher than that in water stressed plants in all fertilization treatments. The positive impact of Si and K on nodule N yield was more pronounced for plants grown under stressed conditions (FC1) than those grown under non-stressed (FC2) which imply a significant response to fertilization. The lowest value (24.6 mg N/pot) was observed in plants grown under water stress treatment (FC1) without fertilization (S'K). Sole applications of Si (Si₂₀₀K) or K fertilizers (Si'K⁺) and Si plus K fertilizer (Si₁₀₀K⁺ and Si₂₀₀K⁺) increased nodule N yield by 67, 28, 81 and 67%, respectively. This result indicated that Si and K had a beneficial effect on nodule N yield in water stressed chickpea particularly in the Si₁₀₀K⁺ treatment.

The effect of soil moisture and fertilizer amendment on whole plant N yield is shown in Fig 2. The pattern of total N yield was relatively similar to that of total dry matter yield. Under water stress conditions (FC1), whole plant N yield ranged between 325 (S'K) and 477 mg N/pot (Si₁₀₀K⁺). Sole application of silicon (Si₅₀K, Si₁₀₀K and Si₂₀₀K) or in combination with potassium (Si₅₀K⁺, Si₁₀₀K⁺ and Si₂₀₀K⁺) significantly increased N yield of the whole plant by 16, 20, 26, 21, 47 and 32%, respectively as compared with the control (S'K). However, K alone

did not significantly affect N yield of chickpea under both watering regimes. Whole plant N yield in well-watered plants was significantly higher than that in water stressed plants in almost all of the fertilized treatments. Under well watered conditions (FC2), whole plant N yield ranged between 412 (S⁻K) and 532 mg N/pot (Si₅₀K⁺). Sole application of silicon (Si₅₀K) or in combination with potassium (Si₅₀K⁺, Si₁₀₀K⁺ and Si₂₀₀K⁺) significantly increased total N yield by 22, 30, 28, and 26%, respectively.

Percentages and Amounts of N₂ Fixed

The fractional contribution of fixed N₂ derived from air (%Ndfa) in different parts of chickpea plants is presented in Table 3. Ndfa% in shoots ranged from 71 to 77% in FC1 and from 73 to 85% in FC2. Sole application of silicon significantly improved %Ndfa particularly when applied at higher rate (Si₂₀₀K). Moreover, silicon in combination with potassium (Si₅₀ K⁺ and Si₂₀₀K⁺) significantly increased %Ndfa. However, K alone did not significantly affect %Ndfa of chickpea's shoots under FC1. %Ndfa in well-watered plants were significantly higher than that in water stressed plants in almost all of the fertilized treatments. The lowest %Ndfa in FC2 was in the non fertilized treatment (S⁻K). However, application of Si and/ or K significantly improved %Ndfa.

The pattern of %Ndfa in pods was relatively similar to that of shoots. Ndfa% ranged from 71 to 81% in FC1 and from 75 to 83% in FC2. In nodulated root, however, %Ndfa values were lower than the aerial parts, and ranged from 43 to 61% % in FC1, and from 57 to 70% in FC2. In the whole plant, %Ndfa ranged from 66 to 75% in FC1 and from 71 to 84% in FC2 (Fig. 3). The lowest %Ndfa (66%) was observed in plants grown without fertilization (S⁻K) under water stress treatment (FC1). Sole or dual applications of silicon and potassium frequently

increased N₂-fixation. The %Ndfa values in well-watered plants were significantly higher than that in water stressed plants in almost all of the fertilized treatments.

Regarding the amounts of N₂ fixed in shoots, the lowest values (141 and 153 mg N/pot, in FC1 and FC2, respectively) were observed in plants grown without fertilization (S⁻K), (Table 3). Under water stress conditions, the highest amount was in the Si₁₀₀K⁺ treatment (21% over the control). Values of amounts of N₂ fixed in well-watered plants were generally higher than those in water stressed plants regardless of fertilization treatments. Positive and significant impacts on N₂ fixed yield in response to Si and/or K were generally observed in plants grown under well watering regime. Application of Si at a lower rate (Si₅₀) with (Si₅₀K⁺) or without K (Si₅₀K) gave high enough amounts of N₂ fixation where the percent increments over the control were 55 and 52%, respectively.

For pods, the lowest amount of N₂ fixation (48 mg/pot) was observed in plants grown without fertilization (S⁻K) under water stress treatment (FC1). Sole application of silicon (Si₅₀K, Si₁₀₀K and Si₂₀₀K) or in combination with potassium (Si₅₀K⁺, Si₁₀₀K⁺ and Si₂₀₀K⁺) significantly increased N₂-fixation of pods by 51, 83, 83, 73, 87 and 82%, respectively. However, no significant effect was observed between S⁻K⁺ and S⁻K. Increasing soil water level from FC1 to FC2 often had a positive impact on N₂ fixed. The highest value was in the K⁺Si₅₀ where the percent increment rate was 26% as compared with the control (S⁻K), (Table 3).

In nodulated root, significant impact on N₂ fixed yield in response to Si and/or K was generally observed in plants grown under both watering regimes. Amount of N₂ fixed in the FC1-Si₁₀₀K⁺ treatment was two-folds more than that in the

control. Moreover, values in well-watered plants were significantly higher than that in water stressed plants in almost all of the fertilized treatments (Table 3).

In the whole plant (Fig. 3), the lowest amount of N_2 fixation (214 mg N/pot) was observed in plants grown without fertilization (S'K) under water stress treatment (FC1). Sole application of silicon ($Si_{50}K$, $Si_{100}K^+$ and $Si_{200}K^+$) or in combination with potassium ($Si_{50}K^+$, $Si_{100}K^+$ and $Si_{200}K^+$) significantly increased N_2 fixation in the whole plant by 23, 29, 43, 35, 51 and 47%, respectively. However, slight but significant increment was observed in sole fed K plants (15%). Increasing soil water level from FC1 to FC2 had a significant impact on N_2 fixed in all fertilizer treatments. Also, dual or separate applications of Si and K significantly increased amounts of N_2 -fixation in chickpea grown under non-stressed conditions. The highest value was in the $Si_{50}K^+$ where the percent increment rate was 47% as compared with the control (S'K).

Nitrogen Derived from Fertilizer (Ndff), Soil (Ndfs) and N- Use Efficiency (%NUE)

The effect of soil moisture and fertilizer amendment on nitrogen derived from fertilizer (Ndff) in the whole plant is shown in Figure 4. Under water stress conditions (FC1), dual application of Si and K ($Si_{100}K^+$) significantly increased amount of Ndff by 52% as compared with the control (S'K). Moreover, $Si_{100}K^+$ and $Si_{200}K^+$ treatments also showed higher values (24% over the control). Under non stress conditions (FC2), the highest amount of Ndff was observed in the $Si_{200}K^+$ treatment (32% over the control); whereas, no significant differences were observed between the control and other fertilizer treatments, excepting the Si^+K^+ which showed the lowest value.

Under water stress conditions (FC1), the highest amount of Ndfs was observed in the $Si_{100}K^+$ treatment (35% over the control). Whereas, the highest value in the well watered plants was in the $Si_{100}K^+$ treatment (19% over the control).

Fertilizer nitrogen use efficiency (%NUE) in the whole plant is shown in Table 4. Under water stress conditions (FC1), NUE% in the non fertilized treatment was 37.4%. Significant increments in NUE % were observed in $Si_{100}K^+$, $Si_{100}K^+$ and $Si_{200}K^+$ treatments (46, 60 and 46.5%, respectively). Under non stress conditions (FC2), the highest NUE% was observed in the $Si_{200}K^+$ treatment (64%), whereas the lowest was in Si^+K^+ (39%)

Plant Nitrogen Balance

Plant N balance is calculated by subtracting the total amount of Ndfs in pods from amounts of Ndfa in plant residues (shoots + nodulated roots). Consequently, estimated amounts of N_2 fixed which may be returned to the soil (N-balance) following pod harvest are shown in Fig. 5. Under water stress conditions (FC1), plant N-balance in the control was +107 mg N/pot. Sole application of silicon ($Si_{200}K$) significantly increased plant N-balance by 48% (+157 mg N/pot). Dual applications of Si and K ($Si_{50}K^+$, $Si_{100}K^+$ and $Si_{200}K^+$) had also positive impact on N- balance (+146, +144 and +169 mg N/pot, respectively). However, no significant effect was observed between S'K⁺ and S'K. Moreover, values in well-watered plants were in most cases higher than that in water stressed plants in almost all of the fertilized treatments. Significant impacts on N-balance in response to Si and/or K were generally observed in plants grown under well watering regime. Application of Si at a lower rate (Si_{50}) with ($Si_{50}K^+$) or without K ($Si_{50}K$) gave high enough N-balance values (231 and 237 mg N/pot respectively). Percent increments in these latter

treatments were 56 and 60% over the control, respectively.

Carbon isotope discrimination ($\text{‰}\Delta^{13}\text{C}$)

Data presented in Figure 6 showed that carbon isotope discrimination ($\text{‰}\Delta^{13}\text{C}$) in chickpea's shoots was affected by the rate of fertilizers and soil water levels. Under water stress conditions (FC1), $\Delta^{13}\text{C}$ value in the control (Si-K) was 18.49‰. Sole application of Si in the Si₅₀K and Si₁₀₀K treatments resulted in a significant decrease in $\Delta^{13}\text{C}$ (17.92 and 17.94‰, respectively). However, no significant differences were observed between the control and

other fertilizer treatments. Moreover, $\Delta^{13}\text{C}$ values in well-watered plants were, in most cases, higher than those in water stressed plants in almost all of the fertilized treatments. Under well watering regime (FC2), $\Delta^{13}\text{C}$ values in the Si₅₀K and Si₁₀₀K treatments (18.21 and 18.36‰, respectively) were also significantly lower than those of the control (19.39‰). Moreover, sole application of K (19.02‰) or in combination with Si₅₀ (18.32‰) showed lower $\Delta^{13}\text{C}$ values in comparison with the control; whereas, no significant decreases were obtained in the other fertilizer treatments.

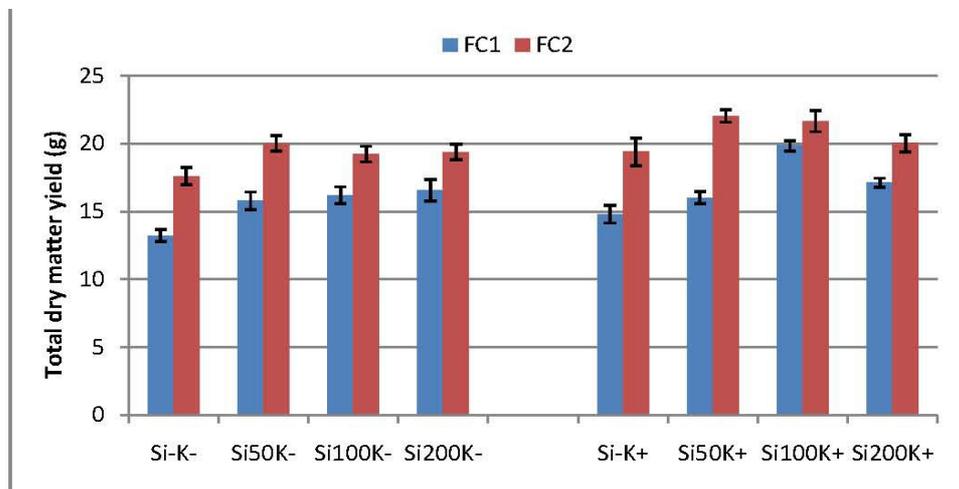


Figure 1. Dry matter yield (g/pot) of the whole plant of chickpea grown under two irrigation regimes (FC) as affected by silicon (Si) and potassium (K) fertilizers

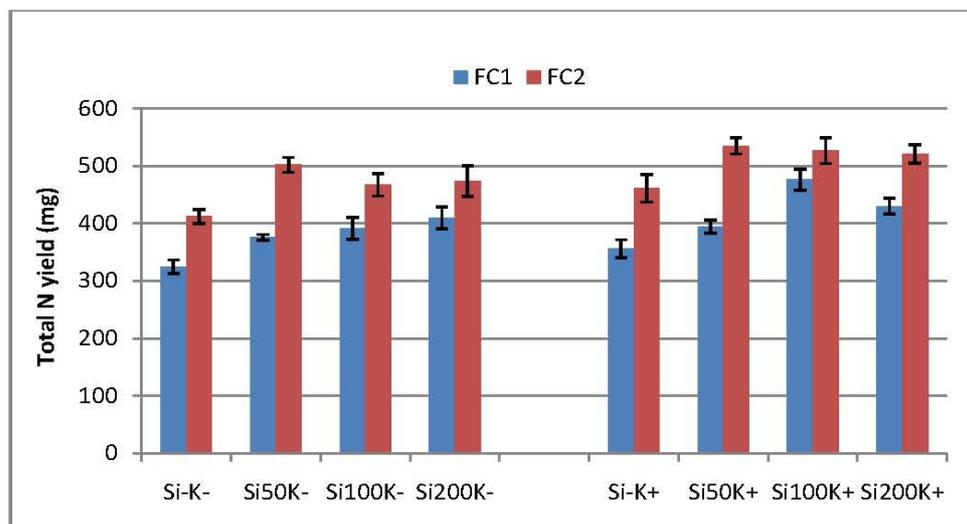


Figure 2. Nitrogen yield (mg N/pot) in the whole plant of chickpea grown under two irrigation regimes (FC) as affected by silicon (Si) and potassium (K) fertilizers

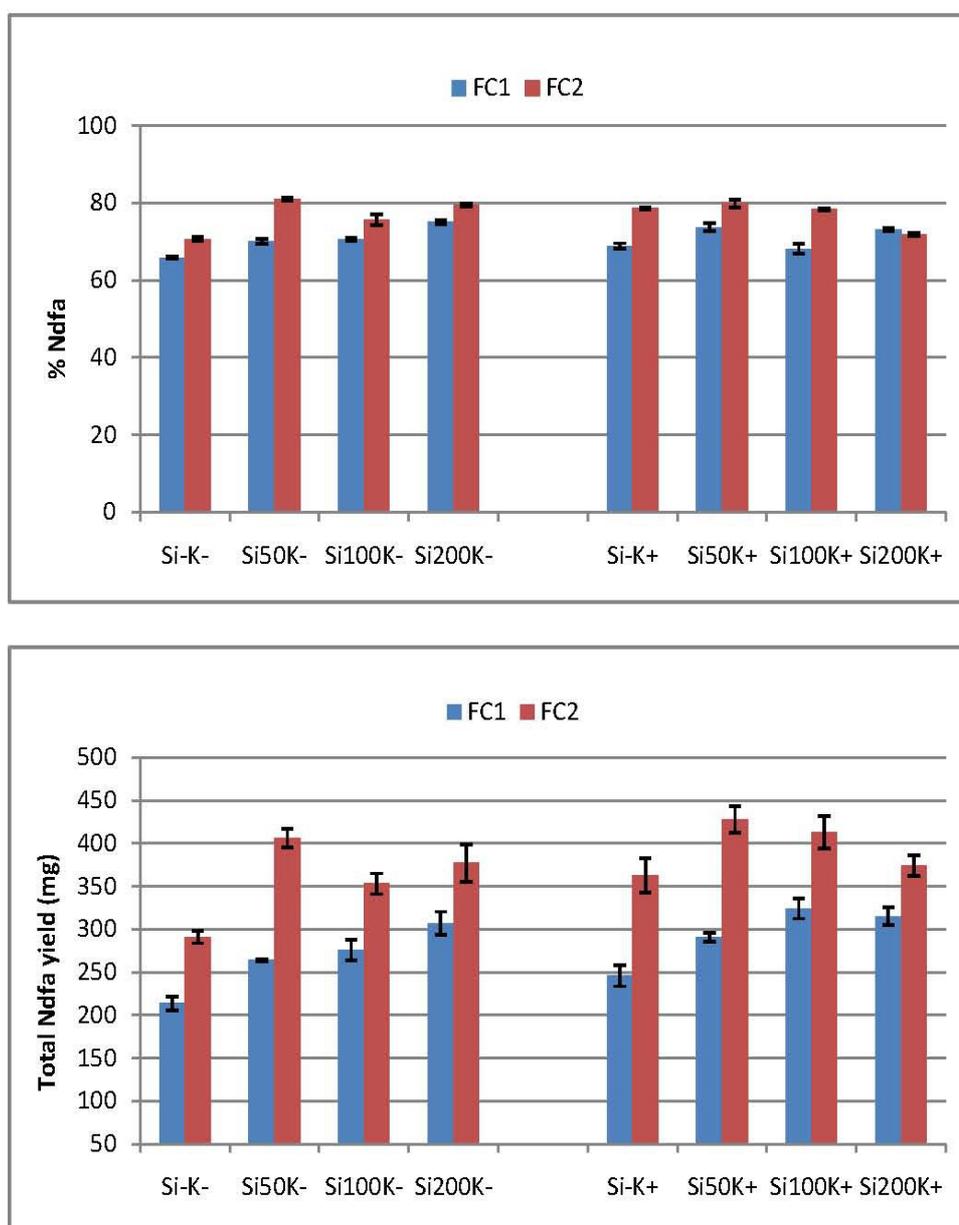


Figure 3. Proportions (%) and amounts of nitrogen derived from atmosphere (Ndfa), (mg N/pot) in the whole plant of chickpea grown under two irrigation regimes (FC) as affected by silicon (Si) and potassium (K) fertilizers.

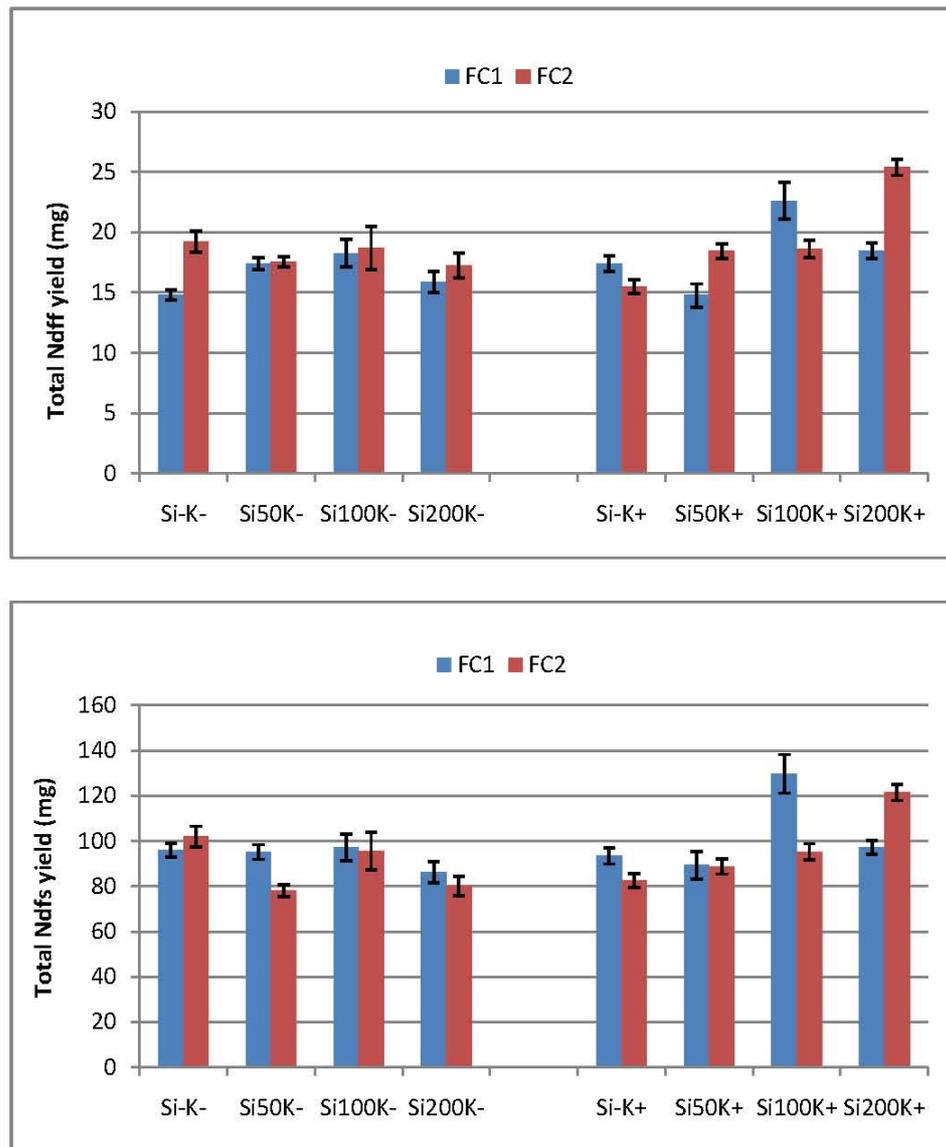


Figure 4. Amounts of nitrogen derived from fertilizer (Ndff) and soil (Ndfs) in the whole plant of chickpea grown under two irrigation regimes (FC) as affected by silicon (Si) and potassium (K) fertilizers

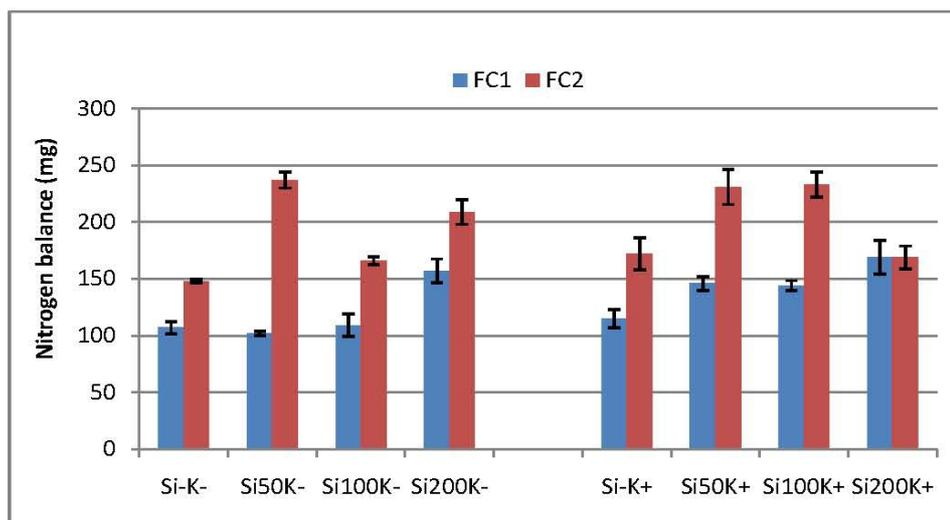


Figure 5. Estimated amounts of N_2 fixed which may be returned to the soil after pod removal (i.e plant N-balance, mg N/pot) in the whole plant of chickpea grown under two irrigation regimes (FC) as affected by silicon (Si) and potassium (K) fertilizers.

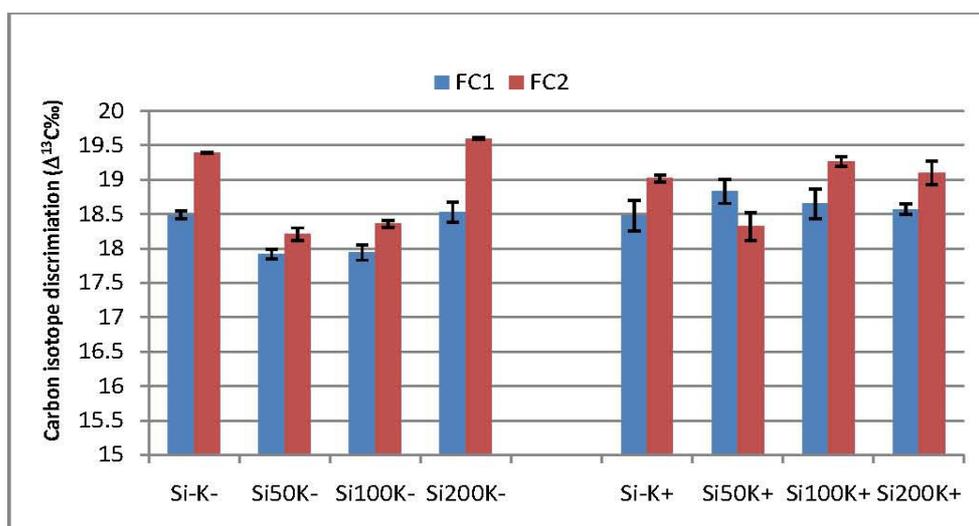


Figure 6. Carbon isotope discrimination ($\Delta^{13}C\%$) in leaves of chickpea grown under two irrigation regimes (FC) as affected by silicon (Si) and potassium (K) fertilizers

Table 1. Dry matter yield (g/pot) in different plant parts of chickpea plants grown under two irrigation regimes (FC) as affected by silicon (Si) and potassium (K) fertilizers.

Treatment	Shoots			Pods			Roots			Nodules		
	FC1	FC2	LSD	FC1	FC2	LSD	FC1	FC2	LSD	FC1	FC2	LSD
Si-K ⁻	8.6c,B	10.2d,A	0.86	2.0d,B	3.2c,A	0.58	1.9b,B	2.6a,A	0.43	0.7b,B	1.6a,A	0.24
Si ₅₀ K ⁻	10.3b,B	11.7bc,A	1.39	2.7bc,B	3.5bc,A	0.72	2.1b,B	3.1a,A	0.74	0.8b,B	1.8a,A	0.36
Si ₁₀₀ K ⁻	10.1b,A	11.3cd,A	N.S	3.2ab,A	3.6bc,A	N.S	2.2b,A	2.8a,A	N.S	0.8b,B	1.6a,A	0.27
Si ₂₀₀ K ⁻	10.3b,A	11.4cd,A	N.S	3.2ab,A	3.6bc,A	N.S	2.2b,B	2.9a,A	0.54	1.1a,B	1.7a,A	0.24
Si-K ⁺	9.6bc,B	11.3cd,A	1.66	2.5cd,B	3.4bc,A	0.57	2.0b,B	3.0a,A	0.75	0.8b,B	1.7a,A	0.34
Si ₅₀ K ⁺	10.2b,B	12.6ab,A	1.71	2.9bc,B	4.5a,A	0.88	2.1b,B	3.1a,A	0.78	0.8b,B	1.8a,A	0.37
Si ₁₀₀ K ⁺	12.1a,B	13.1a,A	0.99	3.5a,A	3.9ab,A	N.S	3.2a,A	3.0a,A	N.S	1.1a,B	1.6a,A	0.36
Si ₂₀₀ K ⁺	10.4b,A	11.7bc,A	N.S	3.5a,A	3.7bc,A	N.S	2.2b,B	3.0a,A	0.64	1.0a,B	1.6a,A	0.26
LSD _{0.05}	1.16	1.21		0.64	0.54		0.45	N.S		0.61	N.S	

Means within a row (capital letters) and within a column (small letters) followed by the same letter are not significantly different ($P > 0.05$).

Table 2. Nitrogen yield (mg N/ pot) in different plant parts of chickpea plants grown under two irrigation regimes (FC) as affected by silicon (Si) and potassium (K) fertilizers.

Treatment	Shoots			Pods			Roots			Nodules		
	FC1	FC2	LSD	FC1	FC2	LSD	FC1	FC2	LSD	FC1	FC2	LSD
SiK	198.7c,A	209.1d,A	N.S	67.7d,B	108.7c,A	20.3	34.2b,B	41.6b,A	6.87	24.6c,B	53.0a,A	6.4
Si ₅₀ K	211.5bc,B	273.5ab,A	22.3	94.3bc,A	114.5bc,A	N.S	40.3b,A	53.7a,A	N.S	30.0bc,B	60.1a,A	12.9
Si ₁₀₀ K	205.8bc,A	244.7bcd,A	N.S	115.2ab,A	118.1bc,A	N.S	42.0b,A	49.1ab,A	N.S	28.4bc,B	55.6a,A	9.7
Si ₂₀₀ K	220.5abc,A	248.2bc,A	N.S	108.8abc,A	115.1bc,A	N.S	39.2b,B	54.5a,A	8.53	41.2a,B	56.8a,A	8.9
SiK ⁺	202.5bc,A	231.2cd,A	N.S	88.8cd,B	115.4bc,A	19.8	33.4b,B	55.6a,A	13.9	31.6b,B	58.8a,A	13.9
Si ₅₀ K ⁺	219.5abc,B	277.5ab,A	52.6	107.5abc,B	138.9a,A	27.0	36.7b,B	55.1a,A	14.3	31.1bc,B	63.6a,A	9.9
Si ₁₀₀ K ⁺	252.7a,A	289.8a,A	N.S	124.1a,A	126.1abc,A	N.S	55.3a,A	51.0ab,A	N.S	44.5a,B	60.3a,A	14.1
Si ₂₀₀ K ⁺	234.4ab,A	275.8ab,A	N.S	115.4ab,A	130.3ab,A	N.S	39.3b,B	52.9a,A	11.9	41.2a,B	61.9a,A	10.9
LSD _{0.05}	35.18	37.80		22.15	19.82		9.05	11.13		6.62	N.S	

Means within a row (capital letters) and within a column (small letters) followed by the same letter are not significantly different ($P > 0.05$).

Table 3. Proportions of nitrogen derived from atmosphere (%Ndfa) in different plant parts of chickpea plants grown under two irrigation regimes (FC) as affected by silicon (Si) and potassium (K) fertilizers.

Treatment	Shoots			Pods			Nodulated roots		
	FC1	FC2	LSD	FC1	FC2	LSD	FC1	FC2	LSD
SiK	70.72d,B	73.31e,A	2.3	71.15c,B	76.91e,A	1.4	43.19c,B	57.40e,A	3.6
Si ₅₀ K	74.36bc,B	84.82a,A	1.0	77.23b,B	83.01a,A	0.9	47.74bc,B	69.45ab,A	5.8
Si ₁₀₀ K	74.78bc,B	80.14c,A	5.2	76.66b,A	75.92e,A	N.S	47.84bc,B	65.36cd,A	7.2
Si ₂₀₀ K	77.42a,B	82.93ab,A	1.2	81.27a,A	80.83c,A	N.S	60.17a,B	70.25a,A	4.0
SiK ⁺	71.47d,B	84.84a,A	1.3	71.31c,B	82.36ab,A	2.5	57.27a,B	62.33d,A	4.7
Si ₅₀ K ⁺	76.53ab,B	85.36a,A	3.7	77.76b,B	81.48bc,A	1.4	58.08a,B	65.31cd,A	7.7
Si ₁₀₀ K ⁺	72.77cd,B	81.05bc,A	3.6	72.71c,B	82.27ab,A	3.1	50.61b,B	66.96bc,A	2.2
Si ₂₀₀ K ⁺	75.70ab,A	75.93d,A	N.S	76.10b,A	75.13e,A	N.S	61.06a,A	58.06e,A	N.S
LSD _{0.05}	2.59	2.50		1.88	0.96		5.44	3.24	

Means within a row (capital letters) and within a column (small letters) followed by the same letter are not significantly different ($P > 0.05$).

Table 4. Amounts of nitrogen derived from atmosphere (mg N/ha) in different plant parts of chickpea plants grown under two irrigation regimes (FC) as affected by silicon (Si) and potassium (K) fertilizers.

Treatment	Shoots			Pods			Nodulated roots		
	FC1	FC2	LSD	FC1	FC2	LSD	FC1	FC2	LSD
SiK	140.5d,B	153.3c,A	12.6	48.2d,B	83.5c,A	15.5	25.4c,B	54.3b,A	6.9
Si ₅₀ K	157.2abcd,B	232.0a,A	17.4	72.8bc,B	95.1bc,A	18.1	33.5b,B	79.1a,A	14.5
Si ₁₀₀ K	153.9bcd,B	195.4b,A	30.6	88.3ab,A	89.7bc,A	N.S	33.7b,B	68.3a,A	8.5
Si ₂₀₀ K	170.7abc,A	205.7ab,A	N.S	88.4b,A	93.0bc,A	N.S	48.3a,B	78.2a,A	8.4
SiK ⁺	144.8cd,B	196.3b,A	32.2	63.3cd,B	95.1bc,A	14.8	37.4b,B	71.6a,A	20.0
Si ₅₀ K ⁺	167.7abc,B	237.2a,A	47.6	83.6ab,B	113.9a,A	22.1	39.2b,B	77.4a,A	8.9
Si ₁₀₀ K ⁺	183.6a,B	234.9a,A	27.1	90.27a,A	103.7ab,A	N.S	50.4a,B	74.7a,A	14.9
Si ₂₀₀ K ⁺	177.8ab,A	209.5ab,A	N.S	87.8ab,A	97.9bc,A	N.S	49.0a,B	66.7ab,A	10.0
LSD _{0.05}	26.41	31.77		16.91	15.98		6.91	12.87	

Means within a row (capital letters) and within a column (small letters) followed by the same letter are not significantly different ($P > 0.05$).

Table 5. Nitrogen use efficiency (%NUE) of added fertilizer in the whole plant of chickpea plants grown under two irrigation regimes (FC) as affected by silicon (Si) and potassium (K) fertilizers.

Treatment	NUE (%)		
	FC1	FC2	LSD
SiK	37.37c,B	48.49b,A	6.0
Si ₅₀ K	43.84bc,A	44.17bc,A	N.S
Si ₁₀₀ K	46.05b,A	47.10b,A	N.S
Si ₂₀₀ K	40.03bc,A	43.47bc,A	N.S
SiK ⁺	43.87bc,A	39.07c,A	N.S
Si ₅₀ K ⁺	37.22c,B	46.44b,A	6.97
Si ₁₀₀ K ⁺	59.97a,A	46.92b,A	N.S
Si ₂₀₀ K ⁺	46.51b,B	63.97a,A	5.75
LSD _{0.05}	6.68	6.74	

Means within a row (capital letters) and within a column (small letters) followed by the same letter are not significantly different ($P > 0.05$).

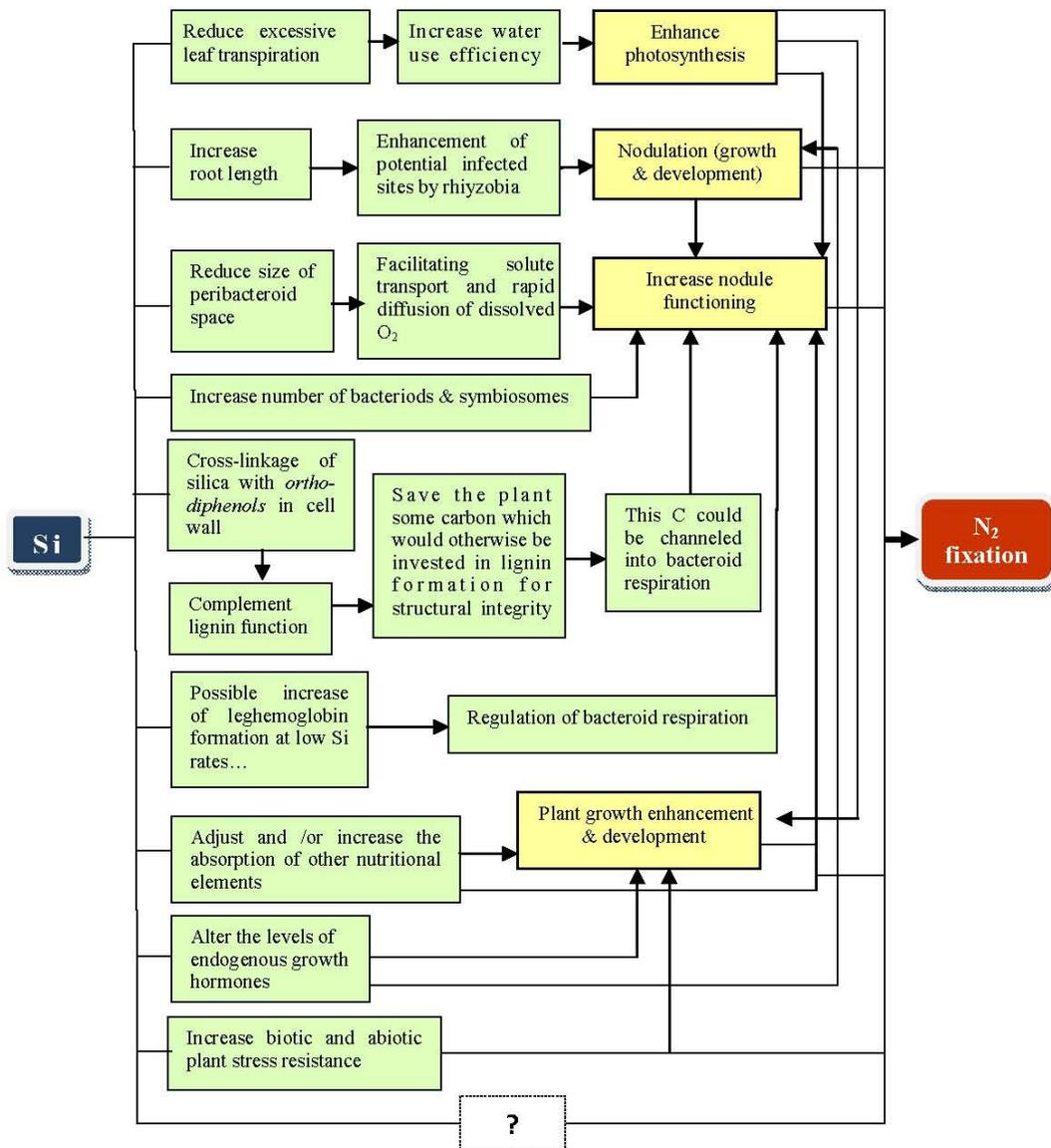


Figure 7. Mechanisms which may be involved in the enhancement of N₂ fixation in silicate treated legumes. See Nelwamondo and Dakora 1999; Nelwamondo et al., 2001; Mali and Aery 2008a; Hamayun et al., 2010; Chen et al., 2011.

DISCUSSION

The results of this study indicate that water restriction during the post-flowering period in chickpea considerably affect growth and N_2 -fixation. Increasing soil water humidity from FC1 to FC2 had a positive impact on chickpea growth with respect to dry matter yield. Sole application of silicon or in combination with potassium to water stressed and well watered chickpeas significantly increased dry matter yield. However, potassium did not significantly affect dry matter yield of chickpea under prevailing experimental conditions. Si in combination with K was often more effective in producing dry matter yield than solely added Si. $Si_{100}K^+$ and $Si_{50}K^+$ treatments gave high enough yield under stressed and non stressed conditions, respectively. The increased dry matter yield in silicon-fed chickpea is consistent with findings of other studies which show that silicon can promote growth, and even increase yields of several agricultural crop plants including legumes such as cowpea (Mali and Aery 2008a, 2009), pigeon pea (Owino-Gerroh et al. 2005) and soybean (Hamayun et al., 2010), and non-legumes such as rice (Chen et al., 2011), wheat (Mali and Aery 2008b), maize (Kaya et al., 2006) and sugarcane (Savant et al., 1999). Many studies suggest that Si exerts its effect on certain metabolic processes in plants. Agarie et al., (1992) reported that the beneficial effect of Si on shoot dry matter yield in rice was attributed to maintenance of water status and photosynthesis along with protection of chlorophyll from destruction. Hamayun et al., (2010) reported that an increase in growth of salt and drought stressed soybean plants with the addition of Si in the growth medium, may be due to the fact that Si improves photosynthesis rate, which was related with leaf ultra-structure, chlorophyll content, and ribulose

biphosphate carboxylase activity.

It has been reported that carbon isotope discrimination ($\Delta^{13}C\text{‰}$) is linearly related to the ratio of intercellular CO_2 partial pressure to ambient CO_2 partial pressure (C_i/C_a), (Farquhar et al., 1982). The lower $\Delta^{13}C$ value in the water stressed chickpeas compared to the non-stressed plants implies that C_i/C_a ratios were lower in the former treatments. A lower C_i/C_a ratio could result either from stomatal closure induced by stress or from higher rates of photosynthetic capacity or a combination of both (Condon et al., 2002). However, considering the lower dry matter yield in the water stressed plants (FC1) compared with well watered plants (FC2), it was unlikely that a higher photosynthetic capacity occurred in FC1. Thus, the decrease in C_i/C_a ratio because of water stress was mainly due to the stomatal closure (i.e. decline in stomatal conductance). Therefore, the higher $\% \Delta^{13}C$ in well watered plants imply that a maximization of yield may occur via a maximization of stomatal opening (Condon et al., 1987).

Potassium has been shown to play a significant role in the opening and closing of leaf stomata which control the movement of CO_2 into the plant and water out into the air, and would therefore have an effect on stomatal conductance (Bednarz et al., 1998). Moreover, silicon nutrition has a beneficial effect on plant water economy by reducing excessive leaf transpiration (Ma, 2004). Silicon alleviates drought stress of rice plants by improving water use efficiency (WUE), photosynthesis and mineral nutrient absorption (Chen et al., 2011). Under dry conditions, Si-supplied ryes showed higher photosynthetic rate, stomatal conductance, water use and leaf water potential than silicon-deficit ones (Hattori et al., 2009). Gao et al., (2004) also observed that the

WUE of Si fed maize was significantly higher than that of the non fed Si plants (Si⁻). It has been generally recognized that WUE is negatively correlated with carbon isotope discrimination ($\Delta^{13}\text{C}$). Therefore, low $\Delta^{13}\text{C}$ is an indicator of high WUE (Farquhar and Richards, 1984). Consequently, it can be suggested that K and/or Si may affect the $\Delta^{13}\text{C}$ in plants. In this study, sole application of silicon (Si₅₀K and Si₁₀₀K) to water stressed and well watered chickpeas significantly reduced shoot's $\Delta^{13}\text{C}$. Such decreases reflect lower C_i/C_a ratios which could result mainly from higher rates of photosynthetic capacity (i.e. higher dry matter yields) and lower stomatal conductance (i.e. lower $\Delta^{13}\text{C}$), and consequently, higher WUE. Transpiration from the leaves occurs mainly through the stomata and partly through cuticle. As Si is deposited beneath the cuticle of the leaves forming a Si-cuticle double layer, the transpiration through the cuticle may decrease by Si deposition (Ma, 2004). Hattori et al., (2007) reported that silicon application could affect stomatal conductance in sorghum through the modification of plant water status but not through any physical changes. Water loss through opened stomata is inevitable for crops to absorb CO_2 from the surrounding atmosphere. This dilemma increases its importance when crops are grown under water-limited conditions (Hattori et al., 2007). In rice, silicon application is useful for overcoming the dilemma through saving water loss from leaves by silicon-induced changes in stomatal response (Agarie et al., 1998). In contrast, silicon application to sorghum could overcome the dilemma through compensating for water loss from leaves by facilitating water uptake and transportation (Hattori et al., 2007). On the light of $\Delta^{13}\text{C}$ data, the undertaken study may suggest that the beneficial effect of Si on WUE in chickpea plants

could be resulted from saving water loss through reducing transpiration rate and facilitating water uptake; consequently, increasing its use efficiency. Additional studies are needed to determine how silicon affects hydraulic resistance in chickpea as well as to clarify the mechanisms for inter-species differences in response to silicon application (Hattori et al., 2007), particularly between C3 and C4 plants. On the other hand, the effect of solely application of Si in reducing $\Delta^{13}\text{C}$ values was more evident than that of K where its significant effect was only occurred in well watered plants. Moreover, dual application of Si and K to well watered chickpea plants had small effects on leave's $\Delta^{13}\text{C}$. The only significant effect occurred in the K⁺Si₅₀ treatment which showed a lower $\Delta^{13}\text{C}$ value (i.e. higher WUE).

Increasing soil moisture level from FC1 to FC2 is generally accompanied with increments of $\Delta^{13}\text{C}$ and N_2 fixation. This observation is consistent with that of Kurdali and Al-Shammaa, (2010) in potassium fed lentil grown under water stress, and with that of Knight et al., (1993) who reported a positive correlation between $\Delta^{13}\text{C}$ and the amount of N_2 fixed in lentil inoculated with different strains of rhizobia.

The present study revealed that increasing soil water humidity from FC1 to FC2 had a positive impact on N_2 - fixation in chickpea plants regardless of the fertilizer treatment employed. The depressing effect of water stress on N_2 fixation has been demonstrated in a range of other grain legumes including common-bean (Calvache and Reichardt, 1999), fababean (Kurdali and Al-Shammaa, 2002) and lentil (Kurdali and Al-Shammaa, 2010). Dual or separate applications of Si and K significantly increased amounts of N_2 -fixation in chickpea grown under non-stressed (high

humidity) and stressed conditions (low humidity). However, the beneficial effect of Si on N_2 -fixation was higher than K added separately. Si in combination with K was often more effective in enhancing N_2 -fixation than solely added fertilizers. As shown for dry matter yield, $Si_{100}K^+$ and $Si_{50}K^+$ treatments gave high enough amounts of N_2 -fixation under stressed and non stressed conditions, respectively. The percent increments of total N_2 -fixed were 51 and 47% over their controls, in the above mentioned treatments, respectively. Under stressed conditions (low humidity), the enhancement of N_2 -fixation in the K^+Si_{100} was found to be associated with higher root mass. The increase in the root mass in K^+Si_{100} may lead to an enhancement of the number of potential infection sites for rhizobial invasions (Mali and Aery, 2008a), which in turn lead to increase nodulation and nitrogen fixation as well as soil N uptake. Potassium is reported to improve plant's resistance against drought stress (Marschner 1995; Moinuddin and Imas, 2007) and can alleviate water shortages in many legume crops (Sangakkara et al., 1996, Kurdali et al., 2002). This is attributed to its role in cell turgor control and metabolic activity (Beringer et al., 1983). Kurdali and Al-Shammaa, 2010) reported a positive role of K on stomata functioning as well as of its vital contribution to effective N_2 fixation in water stressed lentil plants.

Although silicon is not considered to be an essential nutrient for most terrestrial plants, it is beneficial to many plants and can modulate plants' metabolism, alter physiological activity (Sacala 2009) and alter the levels of endogenous growth hormones (Hamayun et al., 2010). Our results demonstrated that exogenous supply of silicon to chickpea plants had beneficial effects on growth and nitrogen fixation. Similarly, Nelwamondo and

Dakora (1999) showed that silicon nutrition promotes nodule formation and nodule function in hydroponically grown cowpea. Also, Mali and Aery, (2008a) reported beneficial effect of Si on nodule growth with respect to nodule number and weight in potted grown cowpea. Mechanisms which may be involved in the enhancement of N_2 fixation in silicate treated legumes are summarized in Figure 7. Nelwamondo et al., (2001) reported that the positive effects of silicon on N_2 fixation in cowpea plants might be due to an increased number of bacteroids and symbiosomes. Moreover, these authors also reported that enhancement of N_2 -fixation in cowpea may be resulted from the reduced size of peribacteroid spaces in silicate treated nodules facilitating solute transport and rapid diffusion of dissolved O_2 . Furthermore, applying silicon to nodulated cowpea plants significantly increased the mechanical strength of stems and peduncles as a consequence of silicon accumulation in cell walls. The marked silica deposition in plant cell walls and possible cross-linkage with *ortho-diphenols* could significantly complement lignin function and thus save the plant some carbon which would otherwise be invested in lignin formation for structural integrity. This saved carbon could potentially be channeled into bacteroid respiration for increased N_2 fixation (Nelwamondo et al., 2001). On the other hand, since N_2 -fixation capacity of legume root nodules is closely correlated with leghemoglobin content, enhancement of N_2 fixation in silicate treated plants may be due to increased leghemoglobin formation (Mali and Aery 2008a). Furthermore, Si was useful to increase drought resistance through adjustment of the mineral absorption (Chen et al., 2011). Thus, Si nutrition may increase the absorption of other nutritional elements that are involved in N_2 -

fixation process. Although the role of Si on nodulation and nitrogen fixation remains ambiguous, our data demonstrated that silicon is an important element for the symbiotic performance of chickpea plants. However, more studies on the basis of N_2 fixation increment in response to Si fertilizer are worthy of further study.

Decreasing water availability under drought generally results in reduced nutrient uptake and frequently reduced concentrations of elements in crop plants (Marschner, 1995). There are many reports that Si is beneficial for crops under drought stress through adjustment of the mineral absorption in many plant species including rice (Chen et al., 2011), sunflower (Gunes et al., 2008), Maize (Kaya et al., 2006) and pigeon pea (Owino-Gerroh et al. 2005). In this study, increasing soil water humidity from FC1 to FC2 had a positive impact on total nitrogen uptake in different plant parts of chickpea plants regardless of the fertilizer treatment employed. Sole application of Si or in combination with K significantly increased total N uptake in the whole plant of chickpea grown under both watering regimes. Si in combination with K was often more effective in enhancing N-uptake than solely added Si. $Si_{100}K^+$ and $Si_{50}K^+$ treatments gave high enough amounts of N yield in FC1 (low humidity) and FC2 (high humidity) treatments, respectively. The contribution of soil, fertilizer and atmospheric nitrogen to total nitrogen accumulated in chickpea plants differed greatly among fertilizer treatments and was affected by watering regime. In water stressed plants, the higher amounts of N-yield in solely added Si treatments were mainly resulted from enhancement of N_2 fixation since no significant differences were found in the amounts of nitrogen derived from soil. However, the high N yield in the $Si_{100}K^+$ resulted from enhancement of

soil, fertilizer and atmospheric nitrogen. Similarly, fertilizer nitrogen use efficiency (%NUE) was the highest in the latter treatment. Under non-stressed conditions (high humidity), high N-uptake in dual fertilizer applications ($Si_{50}K^+$ and $Si_{100}K^+$) was mainly resulted from enhancement of N_2 fixation; whereas, all N sources (soil, fertilizer and atmospheric N) contributed to the enhancement of N yield in the $Si_{200}K^+$ treatment which also showed the highest %NUE. These results highlight that Si is not only involved in amelioration of growth, but can also improve nitrogen uptake. These findings may have important implications in the agronomic practice of improving nutrient efficiency by the application of Si fertilizers (Miao et al., 2010).

A positive soil N balance would be normally expressed when the total amount of N_2 fixed in plant residues exceeded the total amount of N removed. Therefore, it is important to adopt suitable agricultural practices to obtain a high grain yield and a high potential yield of residual N with low C/N ratio (Kurdali and Al-Shammaa, 2010). In this study, when the entire plants were harvested, a negative N balance resulted in all treatments. However, the harvesting of pods without shoots and roots resulted in a net gain of N to the soil. Nevertheless, we did not take into consideration the N contribution from pod external layer, dropped leaves, nodule decay, and exudates (Van Kessel, 1994). Therefore, the total N benefit to the soil from above and below ground parts after pod removal may have been underestimated. Generally, exogenous supply of silicon with or without potassium fertilizer to water stressed and well watered chickpea plants had beneficial effects on soil N balance. The positive impact of Si and K on N balance was more pronounced in plants grown under non-stressed (high humidity) than those

grown under stressed conditions (low humidity). The beneficial effect of Si on soil N balance may constitute an important agricultural practice to the subsequent crops when chickpea is rotated with non-legumes, particularly in semi-arid areas where plants are usually subjected to water stress throughout the reproductive stage (Kurdali et al., 1997).

CONCLUSIONS

This study presents the first report on the response of chickpea plants to silicon and potassium fertilizers with respect to growth, carbon isotope discrimination ($\Delta^{13}\text{C}$) and nitrogen fixation using isotopic techniques. Our data demonstrated that sole application of silicon or in combination with potassium to water stressed and well watered chickpeas significantly increased dry matter production and nitrogen yields besides of their positive impacts on soil N balance. On the other hand, $\Delta^{13}\text{C}$ could be an adequate indicator of water use efficiency in response to the exogenous supply of silicon to chickpea plants. Based on the $\Delta^{13}\text{C}$ values, the undertaken study may suggest that the beneficial effect of Si on WUE in chickpea plants could be resulted from saving water loss through reducing transpiration rate and facilitating water uptake; consequently, increasing its use efficiency. Moreover, silicon is suggested to be an important element for the symbiotic performance of chickpea plants. Si in combination with K was often more effective in enhancing N_2 -fixation than solely added fertilizers. These findings may have important implications in the agronomic practice of improving growth and nitrogen fixation in legumes by the application of Si fertilizers. Further studies are needed to illustrate the role of silicon in terms of growth, yield, water use efficiency and the time

course of N_2 fixation of grain legumes grown under rain-fed conditions in the semi-arid areas.

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REFERENCES

- Agarie, S., Agata, W., Kubota, F. and Kaufman, P.B. (1992) Physiological roles of silicon in photosynthesis and dry matter production in rice plants. I. Effect of silicon and shading treatments. *Jpn. J. Crop Sci.*, **61**, 200-206.
- Agarie, S., Uchida, H., Agata, W., Kubota, F. and Kaufman, P.B. (1998) Effects of silicon on transpiration and leaf conductance in rice plants (*Oryza sativa* L.). *Plant Prod. Sci.*, **1**, 89–95.
- Ashraf M., Rahmatullah, Ahmad, R., Afzal, M., Tahir, M. A., Kanwal, S. and Maqsood, M.A. (2009) Potassium and silicon improve yield and juice quality in sugarcane (*Saccharum officinarum* L.) under salt stress. *J. Agron. Crop Sci.*, **195**, 284-291.
- Bednarz, C.W., Oosterhuis, D.M. and Evans, R.D. (1998) Leaf photosynthesis and carbon isotope discrimination of cotton in response to potassium deficiency. *Environ. Exp. Bot.*, **39**, 131-139.
- Beringer, H., Haeder, H.E. and Lindhauer, M.G. (1983) Water relationships and incorporation of ^{14}C assimilates in tuber of potato plants differing in potassium nutrition. *Plant Physiol.*, **73**(4), 956-960.
- Calvache, M., and Reichardt, K. (1999) Effect of water stress at different plant growth stages in

- common bean (*Phaseolus vulgaris*) on yield and N₂ fixation. In: *Crop Yield Response to Deficit Irrigation*, ed. Kirda, C., Moutonnet, P., Hera, C. and Nielsen, D.R., pp, 121-127. The Netherlands: Kluwer Academic Publishers.
- Chen, W., Yao, X., Cai, K. and Chen, J. (2011) Silicon alleviates drought stress of rice plants by improving plant water status, photosynthesis and mineral nutrient absorption. *Biol. Trace Elem. Res.*, **142(1)**, 67-76.
- Condon, A.G., Richards, R.A. and Farquhar, G.D. (1987) Carbon isotope discrimination is positively correlated with grain yield and dry matter production in field grown wheat. *Crop Sci.*, **27**, 996-1001.
- Condon, A.G., Richards, R.A., Rebetzke, G.J. and Farquhar, G.D. (2002) Improving intrinsic water-use efficiency and crop yield. *Crop Sci.*, **42**, 122-131.
- Epstein, E. (2009) Silicon: its manifold roles in plants. *Annals Appl. Biol.*, **155**, 155-160
- Farooq, M., Wahid, A., Kobayashi, N., Fujita, D. and Basra, A.M.A. (2009) Plant drought stress: effects, mechanisms and management. *Agron. Sust. Develop.*, **29**, 185-212.
- Farquhar, G.D. and Richards, R.A. (1984) Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. *Aust. J Plant Physiol.*, **11**, 537-542.
- Farquhar, G.D., O'Leary, M.H. and Berry, J.A. (1982) On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. *Aust. J Plant Physiol.*, **9**, 121-137.
- Gao, X., Zou, C., Wang, L. and Zhang, F. (2004) Silicon improves water use efficiency in maize plants. *J. Plant Nutr.*, **27**, 1457-1470.
- Gunes, A., Kadioglu, Y.K., Pilbeam, D.J., Inal, A., Coban, S. and Aksu, A. (2008) Influence of silicon on sunflower cultivars under drought stress, II: Essential and nonessential element uptake determined by polarized energy dispersive X-ray fluorescence. *Comm. Soil Sci. Plant Anal.*, **39(13-14)**, 1904-1927
- Hamayun, M., Sohn, E.Y., Khan, S.A., Shinwari, Z.K., Khan, A.L. and Lee, I.J. (2010) Silicon alleviates the adverse effects of salinity and drought stress on growth and endogenous plant growth hormones of soybean (*glycine max* L.). *Pakistan J Bot.*, **42(3)**, 1713-1722.
- Hattori, T., Ishii, K., An, P. and Inanaga, S. (2009) Growth Enhancement of Rye by Silicon Application Under Two Different Soil Water Regimes. *J. Plant Nutr.*, **32(2)**, 187-196
- Hattori, T., Sonobe, K., Inanaga, S., An, P., Tsuji, W., Araki, H., Eneji, A.E. and Morita, S. (2007) Short term stomatal responses to light intensity changes and osmotic stress in sorghum seedlings raised with and without silicon. *Environ. Exp. Bot.*, **60(2)**, 177-182
- Kaya, C., Tuna, L. and Higgs, D. (2006) Effect of silicon on plant growth and mineral nutrition of maize grown under water-stress conditions. *J. Plant Nutr.*, **29**, 1469-1480.
- Knight, J. D., Verhees, F., Van Kessel, C. and Slinkard, A.E. (1993) Does carbon isotope discrimination correlate with biological nitrogen fixation? *Plant and Soil*, **153**, 151-153.
- Kurdali, F. (1996) Nitrogen and phosphorus assimilation, mobilization and partitioning in rainfed chickpea (*Cicer arietinum* L.). *Field Crop Res.*, **47(2-3)**, 81-92.
- Kurdali, F. and Al-Shamma'a, M. (2010) Natural abundances of ¹⁵Nitrogen and ¹³Carbon

- indicative of growth and N₂ fixation in potassium fed lentil grown under water stress. *J. Plant Nutr.*, **33(2)**, 157-174.
- Kurdali, F. Al-Ain, F. and Al-Shamma, M. (2002) Nodulation, dry matter production, and N₂ fixation by fababean and chickpea as affected by soil moisture and potassium fertilizer. *J. Plant Nutr.*, **25**, 355 - 368
- Kurdali, F., Kalifa, K. and Al-Shamma, M. (1997) Cultivar differences in nitrogen assimilation, partitioning and mobilization in rain-fed grown lentil. *Field Crop Res.*, **54(2-3)**, 235-243.
- Liang, Y. 1999. Effect of silicon on enzyme activity and sodium, potassium and calcium concentration in barley under salt stress. *Plant and Soil* **209**, 271-224.
- Liang, Y., Sun, W., Zhu, Y.G. and Christie, P. (2007) Mechanisms of silicon-mediated alleviation of abiotic stresses in higher plants: A review. *Environ. Poll.* **147(2)**, 422-428.
- Ma, J.F. (2004) Role of silicon in enhancing the resistance of plants to biotic and abiotic stresses. *Soil Sci. Plant Nutr.*, **50(1)**, 11 – 18.
- Mali, M. and Aery, N.C. (2008a). Silicon effects on nodule growth, dry matter production and mineral nutrition of cowpea (*Vigna unguiculata*). *J. Plant Nutr. Soil Sci.*, **171(6)**, 835-840.
- Mali, M. and Aery, N.C. (2008b) Influence of silicon on growth, relative water content and uptake of silicon, calcium and potassium in wheat grown in nutrient solution. *J. Plant Nutr.*, **31**, 1867-1876.
- Mali, M. and Aery, N.C. (2009) Effects of silicon on growth, biochemical constituents and mineral nutrition of cowpea. *Comm. Soil Sci. Plant Anal.*, **40**, 1041-1052.
- Marschner, H. (1995) *Mineral nutrition of higher plants*. Second edition. Academic press limited. London U.K. NW1 7DX.
- Miao, B-H., Han, X.G. and Zhang, W.H. (2010) The ameliorative effect of silicon on soybean seedlings grown in potassium-deficient medium. *Annals Bot.*, **105**, 967–973
- Moinuddin and Imas, P. (2007) Evaluation of potassium compared to other osmolytes in relation to osmotic adjustment and drought tolerance of chickpea under water deficit environments. *J. Plant Nutr.*, **30**, 517-535.
- Nelwamondo, A. and Dakora, F.D. (1999) Silicon nutrition promotes nodule formation and nodule function in symbiotic cowpea (*Vigna unguiculata* (L.) Walp.). *New Phytologist*, **142**, 463-467
- Nelwamondo, A., Jaffer, M.A. and Dakora, F.D. (2001) Subcellular organization of N₂-fixing nodules of cowpea (*Vigna unguiculata*) supplied with silicon. *Protoplasma*, **216**, 94-100
- Owino-Gerroh, C., Gascho, G.J. and Phatak, C. (2005) Pigeonpea response to silicon, phosphorus and *Rhizobium* inoculation in an acid coastal plain soil. *J. Plant Nutr.*, **28**, 797-804.
- Sacala, E. (2009) Role of silicon in plant resistance to water stress. *J. Elementology*, **14(3)**, 619-630.
- Sangakkara, U.R., Hartwig, U.A. and Nosberger, J. (1996) Soil moisture and potassium affect the performance of symbiotic nitrogen fixation in faba bean and common bean. *Plant and Soil*, **184(1)**, 123-130.
- Savant, N.K., Korndorfer, G.H., Datnoff, L.E. and Snyder, G.H. (1999) Silicon nutrition and sugarcane production: a review. *J. Plant Nutr.*, **22(12)**, 1853-1903

- Singh, K.K., Singh, K. and Singh, R. (2005) Effect of nitrogen and silicon levels on growth, yield attributes, and yield of rice in Alfisols. *International Rice Res. Notes*, **30(1)**, 40-41
- Van Kessel, C. (1994) Seasonal accumulation and partitioning of nitrogen by lentil. *Plant and Soil*, **164**, 69-76.
- Waraich, E.A., Ahmad, R., Saifullah, Ashraf, M.Y. and Ehsanullah. (2011) Role of mineral nutrition in alleviation of drought stress in plants. *Aust. J. Crop Sci.*, **5(6)**, 746-777.