

# Effects of Exogenous Silicic (Si) and Salicylic acid (SA) Applied Individually or in Combination on Barley Growth and Nitrogen Uptake under Various Watering Regimes

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This pot experiment investigated the effect of treatment with exogenous silicic (Si) and/or salicylic acids (SA) on dry matter (DM), nitrogen yield (NY) and soil N uptake from soil (Ndfs) and from fertilizer (Ndff) nitrogen use efficiency (%NUE) of barley plants (*Hordeum vulgare* L.) grown under varying levels of watering regimes (high stress I1, mild stress I2 and well-watered I3). Results showed that, foliar application of Si and/or SA markedly improved the overall studied growth parameters of barley plants and decreased the water-deficit influences. The synergistic effects of Si + SA application were more effective as compared with Si or SA applied separately indicating that Si and SA supports each other's in improving plant performance which was more pronounced under high water-deficit than other watering regimes. A proper application of Si and SA might represent a suitable agricultural approach and result in increased production of barley, particularly, in the semi-arid areas under rain-fed conditions.

*Key words:* Water stress, Salicylic acid, Silicon; Barley, 15N

Barley (*Hordeum vulgare*) is one of the most important widely grown cereal grain crops worldwide with multipurpose use as animal feed, human food, and brewing material (Badea and Wijekoon 2021). In semiarid areas of the Mediterranean basin, barley is cultivated on a large scale under rain-fed conditions, where rain is scarce, irregularly distributed, and variable from one year to another. Thus, water deficit occurring during the growing periods considered the major environmental constraint affecting the agronomic production of barley (Samarah, 2005).

The improvement of barley's yield could be achieved by several approaches including plant breeding (Cai et al., 2020) and best agricultural practice managements. Among the agricultural practices, the use of beneficial elements such as silicon (Etesami and Jeong 2023, Maghsoudi et al., 2019) or exogenous application of plant growth regulators (PGR) such as salicylic acid which can effectively alleviate the adverse effects of abiotic stresses and consequently enhance plant growth, development and yield (Pirasteh-Anosheh et al., 2015; Al-Chammaa et al., 2019; Ahmad et al., 2021; Mir et al., 2021).

Both Si and SA have been reported to be effective in mitigating the harmful effects of environmental stresses by promoting the endurance of plants against a variety of stresses due to induced changes in many physiological and biochemical processes (Etesami and Jeong 2023, Maruri-López et al. 2019; Gorni et al. 2021; Torun et al., 2020). Silicon (Si) has been reported to be effective in mitigating the harmful effects of environmental constraint including salinity (El-Moukhtari et al., 2022), drought (Kurdali et al., 2019), heavy metal toxicity and nutritional imbalance stresses (Etesami and Jeong 2023) in plants. Moreover, salicylic acid (SA), when sprayed, results in increased photosynthetic activity in plants due to SA-induced changes in physiological and biochemical processes (Bandurska and Stroinski 2005; Maruri-López et al. 2019; Gorni et al. 2021). Moreover, many recent studies intensively focused on the indispensable role of SA as an internal signal arbitrating plant defense behavior against both biotic and abiotic stresses (Liu et al. 2022; Mir et al. 2021; Aldoude et al., 2019). Individual application of Si or SA has been reported to be effective in mitigating

the harmful effects of drought on barley plants. Bandurska and Stroinski (2005) reported that ABA and proline can contribute to the development of anti-stress reactions induced by SA in barley. Also, Pirasteh-Anosheh et al., (2015) reported that growth and yield of barley were improved due to SA applied as a foliar spray in both stressed and non-stressed plants. Habibi (2012) suggests that the improvement of SA on drought tolerance of barley plants was associated with the increase of antioxidant defense abilities and maintenance of photosynthesis under drought, which may elucidate the physiological mechanism of SA in improvement of drought tolerance of barley plants. In regards to Si influences, Kurdali and Al-Chammaa (2013) reported that Si might be involved in saving water loss through reducing transpiration rate and facilitating water uptake, consequently, increasing water use efficiency in barley under water deficit. A number of recent studies showed that application of Si in combination with SA was more effective in enhancing plant growth in many plant species including wheat (Maghsoudi et al. 2019), cotton (Barros et al., 2019) and tomato (Khan et al., 2019). Since Si and SA have been noted for their particular role in enhancing drought tolerance of crops, there are still few studies that address their beneficial effects on growth and N-uptake of barley plants in response to drought tolerance particularly, when they are applied in combination. Hence, a better understanding of the interaction between Si and SA applications and plant responses will contribute to more efficient agricultural practices, particularly under water stress conditions. Therefore, the objective of this study was to assess the effect of exogenous Si and SA applied individually or in combination on dry matter yield, nitrogen uptake and N fertilizer use efficiency in barley plants grown under water-deficit and non-deficit conditions using <sup>15</sup>N.

## MATERIALS AND METHODS

### Site Description, Soil Properties and Plant Materials

A pot experiment was conducted at the research field of Deir AL-Hajar station" located south east of Damascus, Syria (36° 28'E, 33° 21' N; altitude 617 m) in the growing seasons of 2020. The site is located within a dry

Mediterranean semiarid area with hot-dry summer and cold winter. The total annual rainfall is about 120 mm, and most precipitations occur between November and early April. For the last ten years, the average minimum temperature in winter was 1.3°C in January, while it increases to the average maximum temperature of 38.3 °C in July.

Seeds of local genotypes of barley (cv. Arabi Aswad) were sown in plastic pots containing of thoroughly mixed soil (7 kg) collected from the field at the depth of 0 to 20 cm. Soil was air dried before being ground and sieved (2 mm) to remove small rocks and plant litter. The main physical and chemical soil properties were: pH 7.85,  $EC_e$  0.52 dSm<sup>-1</sup>, organic matter 0.54%, cations (Ca<sup>++</sup>4.08, Mg<sup>++</sup> 13.21, K<sup>+</sup>0.01 and Na<sup>+</sup>0.08 mmol L<sup>-1</sup>), anions (SO<sub>4</sub><sup>-</sup> 1.27, HCO<sub>3</sub><sup>-</sup> 4.0 and Cl<sup>-</sup> 1.02 mmol L<sup>-1</sup>), available P (Olsen) 5.6 µgg<sup>-1</sup>, total N 0.02%, NO<sub>3</sub><sup>-</sup> 8.9 µgg<sup>-1</sup>, NH<sub>4</sub><sup>+</sup>19.2 µgg<sup>-1</sup>. Exchangeable cations (Ca<sup>++</sup> 46.08 and Mg<sup>++</sup> 6.33 meq 100 g<sup>-1</sup> soil). The soil is classified as a clay loam, with average 54.2% clay, 32.5% silt, and 13.3% sand.

After germination, plants were thinned to two plants per pot. The pots were placed outdoor under natural climatic conditions, and protected from rainfall by manually operated shelter equipped with movable sheet of transparent flexible plastic. The pots were kept weed-free and any drainage was prevented.

#### **Experimental Procedures and Treatments and <sup>15</sup>N-Application**

A split-plot based on randomized complete block design (RCBD) with three replicates was carried out in a pot experiment. The main factor was soil moisture level (low, 45–50%; medium, 55–60% and high, 75–80% of field capacity) which abbreviated as I1, I2, and I3, respectively. The sub-factor was different spraying compounds consisted of: silicic acid (Si+), salicylic acid (SA+), Si+ plus SA+. For non-treated plants, leaves were sprayed with distilled water and set as control (W). Soil water content in all pots was maintained at around 75% of field capacity from planting up to the tillering stage (i.e., main shoot plus three tillers). Thereafter, plants were subjected to the three above-mentioned soil moisture regimes (i.e., I1, I2 and I3).

Foliar application with silicon (Si) was applied in the form of silicic acid Si(OH)<sub>4</sub> at a rate of 150 mg L<sup>-1</sup>. One hundred mL per plant of Si solution were applied as foliar

spray. First spray was given two weeks after appearance of the first leave, and further 3 sprays were given regularly at 15 days interval. Salicylic acid (SA), C<sub>6</sub>H<sub>4</sub> (OH) COOH, was applied at a rate of 10<sup>-5</sup>mol L<sup>-1</sup> as foliar spray. Spraying of the plants with SA was started at the same time of applying soil moisture regimes, and performed six times at seven days intervals. For untreated Si and SA plants, leaves were sprayed with distilled water and set as control (W). Spraying with Si and/or SA was made using a manual sprayer early in morning, even full wet. On the other hand, equivalent amounts of 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> in the form of phosphoric acid and of 75 kg K<sub>2</sub>O ha<sup>-1</sup> in the form of potassium sulfate were applied to each pot prior planting to all treatments. Moreover, an equivalent rate of 20 kg N ha<sup>-1</sup> of <sup>15</sup>N labeled urea (5% <sup>15</sup>N atom excess) was applied at planting to estimate the fractional contribution of nitrogen derived from soil (Ndfs) and from fertilizer (Ndff), using <sup>15</sup>N isotopic dilution method.

#### **Plant Sampling, Isotopic Composition and Statistical Analysis**

Plants were harvested at the spike formation stage. Shoots and roots were dried at 70 °C for 72 h, weighed for dry matter determinations. Samples were then ground to a fine powder using a mill having a 0.5 mm sieve. Total nitrogen was determined by Kjeldahl procedure, and <sup>15</sup>N/<sup>14</sup>N isotope ratio was measured with an emission spectrometer (Jasco-150, Japan). The N fractions derived from the available sources (i.e., soil Ndfs and fertilizer Ndff) were calculated using the isotopic dilution method by applying equations previously described (Zapata, 1990). Nitrogen fertilizer use efficiency (%NUE) was calculated as fertilizer N recovery in the whole plant.

#### **Statistical Analysis**

Collected data were subjected analysis of variance (ANOVA) using the statistical program Statview, 4.57® Abacus Concepts, Berkley, Canada. Means were compared using the least significant difference (Fisher's LSD) test at the 0.05 probability level.

## **RESULTS AND DISCUSSION**

### **Effect of Si and/or SA on Dry Matter Yield and Nitrogen uptake**

Several studies indicate that exogenous application of silicon (Si) or salicylic acid (SA) may be a sustainable

technique to help mitigate the various environmental stresses in various crop plants (Etesami and Jeong 2023; Mir et al., 2021). This study investigated the effect of treatment with exogenous silicic and/or salicylic acids (SA) in barley plants (*Hordeum vulgare* L.) grown under water-deficit and non-deficit conditions. Exogenous application of Si and SA could positively affect dry matter yield of barley plants by improving the uptake of nitrogen and decreasing the water-deficit influences.

In Si, SA and Si+SA treatments, total dry matter yield (DM) was 13%, 11% and 19% higher, respectively, than with no foliar application under high water stress (I1). However, the effect was less pronounced under mild water stress (I2) where DM was 8%, 8%, and 16% higher, respectively than the control. Under well watering regime (I3), the significant increment was only obtained in the combined treatment of Si+SA (14% over the control), (Fig. 1). These results were in harmony with those of Maghsoudi et al., (2019) who reported that in Si, SA and Si+SA treatments, grain yield of two wheat cultivars was 10-16%, 16-17% and 19-24 % higher, respectively than with no foliar application under water stress in two wheat cultivars. Similarly, data of total N yield were consistent with those of DM. In Si, SA and Si+SA treatments, NY was 27, 23 and 33% higher, respectively than with no foliar application under high water stress. Under mild water stress (I2), although separate application of Si and SA enhanced NY, the dual application of Si+SA resulted in a significant increase of NY by 15% over the control. In the well irrigated plants, NY yield increased by 16%, 20% and 40%, over the control in Si, SA and Si+SA treatments, respectively (Fig.1). Shoot DM data were in consistence with those of total DM. Si and /or SA treated plants resulted in significant greater shoot DM than the control in stressed and non-stressed plants (Table 1), whereas, root DM was significantly higher in Si and/or SA treatments than with no foliar application under high water stress (I1). However, under mild water stress (I2) and in well-watered plants (I3), no apparent and significant increases were found in root DM. Also, it can be noticed that the dual application of both compounds was more effective than those of sole applications. Our results are similar to findings reported by other workers demonstrating that foliar application of Si, SA and especially their

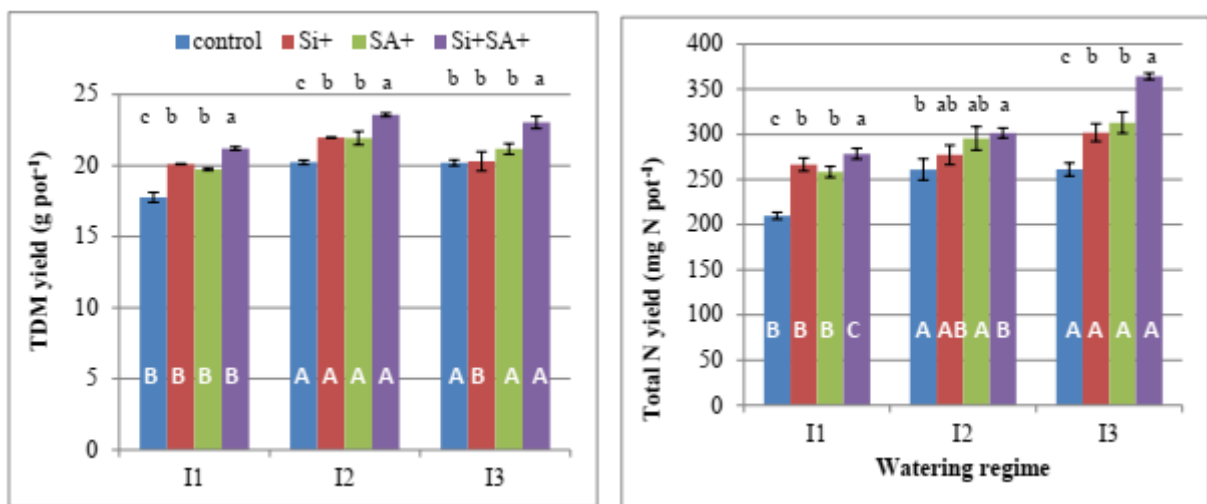
combination, markedly improved yield components in a variety of plant species under environmental stresses including drought (Maghsoudi et al., 2019; Li et al. 2009; Zhang et al. 2017). Moreover, the present investigation demonstrated that synergistic effects of Si+SA application on plant performance were greater than of Si or SA applied separately. Similarly, de Souza Junior et al. (2021) reported that, applying SA, especially when associated with foliar Si, favored dry weight production in peanut plants. Moreover, Barros et al. (2019) reported that Si foliar application associated with SA favors the physiological variables, increasing the photosynthesis, stomatal conductance and water use efficiency reflecting on the increase of cotton yield. Likewise, Maghsoudi et al. (2019) reported that synergistic effects of Si+SA application on physiological parameters and yield of water stressed wheat plants were apparent compared with Si or SA applied separately. Khan et al. (2019) reported that exogenously applied Si and SA enhance the activation of the antioxidant defense system, modulate key hormones, increase the potassium concentration and stimulate the expression of the critical genes involved, which confers successful tolerance of tomato plants to alkaline conditions. Therefore, it can be concluded that the dual application of Si and SA to barley plants is more effective than those of sole applications, indicating that Si and SA underpins each other's in improving plant growth performance which was more pronounced under high water-deficit than the other watering regimes.

#### **Effect of Si and/or SA on nitrogen derived from soil, fertilizer and its use efficiency**

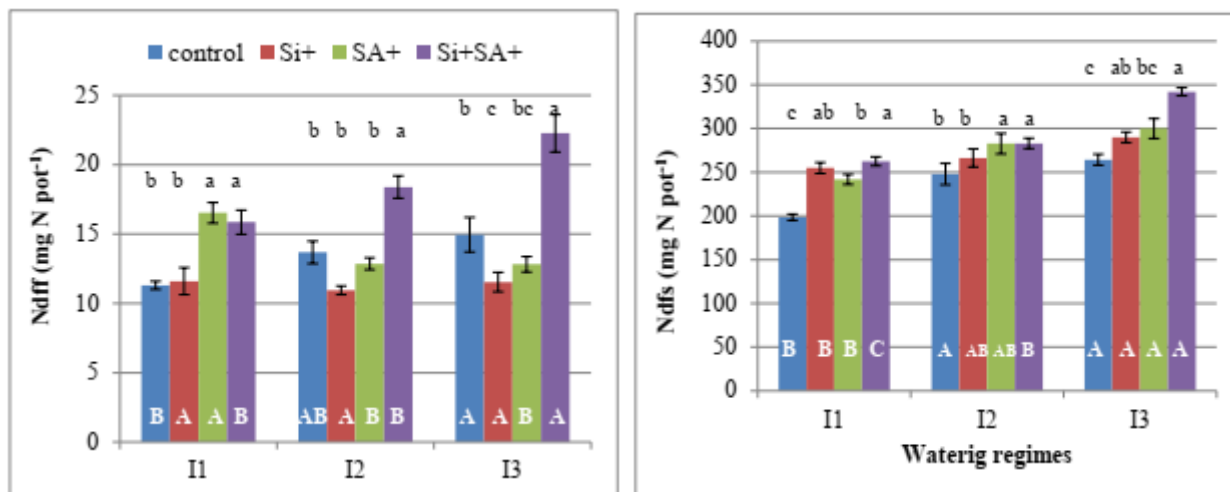
Decreasing water availability under drought generally results in reduced nutrient uptake and frequently reduced concentrations of elements in crop plants (Marschner, 1995). In this study, both Ndfs and Ndff increased with increasing soil moisture content under various spraying treatments (Fig. 2). Positive and significant impacts on soil nitrogen uptake (Ndfs) and nitrogen derived from fertilizer (Ndff) in response to Si and/or SA were generally observed in plants grown under various watering regimes (Fig. 2). On percentage basis, amount of Ndfs in water stressed plants (I1) increased by 28%, 22 and 32% in response to Si, SA and Si+SA treatments, respectively. Under mild water stress (I2), although separate application

of Si enhanced Ndfs, solely applied SA or in combination with Si significantly increased by 14% as compared with no foliar application. Under well irrigated conditions, Ndfs increased by 18%, 22% and 39%, over the control in Si, SA and Si+SA treatments, respectively. The efficient use of N fertilizer (%NUE) ranged between 19% in the control (i.e., no foliar application under high water stress) and 37% in well irrigated plants of Si+SA treatment (Fig. 3). The synergistic effects of Si and SA application on %NUE were greater than that of Si or SA applied separately. These results are similar to findings reported by other workers demonstrating that foliar application of Si or SA improved plant growth under stresses by stimulating the accumulation of mineral elements including nitrogen (Gunes et al. 2005; Yildirim et al. 2008; Al-Chammaa et al. 2019; Kurdali and Al-Chammaa 2013). Etesami and Jeong (2023) reported that silicon interferes with the absorption of essential nutrients by plants and could affect the nutrient availability by optimizing soil fertility, making nutrients more available to plants. Guo et al. (2006) reported that the increase in root growth with Si application would have the potential to enhance the root's absorptive capacity and nutrient uptake. Likewise, the

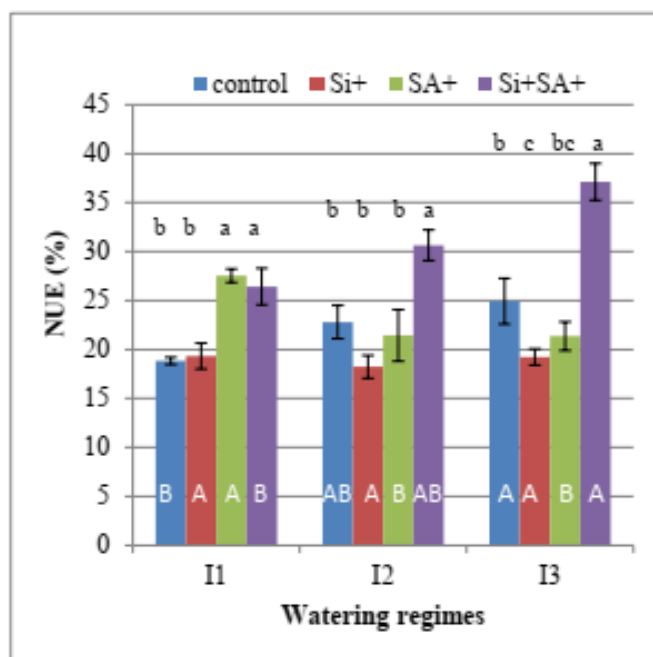
enhancement of endogenous abscisic acid (ABA) concentration in Si-fed plants could lead to lateral root growth stimulation (Agarie et al. 1992) which in turn increase nutrient uptake. On the other hand, there are some evidences that SA can induce the synthesis of nitrite reductase enzyme, which plays a key role in nitrogen metabolism and assimilation (Ghasemzadeh and Jaafar, 2013). Al-Chammaa et al. (2019) reported that applied SA to water-stressed chickpea resulted in increments of soil nitrogen uptake (Ndfs) as well as amount of N derived from fertilizer (Ndff) and its use efficiency (%NUE). In this study, Si and/or SA treated plants under high water stress resulted in significant greater root DM than the control, with the highest value being obtained in Si+SA treatment (Table 1). Consequently, the enhanced soil nitrogen uptake by water-stressed plants following Si+SA applications may improve biomass accumulation and nitrogen yield. Thus, the co-application of these two compounds (i.e., Si+SA) showed positive effects on soil N uptake and %FUE more than separate application indicating that Si and SA supports each other's in improving N uptake under different watering regimes.



**Figure 1.** Dry matter ( $\text{g pot}^{-1}$ ) and amounts ( $\text{mg N pot}^{-1}$ ) of nitrogen in the whole plant of barley grown under different water regimes (I1, high stress; I2, mild stress and I3, well-watering) as affected by foliar spraying with salicylic acid (SA) and/or silicic acid (Si). Columns followed by the same letter are not significantly different ( $P < 0.05$ ). Small letters indicate comparisons amongst treatments for each water regime. Capital letters denote comparison amongst water regimes for each applied treatment



**Figure. 2.** Amounts (mg N pot<sup>-1</sup>) of nitrogen derived from soil (Ndfs) and fertilizer (Ndff) in the whole plant of barley grown under different water regimes (I1, high stress; I2, mild stress and I3, well-watering).as affected by foliar spraying with salicylic acid (SA) and/or silicic acid (Si). Columns followed by the same letter are not significantly different (P<0.05). Small letters indicate comparisons amongst treatments for each water regime. Capital letters denote comparison amongst water regimes for each applied treatment.



**Figure. 3.** Nitrogen use efficiency of added fertilizer (%) in the whole plant of barley grown under different water regimes (I1, high stress; I2, mild stress and I3, well-watering).as affected by foliar spraying with salicylic acid (SA) and/or silicic acid (Si). Columns followed by the same letter are not significantly different (P<0.05). Small letters indicate comparisons amongst treatments for each water regime. Capital letters denote comparison amongst water regimes for each applied treatment

**Table 1:** Dry matter yield (g pot<sup>-1</sup>) and nitrogen uptake (mg N pot<sup>-1</sup>) in shoots and roots of barley plants grown under different water regimes as affected by foliar spraying with salicylic acid (SA) and/or silicic acid (Si).

Dry matter yield (g pot <sup>-1</sup> )					
Shoots					
Treatments	W (Si+SA)	Si+	SA+	Si+SA+	L.S.D
I1	11.53±0.26c, B	13.10±0.06b,B	12.97±0.09b,C	13.90±0.06a,C	0.46
I2	14.30±0.68c,A	15.67±0.33b,A	16.30±0.33b,A	17.90±0.06a,A	1.35
I3	14.03±0.15b,A	13.97±0.55b,B	14.97±0.03b,B	16.57±0.30a,B	1.05
LSD 0.05	1.48	1.29	0.69	0.61	
Roots					
I1	6.23±0.35cA	6.97±0.03b,A	6.77±0.13, b, A	7.30±0.15a,A	0.36
I2	5.93±0.18ab,A	6.30±0.20a, B	5.60±0.20b, B	5.67±0.12ab,C	0.68
I3	6.17±0.36a,A	6.37±0.13a,AB	6.20±0.36a, AB	6.47±0.17a,B	N.S
LSD 0.05	N.S	0.66	0.87	0.51	
N-uptake (mg N pot <sup>-1</sup> )					
Shoots					
I1	160.6±4.4b,B	218.4±8.98a,B	208.7±0.46a,B	208.5±7.4a,C	20.23
I2	205.4±9.85b,A	232.4±9.5ab,AB	243.7±13.8a,A	258.4±1.3a,B	31.76
I3	218.9±0.97c,A	260.6±9.2,b	267.4±7.8b,A	308.9±4.3a,A	20.93
LSD 0.05	21.60	31.92	31.69	17.20	
Roots					
I1	49.2±0.96b,A	48.1±5.5b,A	49.60±5.9b,A	69.85±3.6a,A	14.49
I2	56.1±4.6a,A	44.8±2.1a,A	51.81±4.0a,A	43.03±6.6a,B	N.S
I3	42.3±6.6ab,A	40.5±3.5b,A	45.39±3.9ab,A	55.65±2.8a,AB	14.7
LSD 0.05	N.S	N.S	N.S	16.06	

Means ±SE within a column (capital letter) and within a row (small letter) followed by the same letter are not significantly different ( $P < 0.05$ ). I: water regimes, expressed as % of field capacity (I1, high stress 45-50%; I2, mild stress 55-60% and I3, well-watering 75-80%). W: spraying with demineralized water as a control (Si+SA); SA+: spraying with salicylic acid at  $10^{-5}$  Mol l<sup>-1</sup>; Si+ : spraying with 100 mg silicic acid (H<sub>4</sub>SiO<sub>4</sub>)/L.

## CONCLUSION

- Exogenous foliar application of Si and/or SA markedly improved the overall growth parameters (DM, NY, Ndfs, Ndff and %NUE) of barley plants under both stressed and non-stressed conditions.

- Synergistic effects of Si+SA application were apparent compared with Si or SA applied separately indicating that Si and SA underpins each other's in improving plant performance which was more pronounced under high water-deficit than the other watering regimes.

- A proper application of Si and SA might result in increased production of barley, particularly in the semi-arid areas under rain-fed conditions.

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## CONFLICT OF INTERESTS

The author declares that he has no competing interests.

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