



Improvement of drought tolerance in five different cultivars of *Vicia faba* with foliar application of ascorbic acid or silicon

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Abstract

Aim of study: To explore the role of ascorbic acid (AsA) or silicon (Si) in improving drought tolerance in five faba bean cultivars under irrigation water deficit (IWD).

Area of study: The experimental farm; 30° 36' N, 32° 16' E, Egypt.

Material and methods: Three drip irrigation regimes (WW, well-watered, 4000 m³ water ha⁻¹; MD, moderate drought, 3000 m³ water ha⁻¹; and SD, severe drought, 2000 m³ water ha⁻¹) were applied to plants, which were sprayed 25, 40, and 55 days after sowing with 1.5 mM AsA or 2.0 mM Si vs distilled water as a control.

Main results: TDrought negatively affected physiological attributes (photosynthetic pigments, gas exchange parameters, relative water content, membrane stability index, electrolyte leakage (EL), and lipid peroxidation), which restricted plant growth and yields, and stimulated alterations in both enzymatic and non-enzymatic antioxidant activities. However, AsA or Si application mitigated drought effects on physiological attributes, improving growth, yields and water use efficiency by raising antioxidant activities and suppressing lipid peroxidation and EL in stressful cultivars. The mitigating effects of AsA and Si were more pronounced under MD.

Research highlights: 'Nubaria-2', 'Giza-843', and 'Sakha-3' were more tolerant than 'Giza-716' and 'Sakha-4', suggesting the use of AsA or Si to ameliorate the IWD effects on stressful cultivars. Certain physiological traits exhibited positive association with growth and seed yield, demonstrating their importance in enhancing seed yield under irrigation treatments.

Additional keywords: irrigation regimes; antioxidants; relative water content; yield; contributing traits.

Abbreviations used: APX (ascorbate peroxidase); AsA (ascorbic acid); CAT (catalase); DHA (dehydroascorbic acid); DW (dry weight); EL (electrolyte leakage); Fv/Fm (photosynthetic efficiency); FW (fresh weight); GR (glutathione reductase); gs (stomatal conductance); GSH (glutathione); IWD (irrigation water deficit); MD (moderate drought); MDA (lipid peroxidation measured as malondialdehyde content); MSI (membrane stability index); Pn (net photosynthesis rate); POD (peroxidase); PRP (pigments related to photosynthesis); PSI (photosystem 1); RWC (relative water content); SD (severe drought); Si (silicon); SOD (superoxide dismutase); Tr (transpiration rate); WUE (water use efficiency); WW (well-watered).

Authors' contributions: Conceived, designed and performed the experiments: EMD, EM, MATY and EEAE. Analyzed the data: EMD, EM and MATY. Contributed reagents/materials/analysis tools: EMD, EEAE and MMR. All authors wrote and approved the final manuscript.

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Introduction

Climate change is the major problem that threatens agricultural production in the 21st century. Extreme changes in temperature, rainfall, light, and other climatic factors excessively reduce plant performances (e.g., growth and productivity) of many horticultural and field crops

(Farooq *et al.*, 2017; Chhogyel & Kumar, 2018; Conesa *et al.*, 2018; Ray *et al.*, 2019). Increased temperature and reduced precipitation lead to heat and drought stresses in many regions, especially in arid and semi-arid regions (Papworth *et al.*, 2015; Li P. *et al.*, 2018; Li J. *et al.*, 2019). Irrigation water deficit (IWD) minimizes water content of plant tissues, leading to decrease in cell turgor and

increase in cytosol solute concentrations and extracellular substances. It has significant negative impacts on the uptake, transport, and metabolism of various nutrients, minimizing the leaf area and altering the partitioning of assimilates for different plant organs (Mitchell *et al.*, 2008; Krouma, 2010; Bista *et al.*, 2018; Ghaffari *et al.*, 2019). Under shortage of water for long time, water scarcity-sensitive crops are dehydrated and eventually die. For this reason, it is necessary to find suitable techniques to improve tolerance to drought in field crops to enhance plant growth and reduce drought negative effects (Saikia *et al.*, 2018; Wu *et al.*, 2018; Shirinbayan *et al.*, 2019). The development of drought-tolerant genotypes through classical or modern breeding techniques requires great efforts, long time and funds because tolerance to drought is a complex sign that includes groups of gene networks that are constraints to rapid solutions (Ashraf & Akram, 2009). On the other hand, there is a great alternative approach in which promoting substances (*e.g.*, antioxidants and osmoprotectants) are used to enhance growth and yield of crop plants under adverse conditions. Such approach will help produce acceptable crop yields in limited water resources environments.

Currently, many antioxidants/osmoprotectants are effectively used to alleviate the negative impacts of IWD on field crops. These compounds are organic or inorganic substances likely to improve tolerance to drought in plants under adverse conditions (Qirat *et al.*, 2018). Among them, ascorbic acid (AsA) and silicon (Si) can be used to minimize disadvantageous impacts of oxidative injurious on crop plants (Yang *et al.*, 2009; Rady *et al.*, 2019). In cytosol and stroma of chloroplasts, plants biosynthesize antioxidants, including AsA to buffer redox reactions by interacting with various components in the cell with respect to their influences on cellular expansions and thus plant growth (Sharma *et al.*, 2012). AsA plays several vital roles in cell metabolism (Akram *et al.*, 2017). It is involved in the regulation of growth and development of crop plants, including cellular divisions and expansions (Pignocchi & Foyer, 2003). It helps the plant recover from environmental stress-induced damages, including drought stress (Baghizadeh *et al.*, 2009). In addition, Si can improve the ability of plants to tolerate different stresses due to its basic mechanical-physical functions (Alzahrani *et al.*, 2018; Merwad *et al.*, 2018). Although Si is precipitated on cellular walls, it catalyzes most pathways of plant physiology (Parveen & Ashraf, 2010) to alleviate the stress effects by enhancing the tolerance in plants to stress (Alzahrani *et al.*, 2018). It enhances photosynthesis activity (Shi *et al.*, 2016). This Si-mediated positive impact might be due to that it elevates leaf rigidity by making it rougher in texture (Ouzounidou *et al.*, 2016), holding more horizontally, delaying leaf senescence, and increasing chlorophyll content (Merwad *et al.*, 2018) and ribulose-bisphosphate carboxylase ac-

tivity (Fadzilla & Burdon, 1997), and/or facilitating light penetration, and consequently higher photosynthesis (Gong *et al.*, 2003). It also ameliorates nutritional imbalances and minimizes elemental toxicities (Shi *et al.*, 2016). Improved plant growth and production have been reported by applying Si to various crops growing under IWD stress (Shi *et al.*, 2016; Alzahrani *et al.*, 2018; Merwad *et al.*, 2018).

Faba bean (*Vicia faba* L.) is the major pulse crop grown in Mediterranean region due to high nutritional value for human and animal consumptions (*e.g.*, 20–34% proteins, 50–60% carbohydrates, amino acids, mineral nutrients, dietary fibers, vitamins, and antioxidant compounds) (Crépon *et al.*, 2010; Kumar *et al.*, 2015). In addition, faba bean is an important crop used for crop rotation because of its fixation of the atmospheric N, enriching the soil with N and organic matter and resulting in improved water use efficiency (WUE) in the crop system and increased soil productivity, particularly in dry regions (Pala *et al.*, 2000; Köpke & Nemecek, 2010).

Worldwide, the total area cultivated with faba bean was 2.46 million hectares in 2017. The total yield obtained from this area was 4.84 million tons (FAOSTAT, 2019; <http://www.fao.org/faostat/en/>). In Egypt, the total area planted with this crop in the same year was low, 32,532 ha, which yielded 112,871 tons. This amount of production is insufficient to cover domestic consumption, where 549,200 tons were imported. Thus, there is a great need to maximize the production of faba beans through additional reclaimed areas, representing hope for future cultivated land.

Legumes are drought- and salt-sensitive crops, so farmers/producers do not grow them under these stresses (Amede & Schubert, 2003; Ashraf & Foolad, 2007). Under drought stress, faba bean genotypes display different responses in the production of acceptable seed yield. As a result, it is important to assess the potential of genotypes of faba bean under different irrigation regimes (Alghamdi *et al.*, 2015; Kabbadj *et al.*, 2017; Ribeiro *et al.*, 2019). Breeders have a great ability to assess breeding materials to withstand IWD under open field conditions, considering agronomic traits in drought-prone locations (Link *et al.*, 1999). Although the difference in genetic response to drought has been documented, the physiological processes related to drought tolerance still need more understanding.

Therefore, the present study aimed at assessing the potential beneficial roles of AsA and Si in enhancing the tolerance to IWD stress in five different cultivars of commercial faba bean by exploring the improving effect of AsA or Si on maintaining plant water content, growth, and production, in addition to the protection of plant cells from oxidative damages by boosting the antioxidative defense system components under IWD stress. Moreover,

this study aimed at providing a possible additional insight into the promoting role of the two applied promoters (*i.e.*, AsA and Si) in improving drought tolerance and mitigating the drought-induced damage in faba bean plants by mechanisms of their action on the attributes of physio-biochemistry and the components of antioxidative defense system.

Material and methods

Experimental site and cultural practices

In the two growing seasons of 2017/2018 and 2018/2019, two field experiments were conducted using the production area of faba bean on the experimental farm of the Faculty of Agriculture, Zagazig University; 30° 36' N, 32° 16' E, Egypt. Before sowing, soil samples were taken at a depth of 0–60 cm to analyze some chemical and physical properties. These analyses indicated that the soil was sandy throughout the profile (95.1% sand, 3.5% silt, and 1.4% clay). Soil field capacity was 9.7%, calcium carbonate 6.3 g kg⁻¹, organic matter 5.4 g kg⁻¹, pH 8.1, and electrical conductivity 0.6 dS m⁻¹. Soluble cations and anions were 1.8 mmol L⁻¹ for K⁺, 0.3 mmol L⁻¹ Mg²⁺, 1.9 mmol L⁻¹ Ca²⁺, 1.1 mmol L⁻¹ Na⁺, 1.7 mmol L⁻¹ Cl⁻, and 2.3 mmol L⁻¹ HCO₃⁻. In addition, available nutrients were 75.3, 6.0, and 22.5 mg kg⁻¹ soil for K, P, and N, respectively. Depending on the optimal period for growing faba beans in the region, planting was performed in the first week of November for both seasons. Standard agronomic practices including drip irrigation, pest and disease control were applied as recommended for the commercial production of faba beans. Before sowing, 75 kg of P₂O₅ [Ca(H₂PO₄)₂; 15.5% P₂O₅] and 60 kg of K₂O [K₂SO₄; 48% K₂O] were added per hectare. An amount of 45 kg of N ha⁻¹ [(NH₄)₂SO₄; 21% N] was added once at sowing.

Plant material and irrigation regimes

For this study, five faba bean (*Vicia faba*) cultivars were used: 'Giza-716', 'Giza-843', 'Nubaria-2', 'Sakha-3', and 'Sakha-4'. The pedigrees of these five cultivars were 83/453/503 × 83/824/461; 461/845/83 × 561/2076/85; Hybrid 735 (Radiation 2095/76 × ILB 1550); promising line-derived individual selection from 'Giza-716'; and 'Sakha 1' × 'Giza 3', respectively. The design applied to the experiments was a split-split-plot with three replicates. The main plots were randomly occupied by irrigation regimes. In the sub- and sub-sub-plots, foliar treatments and tested cultivars were randomly applied, respectively. In each plot, four rows were designed with a length of 3.00 m each and the distance

among them was 0.70 m. Each row was sown in hills at distances of 0.15 m. The seeding rate was 20 seeds m⁻² and the average population of plants was approximately 190,500 plants ha⁻¹. On all rows, each hill was received four seeds, which were thinned to two seedlings at full emergence. The cultivars were evaluated for three water irrigation regimes (described in Table S1 [suppl.]): WW, well-watered, 4000 m³ ha⁻¹; MD, moderate drought, 3000 m³ ha⁻¹; and SD, severe drought, 2000 m³ ha⁻¹. These amounts were specified considering the amount of irrigation water required for faba beans in the study area. Drought conditions were induced beginning from seedling establishment to maturity. A drip irrigation system was used for the experiments. The drip laterals and emitters were spaced at 0.7 and 0.30 m, respectively. The operating pressure and emitter flow rate were maintained at 1 bar and 4 L h⁻¹, respectively, by specifying a valve and pressure gauge to each irrigation sector. For each irrigation regime, the targeted amount of irrigation water was measured by using a flow meter. Irrigation was applied once a week from the full emergence of seedlings to flowering and twice a week starting from flowering to maturity. Two weeks before harvesting (*i.e.*, in mid-April), irrigation was terminated in both growing seasons.

Foliar applications

Three foliar sprays with AsA or Si (potassium silicate, K₂O₃Si) were applied at a rate of 1.5 or 2 mM, respectively. The sprays were applied at 25, 40, and 55 days of sowing and the control plants were sprayed with DW. These applied concentrations, times, and dates were determined according to our preliminary studies (data not shown). The spray solutions were added to plant foliage until they dripped using Dorsal Spray Machine (20 L). Tween-20 was added at a concentration of 0.1% to the spray solutions as a surfactant to ensure the permeation of spray solutions into plant leaf tissues.

Determination of physio-biochemical constituents

The fully-expanded upper leaves were collected from all treatments to assess all physio-biochemical constituents that were tested in this study. Using pure acetone, chlorophyll a and chlorophyll b were extracted and evaluated (Fadeels, 1962). The contents (mg g⁻¹ FW) of these pigments were calculated according to the modified formula of Von Wettstein (1957). The procedures outlined in Bates *et al.* (1973) were used to evaluate the contents (μmol g⁻¹ DW) of proline using a freshly-prepared standard of pure L-proline. By using ethyl alcohol (96%, v/v),

the procedure of Irigoyen *et al.* (1992) was followed to extract and determine the content (mg g^{-1} DW) of total soluble sugars. Freshly-prepared anthrone reagent (0.15 g anthrone + 0.1 L of H_2SO_4 ; 72%, v/v) was immediately reacted with 100 μL of extract. The reacted mixture was boiled for 10 min in a water-bath and then cooled. Absorbance readings were recorded using Spectronic (Bausch and Lomb-2000) Spectrophotometer at 625 nm. Net photosynthetic rate (Pn), transpiration rate (Tr), and stomatal conductance (gs) were determined for photosynthetic system using a portable LF6400XTR, LI-COR photosynthesis system (USA). Values were recorded every hour from 09:00 to 11:00 morning. The formula $[\text{Fv}/\text{Fm} = (\text{Fm}-\text{F0})/\text{Fm}]$ suggested by Maxwell & Johnson (2000) was used to calculate the maximum quantum yield of PSII (Fv/Fm). Physiological parameters such as relative water content (RWC; Osman & Rady, 2012), membrane stability index (MSI; Rady, 2011), and electrolyte leakage (EL; Weatherly, 1950), were evaluated using blades of fully-expanded upper leaves after excluding the main midribs. Lipid peroxidation was determined by evaluating the content ($\mu\text{mol g}^{-1}$ FW) of MDA. The content of MDA was evaluated using the extracts prepared by Heath & Packer (1968) for H_2O_2 assessment. A coefficient of molar extinction ($0.155 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$) was used to calculate the MDA content.

Determination of antioxidants activities

The method described by Vitória *et al.* (2001) was followed to obtain enzymatic extract. The methods suggested by Chance & Maehly (1955), Fielding & Hall (1978), Thomas *et al.* (1982), Rao *et al.* (1996), and Sairam *et al.* (2002) were followed to assay the activities of catalase (CAT), ascorbate peroxidase (APX), peroxidase (POD), glutathione reductase (GR), and superoxide dismutase (SOD), respectively. Activities of CAT, POD, and APX were assayed spectrophotometrically. Activity of SOD was assayed considering the drop in absorbance readings of superoxide-nitro blue tetrazolium complex caused by the enzyme. Activity of GR was assayed through three absorbance readings, which were recorded at 340 nm as a result in NADPH oxidation. Activities were expressed as $\text{Avalue min}^{-1} \text{ mg}^{-1} \text{ protein}$.

Growth and yield parameters measurements

At the physiological maturity, plant height (cm) was assessed for 10 plants in each plot. Measurements were taken from the ground level to the top of the plant. Pod number plant^{-1} , seeds number pod^{-1} , and 100-seed weight have been assessed. In addition, total yield of seeds and aboveground biomass were determined per hectare.

The weight of 100 seeds was evaluated with an average weight of three sets of 100 seeds. The total yield of seeds and aboveground biomass of plants occupying the two central rows were measured within a total area of 4.2 m^2 per plot. The measurement values were converted into kg ha^{-1} .

Water use efficiency

WUE ($\text{kg ha}^{-1} \text{ mm}^{-1}$) for seed yield (WUE_y) and aboveground biomass (WUE_{ab}) were calculated as follows:

$$\text{WUE}_y = \frac{\text{SY}}{\text{TWU}}$$

$$\text{WUE}_{bm} = \frac{\text{AB}}{\text{TWU}}$$

where SY is the seed yield (kg ha^{-1}), TWU is the total water used (mm), and AB is the aboveground biomass (kg ha^{-1}).

The total water used was calculated using the following equation:

$$\text{TWU} = \text{P} + \text{I} + \Delta\text{W} - \text{R} - \text{D} + \text{CR}$$

where P is the precipitation (mm), I is the irrigation water amount (mm), ΔW is the soil moisture change at planting and harvest (mm), R is the surface runoff (mm), D is the soil water drainage below the crop root zone (mm), and CR is the capillary rise.

Soil moisture content was measured by the oven drying method. Soil samples were taken at two soil depths (0.3 and 0.6 m) before planting and at harvest to determine the initial and final soil moisture content, respectively. The values were converted to a volumetric basis by multiplying them by the bulk density of the respective layer (1.57 g cm^{-3}) and soil depth of the sample. Surface runoff and drainage were neglected because of using drip irrigation system (Aydinsakir *et al.*, 2013). Capillary rise was also considered negligible as the groundwater table was 10 m deep (Dong *et al.*, 2011).

Biplot of principal components analysis was constructed using R to estimate the association between evaluated traits.

Statistical analysis

The R software was utilized to statistically analyze all data of this study. Differences found among all treatments; irrigation regimes, foliar applications, cultivars, and their interactions were separated by the Least Significant Difference (LSD) at $p \leq 0.05$.

Results

Leaf photosynthetic pigments, proline, and soluble sugars

SD and MD significantly ($p \leq 0.05$) decreased the contents of pigments related to photosynthesis (PRP: chlorophyll a and chlorophyll b) and increased the contents of osmoprotectants (e.g., total soluble sugars and proline) in the five faba bean cultivars compared to WW condition (the control) (Table 1). Reductions observed in the contents of PRP and increases noted in the contents of osmoprotectants were more pronounced with SD compared to MD. However, the application of AsA or Si significantly increased the contents of PRP and further increased the contents of osmoprotectants compared to the control. The mitigating effects of AsA or Si were more pronounced under MD than SD. The best response of these attributes was recorded by cv. 'Nubaria-2' treated with AsA, which conferred the best results of photosynthetic pigments under WW, while the highest values of proline and total soluble sugar were obtained under SD compared to other water regimes.

Gas exchange and photosynthetic efficiency (Fv/Fm)

The data in Table 2 show decreases in the criteria of gas exchange (e.g., Pn, Tr, and gs) and Fv/Fm in all faba bean cultivars under IWD stress. The reductions occurred in gas exchange criteria and Fv/Fm was gradually raised as the level of IWD increased. 'Nubaria-2' showed the highest values, while 'Sakha-4' showed the minimal values for these criteria in comparison with other tested cultivars under all water regimes. On the other hand, the application of AsA or Si significantly increased all gas exchange parameters and Fv/Fm in comparison with the control. The higher response of all tested parameters was noted with all cultivars treated with AsA. The interactive treatments (promoter; AsA or Si \times irrigation regime; WW, MD, or SD) showed variable results according to the applied water regime and promoter. The best results of all tested criteria were obtained with AsA application under all water regimes compared to all other treatments.

Relative content of water (RWC), stability index of cellular membranes (MSI), leakage of electrolytes (EL), and lipid peroxidation

SD and MD considerably ($p \leq 0.05$) decreased RWC and MSI, and considerably increased EL and MDA content in all tested cultivars compared to the control

(Table 3). The reductions in RWC and MSI and the rises in EL and MDA were more pronounced with SD compared to MD. However, the application of AsA or Si considerably elevated RWC and MSI and decreased EL and MDA in comparison with the control. By using AsA treatment, 'Nubaria-2' displayed the highest response for all examined attributes. Under WW conditions, AsA treatment generated the best results of the above parameters compared to other water regimes (MD and SD). In addition, the best results of all tested criteria were obtained with AsA application under all water regimes.

Enzymatic antioxidant activities

Plants of all faba bean cultivars exposed to IWD stress conditions (MD or SD) displayed significant ($p \leq 0.05$) elevations in all enzymatic activities (CAT, SOD, POD, APX, and GR) compared to WW plants (Fig. 1). The highest enzyme activities were observed under SD in all cultivars. However, foliar application of AsA or Si further increased leaf enzymatic activity compared to the control. These increases in enzymatic activities were gradually elevated with the gradual rise in severity of IWD stress. AsA treatment showed better results than Si treatment. Under WW conditions, AsA application produced the best results in all cultivars in comparison with other water regimes (MD and SD). In addition, the best results of all tested criteria were obtained with AsA application under all water regimes.

Seed yield and contributing traits

All measured agronomic traits (average plant height, pod number per plant, seed number per pod, 100-seed weight, and total seed and aboveground biomass yields per hectare) of all investigated faba bean cultivars were significantly reduced under the two drought levels compared to the control (Fig. 2). Reductions occurred in all agronomic traits were more pronounced under SD compared to MD. However, the application of AsA or Si significantly enhanced all agronomic traits in all investigated cultivars compared to untreated plants. In addition, faba bean cultivars offered varying responses to IWD stress. Generally, 'Nubaria-2', 'Giza-843', and 'Sakha-3' (with preference for 'Nubaria-2') showed the highest agronomic traits under different irrigation regimes, while 'Giza-716' and 'Sakha-4' showed the lowest ones. Plant height showed the highest response to Si treatment in 'Sakha-3', which presented 8.5 and 5.9% more than the untreated plants under MD and SD stress, respectively (Fig. 2A). Likewise, number of pods per plant showed the highest response to AsA treatment with 'Nubaria-2' under MD

Table 1. Influence of ascorbic acid (AsA) or silicon (Si) application on the contents of leaf photosynthetic pigments (chlorophyll a and b), soluble sugar, and proline of five faba bean cultivars grown under three irrigation regimes

Cultivar	Chlorophyll a (mg g ⁻¹ FW)				Chlorophyll b (mg g ⁻¹ FW)				Soluble sugars (mg g ⁻¹ DW)				Proline (μmol g ⁻¹ DW)				
	Cont.	AsA	Si	Mean	Cont.	AsA	Si	Mean	Cont.	AsA	Si	Mean	Cont.	AsA	Si	Mean	
Well-watered																	
G1	2.01	2.33	2.31	2.22 ^c	0.73	0.85	0.82	0.80 ^d	12.12	14.02	13.95	13.36 ^l	7.92	9.83	9.76	9.17 ^l	
G2	2.10	2.46	2.44	2.33 ^{ab}	0.76	0.93	0.92	0.87 ^b	12.95	15.34	14.97	14.42 ^k	8.76	11.14	10.78	10.23 ^k	
G3	2.15	2.56	2.50	2.40 ^a	0.78	0.98	0.98	0.91 ^a	13.47	16.17	15.80	15.15 ⁱ	9.28	11.98	11.6	10.95 ^j	
G4	2.02	2.38	2.35	2.25 ^{bc}	0.75	0.88	0.86	0.83 ^c	12.82	14.61	14.43	13.95 ^k	8.62	10.42	10.24	9.76 ^k	
G5	2.00	2.25	2.16	2.14 ^c	0.71	0.81	0.80	0.78 ^c	11.37	13.88	13.66	12.97 ^l	7.17	9.68	9.47	8.77 ^l	
Mean	2.06 ^B	2.40 ^A	2.35 ^A	2.27^A	0.75 ^C	0.89 ^A	0.88 ^B	0.84^A	12.55 ^G	14.80 ^F	14.56 ^F	13.97^C	8.35 ^G	10.61 ^F	10.37 ^F	9.78^C	
Moderate drought																	
G1	1.43	1.69	1.68	1.60 ^{fg}	0.51	0.63	0.62	0.58 ⁱ	15.89	19.30	18.69	17.96 ^h	18.11	21.52	20.91	20.18 ^h	
G2	1.49	1.87	1.86	1.74 ^{de}	0.54	0.68	0.66	0.62 ^g	16.49	19.49	20.86	18.95 ^e	18.71	21.71	22.93	21.12 ^e	
G3	1.53	1.92	1.90	1.78 ^d	0.56	0.70	0.68	0.65 ^f	16.69	22.89	22.16	20.58 ^f	18.91	25.12	24.38	22.80 ^f	
G4	1.48	1.72	1.70	1.63 ^{ef}	0.52	0.64	0.63	0.59 ^h	16.29	20.29	20.26	18.95 ^e	18.50	22.51	22.48	21.16 ^e	
G5	1.41	1.53	1.53	1.49 ^g	0.50	0.61	0.60	0.57 ^j	15.29	18.23	17.43	16.98 ⁱ	17.51	20.44	19.64	19.20 ^j	
Mean	1.47 ^D	1.75 ^C	1.73 ^C	1.65^B	0.52 ^F	0.65 ^D	0.64 ^E	0.60^B	16.13 ^E	20.04 ^D	19.88 ^D	18.68^B	18.35 ^E	22.26 ^D	22.07 ^D	20.89^B	
Severe drought																	
G1	0.99	1.17	1.13	1.10 ^{ij}	0.27	0.39	0.38	0.35 ⁿ	21.49	29.52	28.55	26.52 ^d	28.14	36.18	35.21	33.18 ^d	
G2	1.02	1.29	1.18	1.16 ^{hi}	0.29	0.46	0.44	0.40 ^l	23.45	31.55	31.15	28.72 ^b	30.11	38.21	37.81	35.38 ^b	
G3	1.02	1.38	1.31	1.24 ^h	0.31	0.48	0.46	0.42 ^k	24.25	32.25	31.89	29.46 ^a	30.91	38.91	38.54	36.12 ^a	
G4	0.99	1.16	1.15	1.10 ^{ij}	0.28	0.42	0.40	0.37 ^m	22.55	30.42	30.09	27.69 ^c	29.21	37.08	36.74	34.34 ^c	
G5	0.98	1.09	1.07	1.05 ^j	0.25	0.35	0.33	0.31 ^o	19.65	27.32	26.69	24.55 ^c	26.31	33.98	33.34	31.21 ^c	
Mean	1.00 ^F	1.22 ^E	1.17 ^E	1.13^C	0.28 ^I	0.42 ^G	0.40 ^H	0.37^C	22.28 ^C	30.21 ^A	29.67 ^B	27.39^A	28.94 ^C	36.87 ^A	36.33 ^B	34.04^A	
Mean (F)	1.51^C	1.79^A	1.75^B		0.52^C	0.65^A	0.64^B		16.99^C	21.69^A	21.37^B		18.54^C	23.24^A	22.92^B		
ANOVA	df																
Irrigation (I)	2	<0.001				<0.001				<0.001				<0.001			
Foliar (F)	2	<0.001				<0.001				<0.001				<0.001			
Cultivar (C)	4	<0.001				<0.001				<0.001				<0.001			
Year	1	0.129				0.042				0.046				0.730			
I × F	4	<0.001				<0.001				<0.001				<0.001			
I × C	8	0.031				<0.001				<0.001				<0.001			
F × C	8	0.005				<0.001				<0.001				<0.001			
I × F × C	16	0.047				<0.001				<0.001				<0.001			

G1 is 'Giza-716', G2 is 'Giza-843', G3 is 'Nubaria-2', G4 is 'Sakha-3' and G5 is 'Sakha-4'. Bold and italic refers to the three irrigation regimes, bold and not italic refers to foliar applications. Means followed by different letters in the same direction differ significantly by LSD ($p \leq 0.05$).

Table 2. Influence of ascorbic acid (AsA) or silicon (Si) application on leaf net photosynthetic rate (Pn), stomatal conductance (gs), rate of transpiration (Tr) and photosynthetic efficiency (Fv/Fm) of five faba bean cultivars grown under three irrigation regimes

Cultivar	Pn ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)				gs ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)				Tr ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)				Fv/Fm				
	Cont.	AsA	Si	Mean	Cont.	AsA	Si	Mean	Cont.	AsA	Si	Mean	Cont.	AsA	Si	Mean	
Well-watered																	
G1	10.45	11.47	11.22	11.04 ^d	0.68	0.81	0.79	0.76 ^d	5.10	6.12	5.70 ^d	5.70 ^d	0.83	0.87	0.86	0.85 ^c	
G2	10.60	12.56	12.37	11.84 ^b	0.71	0.89	0.87	0.82 ^b	5.25	7.22	6.50 ^b	6.50 ^b	0.90	0.92	0.97	0.93 ^b	
G3	10.67	13.13	12.78	12.19 ^a	0.73	0.92	0.90	0.85 ^a	5.32	7.79	6.85 ^a	6.85 ^a	0.97	0.98	0.96	0.97 ^a	
G4	10.53	11.99	11.69	11.40 ^c	0.70	0.85	0.83	0.79 ^c	5.18	6.64	6.06 ^c	6.06 ^c	0.88	0.84	0.86	0.86 ^c	
G5	10.39	11.04	10.94	10.79 ^e	0.66	0.78	0.76	0.73 ^e	5.04	5.69	5.44 ^e	5.44 ^e	0.83	0.84	0.91	0.86 ^c	
Mean	10.52^C	12.04^A	11.80^B	11.45^A	0.69^C	0.85^A	0.83^B	0.79^A	5.18^C	6.69^A	6.11^A	6.11^A	0.88^A	0.89^A	0.91^A	0.89^A	
Moderate drought																	
G1	8.14	9.47	9.40	9.00 ⁱ	0.42	0.55	0.53	0.50 ⁱ	3.28	4.61	4.54	4.15 ⁱ	0.72	0.72	0.78	0.74 ^c	
G2	8.54	9.88	9.80	9.41 ^g	0.45	0.63	0.61	0.56 ^g	3.68	5.02	4.95	4.55 ^g	0.78	0.73	0.70	0.73 ^c	
G3	8.76	10.09	9.96	9.60 ^f	0.47	0.63	0.64	0.58 ^f	3.90	5.23	5.11	4.75 ^f	0.79	0.77	0.75	0.77 ^d	
G4	8.27	9.67	9.56	9.17 ^h	0.44	0.59	0.57	0.53 ^h	3.41	4.81	4.71	4.31 ^h	0.77	0.74	0.71	0.74 ^c	
G5	8.00	9.30	9.12	8.80 ^j	0.40	0.52	0.50	0.47 ^j	3.14	4.44	4.26	3.95 ^j	0.71	0.75	0.73	0.73 ^c	
Mean	8.34^F	9.68^D	9.57^E	9.20^B	0.43^F	0.58^D	0.57^E	0.53^B	3.48^F	4.82^D	4.71^E	4.34^B	0.75^B	0.74^B	0.73^B	0.74^B	
Severe drought																	
G1	5.37	6.29	6.16	5.94 ⁿ	0.28	0.41	0.39	0.36 ⁿ	1.77	2.68	2.56	2.33 ⁿ	0.40	0.42	0.60	0.47 ^g	
G2	5.67	6.63	6.54	6.28 ^l	0.31	0.49	0.47	0.42 ^l	2.06	3.03	2.94	2.67 ^l	0.53	0.57	0.51	0.54 ^f	
G3	5.85	6.75	6.69	6.43 ^k	0.33	0.49	0.50	0.44 ^k	2.25	3.14	3.08	2.82 ^k	0.53	0.57	0.37	0.49 ^g	
G4	5.57	6.48	6.44	6.17 ^m	0.30	0.45	0.43	0.39 ^m	1.97	2.88	2.84	2.56 ^m	0.44	0.48	0.38	0.43 ^h	
G5	5.12	6.38	6.33	5.94 ⁿ	0.26	0.38	0.36	0.33 ^o	1.51	2.77	2.72	2.33 ⁿ	0.39	0.47	0.42	0.43 ^h	
Mean	5.52^I	6.50^G	6.43^H	6.15^C	0.29^H	0.44^G	0.43^G	0.39^C	1.91^I	2.90^G	2.83^H	2.55^C	0.46^D	0.50^C	0.46^D	0.47^C	
Mean (F)	8.13^C	9.41^A	9.27^B		0.47^C	0.63^A	0.61^B		3.52^C	4.80^A	4.66^B		0.70^A	0.71^A	0.70^A		
ANOVA	df																
Irrigation (I)	2	<0.001				<0.001				<0.001				<0.001			
Foliar (F)	2	<0.001				<0.001				<0.001				0.225			
Cultivar (C)	4	<0.001				<0.001				<0.001				<0.001			
Year	1	0.011				0.074				0.348				0.064			
I × F	4	<0.001				0.032				<0.001				0.006			
I × C	8	<0.001				0.002				<0.001				<0.001			
F × C	8	<0.001				<0.001				<0.001				<0.001			
I × F × C	16	<0.001				0.004				<0.001				<0.001			

G1 is 'Giza-716', G2 is 'Giza-843', G3 is 'Nubaria-2', G4 is 'Sakha-3' and G5 is 'Sakha-4'. Bold and italic refers to the three irrigation regimes, bold and not italic refers to foliar applications. Means followed by different letters in the same direction differ significantly by LSD ($p \leq 0.05$).

Table 3. Influence of ascorbic acid (AsA) or silicon (Si) application on relative water content (RWC), membrane stability index (MSI), electrolyte leakage (EL) and malondialdehyde (MDA) of five faba bean cultivars grown under three irrigation regimes

Cultivar	RWC (%)				MSI (%)				EL (%)				MDA ($\mu\text{mol g}^{-1}$ FW)				
	Cont.	AsA	Si	Mean	Cont.	AsA	Si	Mean	Cont.	AsA	Si	Mean	Cont.	AsA	Si	Mean	
Well-watered																	
G1	51.90	55.93	55.51	54.45 ^d	48.83	52.86	52.44	51.38 ^d	21.19	23.77	23.43	22.79 ^k	47.24	42.04	43.00	44.09 ^l	
G2	53.08	59.40	58.57	57.02 ^b	50.00	56.31	55.47	53.93 ^b	20.77	22.31	22.14	21.74 ^l	45.28	37.74	38.33	40.45 ⁿ	
G3	53.52	60.90	60.70	58.37 ^a	50.45	57.82	57.62	55.30 ^a	20.67	21.37	21.21	21.08 ^m	44.83	35.76	36.72	39.10 ^o	
G4	52.38	57.61	59.61	56.53 ^c	49.28	54.51	56.51	53.43 ^c	20.91	23.01	24.09	22.67 ^k	45.84	39.68	40.90	42.14 ^m	
G5	51.49	53.82	53.37	52.89 ^e	48.39	50.72	50.27	49.79 ^e	21.35	24.35	24.34	23.35 ^j	48.52	43.67	44.14	45.44 ^k	
Mean	52.47 ^B	57.53 ^A	57.55 ^A	55.85^A	49.39 ^B	54.44 ^A	54.46 ^A	52.76^A	20.98 ^F	22.96 ^E	23.04 ^E	22.33^C	46.34 ^G	39.78 ^I	40.62 ^H	42.24^C	
Moderate drought																	
G1	41.38	45.41	44.99	43.93 ⁱ	38.28	42.31	41.90	40.83 ⁱ	24.80	27.38	27.05	26.41 ^f	59.64	55.66	56.35	57.21 ^g	
G2	42.56	48.88	48.05	46.50 ^g	39.47	45.79	44.96	43.41 ^g	24.38	25.92	25.76	25.35 ^h	59.31	51.90	52.94	54.72 ⁱ	
G3	43.00	50.38	50.18	47.85 ^f	39.91	47.29	47.09	44.76 ^f	24.26	24.96	24.80	24.67 ⁱ	59.05	49.72	50.27	53.01 ^j	
G4	41.86	47.09	49.09	46.01 ^h	38.73	43.93	45.93	42.86 ^h	24.50	26.60	27.68	26.26 ^g	59.54	54.19	54.83	56.19 ^h	
G5	40.97	43.30	42.85	42.37 ^j	37.81	40.14	39.69	39.21 ^j	24.97	27.97	27.96	26.96 ^e	59.99	57.13	58.15	58.42 ^f	
Mean	41.95 ^D	47.01 ^C	47.03 ^C	45.33^B	38.84 ^D	43.89 ^C	43.91 ^C	42.21^B	24.58 ^D	26.56 ^C	26.65 ^C	25.93^B	59.51 ^D	53.72 ^F	54.51 ^E	55.91^B	
Severe drought																	
G1	30.50	34.53	34.11	33.05 ^a	27.34	31.37	30.95	29.89 ^a	28.27	30.84	30.50	29.87 ^b	75.11	66.67	67.34	69.71 ^b	
G2	31.68	38.00	37.17	35.62 ^l	28.52	34.84	34.01	32.46 ^l	27.85	29.40	29.23	28.82 ^c	73.98	63.73	64.28	67.33 ^d	
G3	32.12	39.50	39.30	36.97 ^k	28.96	36.34	36.14	33.81 ^k	27.75	28.45	28.29	28.16 ^d	73.59	62.58	63.00	66.39 ^e	
G4	30.98	36.21	38.21	35.13 ^m	27.82	33.05	35.03	31.97 ^m	27.98	30.09	31.17	29.75 ^b	74.73	65.17	65.98	68.62 ^c	
G5	30.09	32.42	31.97	31.49 ^o	26.91	29.24	28.79	28.31 ^o	28.44	31.43	31.42	30.43 ^a	76.21	71.33	72.55	73.36 ^a	
Mean	31.07 ^F	36.13 ^E	36.15 ^E	34.45^C	27.91 ^F	32.97 ^E	32.98 ^E	31.29^C	28.06 ^B	30.04 ^A	30.12 ^A	29.41^A	74.72 ^A	65.90 ^C	66.63 ^B	69.08^A	
Mean (F)	41.83^B	46.89^A	46.91^A		38.71^B	43.77^A	43.79^A		24.54^C	26.52^B	26.6^A		60.19^A	53.13^B	53.92^C		
ANOVA	df																
Irrigation (I)	2	<0.001				<0.001				<0.001				<0.001			
Foliar (F)	2	<0.001				<0.001				<0.001				<0.001			
Cultivar (C)	4	<0.001				<0.001				<0.001				<0.001			
Year	1	0.057				0.266				0.073				0.350			
I × F	4	0.011				0.045				0.042				<0.001			
I × C	8	0.046				0.003				0.035				<0.001			
F × C	8	<0.001				<0.001				<0.001				<0.001			
I × F × C	16	0.081				0.002				0.054				<0.001			

G1 is 'Giza-716', G2 is 'Giza-843', G3 is 'Nubaria-2', G4 is 'Sakha-3' and G5 is 'Sakha-4'. Bold and italic refers to the three irrigation regimes, bold and not italic refers to foliar applications. Means followed by different letters in the same direction differ significantly by LSD ($p \leq 0.05$).

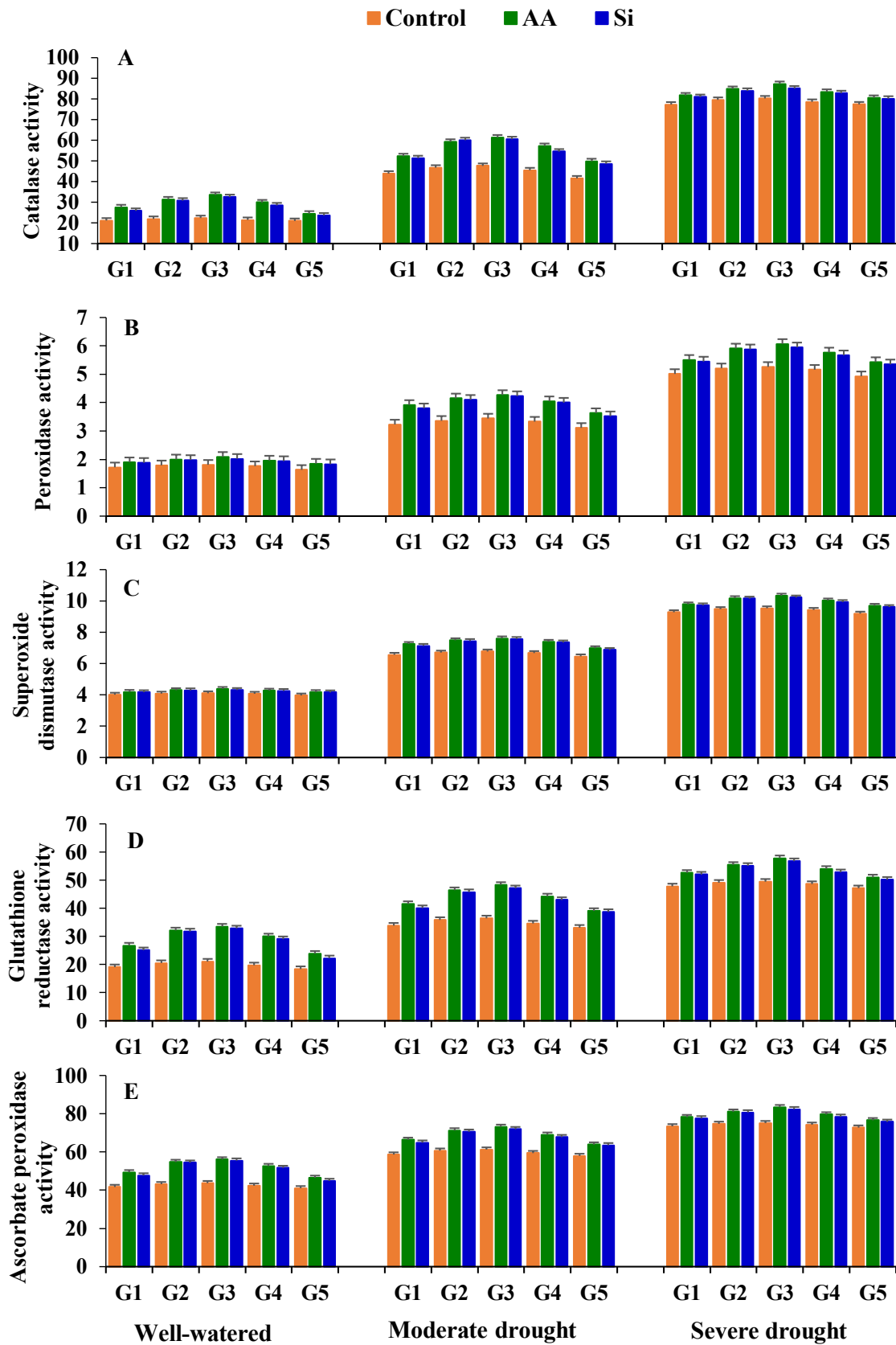


Figure 1. Effect of ascorbic acid (AsA) or silicon (Si) application on the activities ($A_{\text{value}} \text{ min}^{-1} \text{ g}^{-1} \text{ protein}$) of catalase (A), peroxidase (B), superoxide dismutase (C), glutathione reductase (D), and ascorbate peroxidase (E) of five faba bean cultivars (G1 is ‘Giza-716’, G2 is ‘Giza-843’, G3 is ‘Nubaria-2’, G4 is ‘Sakha-3’ and G5 is ‘Sakha-4’) grown under three irrigation regimes. The bars on the top of the columns represent the LSD ($p \leq 0.05$).

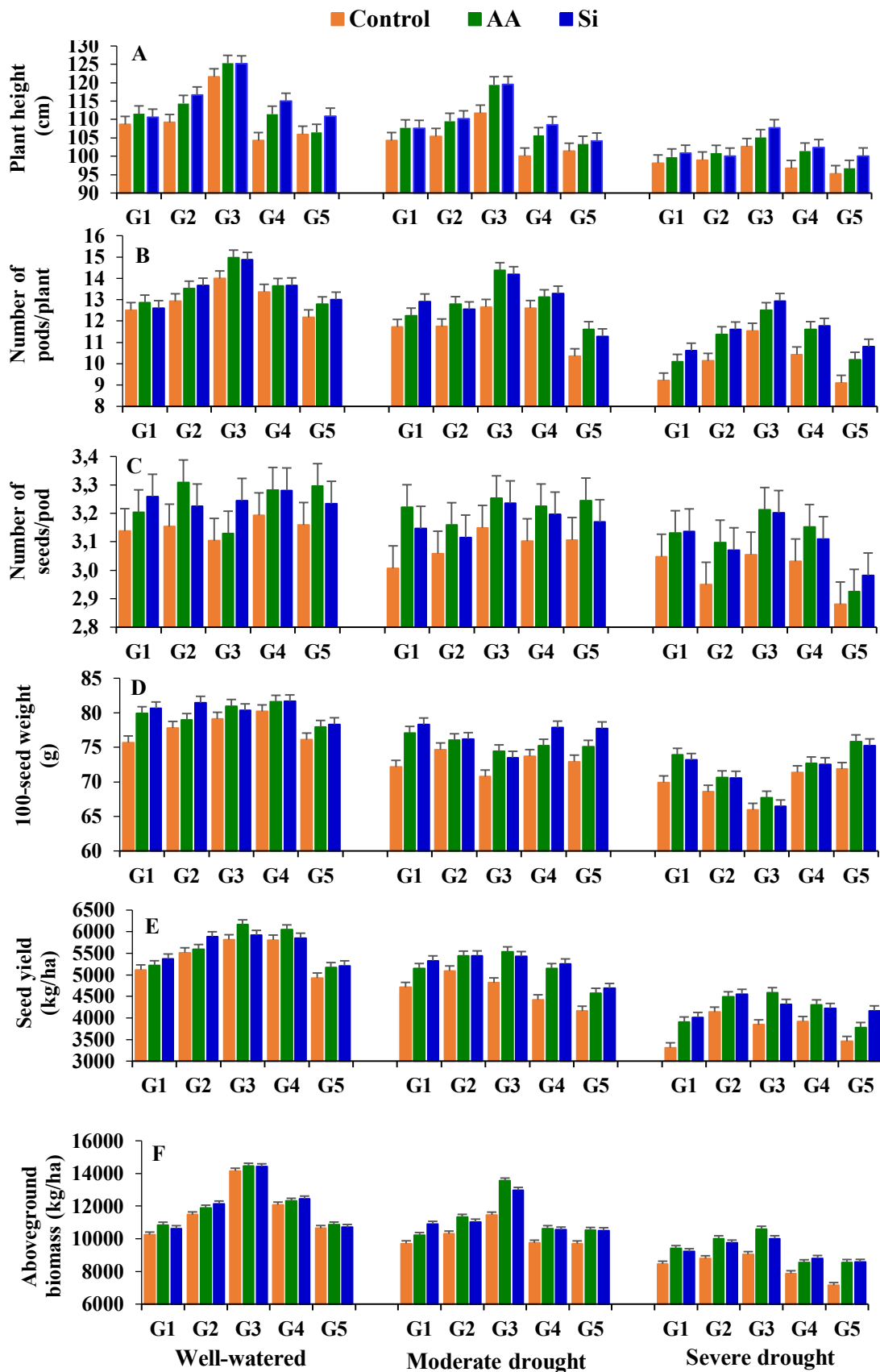


Figure 2. Impact of ascorbic acid (AsA) or silicon (Si) application on plant height (A), number of pods plant⁻¹ (B), number of seeds pod⁻¹ (C), 100-seed weight (D), seed yield (E) and aboveground biomass (F) of five faba bean cultivars (G1 is ‘Giza-716’, G2 is ‘Giza-843’, G3 is ‘Nubaria-2’, G4 is ‘Sakha-3’ and G5 is ‘Sakha-4’) grown under three irrigation regimes. The bars on the top of the columns represent the LSD ($p \leq 0.05$).

and SD stress (13.6 and 8.4%, respectively, Fig. 2B). On the other hand, number of seeds pod⁻¹ showed the highest response to AsA treatment with ‘Giza-843’ under MD and SD conditions (3.3 and 5.0%, respectively, Fig. 2C). Additionally, 100-seed weight showed the highest response to Si treatment with ‘Giza-716’ under MD and SD conditions (8.5 and 4.6%, respectively, Fig. 2D). Similarly, seed yield showed the highest response to AsA treatment with ‘Nubaria-2’ under MD and SD conditions (14.9 and 19.4%, respectively, Fig. 2E). Finally, aboveground biomass showed the highest response to AsA treatment with ‘Nubaria-2’ under MD and SD conditions (18.2 and 17.2%, respectively, Fig. 2F).

Water use efficiency (WUE)

The results of WUE for seed yield (WUE_s) and aboveground biomass (WUE_{ab}) as affected by IWD, genotypes, and foliar application are presented in Table 4. Generally, WUE_s ranged from 9.92 to 15.47 kg⁻¹ ha⁻¹ mm⁻¹ and WUE_{ab} varied from 20.62 to 35.72 kg⁻¹ ha⁻¹ mm⁻¹. Under MD and SD conditions, the genotypes showed higher WUE_s (in average 12.63 and 13.71 kg⁻¹ ha⁻¹ mm⁻¹, respectively) and WUE_{ab} (in average 28.21 and 31.25 kg⁻¹ ha⁻¹ mm⁻¹, respectively) than under WW conditions (11.22 and 24.06 kg⁻¹ ha⁻¹ mm⁻¹ for WUE_s and WUE_{ab}, respectively). The application of AsA or Si significantly improved WUE_s (in average 12.86 or 12.96 kg⁻¹ ha⁻¹ mm⁻¹, respectively) and WUE_{ab} (28.14 and 27.92 kg⁻¹ ha⁻¹ mm⁻¹, respectively) in all cultivars compared to the untreated ones (11.74 and 25.70 kg⁻¹ ha⁻¹ mm⁻¹, respectively for WUE_s and WUE_{ab}). Moreover, the evaluated cultivars displayed different WUE_s and WUE_{ab} under IWD. ‘Nubaria-2’, ‘Giza-843’, and ‘Sakha-3’ showed highest values of WUE_s and WUE_{ab} compared to other cultivars under different conditions.

Interrelationship among evaluated traits

The main components were estimated to explore the correlation between evaluated traits. The first two main components accounted for most of the variability, about 92.1% (78.0% by PC1 and 14.1% by PC2), and, accordingly, were used to construct the biplot (Fig. 3). Attributes were represented by parallel or closely related vectors that revealed a strong positive correlation, while the vectors approximately close (at 180 °) showed a very negative correlation. In addition, the vectors toward sides had expressed little correlation. The evaluated traits in this study could be divided into three groups. The first group consisted of agronomic traits: chlorophylls contents, gs, Pn, Tr, Fv/Fm, RWC, and MSI. The second group included soluble sugars and proline

contents, antioxidant enzymes activities (CAT, POD, SOD, GR, and APX), EL and WUE for seed and aboveground biomass yields. The third group contained only MDA. The traits within the first and second groups displayed high correlation among each other while intermediate correlation was detected among traits of these two groups. On the other hand, the first group exhibited negative correlation with the third group.

Discussion

Drought stress is a major serious threat to security of food worldwide. Climate change increases temperatures and decreases rainfall, and therefore increases the incidence of SD, especially in arid and semi-arid regions (Dai, 2013), causing water shortages and severely impairing plant growth and productivity. Drought stress causes various negative impacts on plant growth and total yields of different crops through reduced leaf absorption of photosynthetic active radiation and decreased efficiency of radiation-use (Earl & Davis, 2003). It significantly depresses MSI, chlorophyll content, Pn, and protein synthesis (Merwad *et al.*, 2018). Moreover, it leads to accumulations of toxic ions, and disturbances in gas exchange attributes, thereby inhibiting development, growth, and production of different crop plants (Anjum *et al.*, 2011; Kusvuran *et al.*, 2016).

Many biological processes are activated when plants encounter drought stress (Seki *et al.*, 2007), including processes related to photosynthesis, which are the most sensitive to IWD stress (Huo *et al.*, 2016). Globally, photosynthetic attributes have therefore been employed to evaluate drought tolerance in crop plants (Chaves *et al.*, 2008). In this study, IWD stress induced lower contents of chlorophylls related to photosynthesis, gas exchange (Pn, Tr, and gs), and Fv/Fm respect to normal conditions. The reduction in these attributes was closely related to the degree (MD and SD) of stress (Tables 1 & 2). All these parameters were found to have a similar trend to tissue health status (RWC and MSI, Table 3). Reducing gs under IWD stress can protect the plant because it allows to conserve water and improve WUE (Table 4). Zhou *et al.* (2013) concluded that reducing photosynthesis is commonly due to limitation of stomata under drought stress conditions due to reducing gs and intercellular CO₂ concentration. In the current study, the reduction in RWC under IWD was accompanied with a reduction in gs and Tr, demonstrating that limitation of stomata mainly led to decrease of Pn. Undesirable changes in gas exchange parameters implied the decrease in photosynthetic activity, which may be due to non-stomatal limitation rather than to reduction in stomata. The Fv/Fm, defined as the maximum quantum yield of primary photochemistry,

Table 4. Impact of ascorbic acid (AsA) or silicon (Si) application on water use efficiency of seed yield (WUEs) and aboveground biomass (WUEab) as affected by irrigation regimes of five faba bean cultivars under three irrigation regimes

Cultivar	WUE _s (kg ha ⁻¹ mm ⁻¹)				WUE _{ab} (kg ha ⁻¹ mm ⁻¹)			
	Cont.	AsA	Si	Mean	Cont	AsA	Si	Mean
Well-watered								
G1	10.30	10.49	10.81	10.53 ^k	20.62	21.84	21.41	21.29 ^k
G2	11.10	11.25	11.85	11.40 ^j	23.10	23.93	24.45	23.83 ^j
G3	11.71	12.40	11.91	12.01 ^h	28.49	29.10	29.03	28.87 ^e
G4	11.19	12.17	11.78	11.71 ⁱ	24.31	24.78	25.06	24.72 ⁱ
G5	9.92	10.41	10.49	10.27 ^l	21.43	21.85	21.55	21.61 ^k
Mean	10.84 ^E	11.34 ^D	11.37 ^D	<i>11.19^C</i>	23.59 ^F	24.30 ^E	24.30 ^E	<i>24.06^C</i>
Moderate drought								
G1	11.88	12.98	13.03	12.63 ^f	24.47	25.74	27.47	25.89 ^h
G2	12.84	13.22	13.21	13.09 ^{de}	25.97	28.55	27.81	27.44 ^g
G3	12.14	13.55	13.68	13.12 ^d	28.99	34.15	32.71	31.95 ^e
G4	11.25	12.97	12.85	12.36 ^g	24.56	26.81	26.59	25.99 ^h
G5	10.49	11.53	11.81	11.28	24.45	26.53	26.48	25.82 ^h
Mean	11.72 ^C	12.85 ^B	12.92 ^B	<i>12.50^B</i>	25.69 ^D	28.36 ^C	28.21 ^C	<i>27.42^B</i>
Severe drought								
G1	12.16	13.18	13.53	12.96 ^e	28.50	31.73	31.08	30.44 ^d
G2	13.95	15.15	15.34	14.81 ^a	30.63	33.75	32.86	32.41 ^b
G3	13.19	15.47	14.55	14.40 ^b	30.48	35.72	33.76	33.32 ^a
G4	13.21	14.52	14.23	13.99 ^c	26.54	28.80	29.68	28.34 ^f
G5	11.96	12.74	14.05	12.92 ^e	24.69	28.85	28.88	27.47 ^g
Mean	12.89 ^B	14.21 ^A	14.34 ^A	<i>13.82^A</i>	28.17 ^C	31.77 ^A	31.25 ^B	<i>30.40^A</i>
Mean (F)	11.82^B	12.80^A	12.87^A		25.82^C	28.14^A	27.92^B	
ANOVA	df							
Irrigation (I)	2		<0.001			<0.001		
Foliar (F)	2		0.002			<0.001		
Cultivar (C)	4		<0.001			<0.001		
Year	1		0.332			0.150		
I × F	4		0.006			0.001		
I × C	8		<0.001			<0.001		
F × C	8		<0.001			<0.001		
I × F × C	16		<0.001			<0.001		

G1 is 'Giza-716', G2 is 'Giza-843', G3 is 'Nubaria-2', G4 is 'Sakha-3' and G5 is 'Sakha-4'. Bold and italic refers to the three irrigation regimes, bold and not italic refers to foliar applications. Means followed by different letters in the same direction differ significantly by LSD ($p \leq 0.05$).

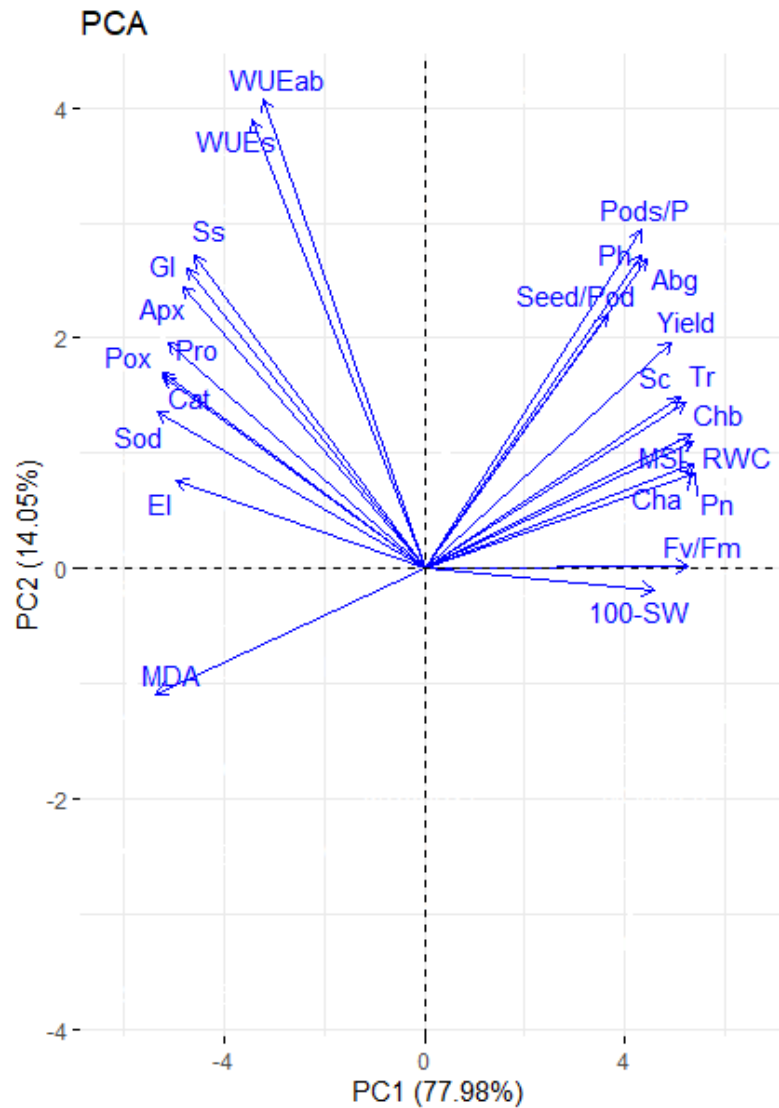


Figure 3. Biplot of principal components analysis demonstrating the relationship among the evaluated traits: Cha, chlorophyll a content; Chb, chlorophyll b; Ss, soluble sugar; Pr, proline content; Pn, net photosynthetic rate; Sc, stomatal conductance; Tr, rate of transpiration; Fv/Fm, photosynthetic efficiency; RWC, relative water content; MSI, membrane stability index; EL, electrolyte leakage; MDA, malondialdehyde; Cat, catalase; Pox, peroxidase; Sod, superoxide dismutase; Gl, glutathione reductase; Apx, ascorbate peroxidase; WUEs, water use efficiency of seed yield; WUEab, aboveground biomass; Ph, plant height; Pods/P, number of pods/plant; S/Pod, number of seeds/pod; 100-SW, 100-seed weight; Yield, seed yield; Abg, aboveground biomass.

can issue a simple and rapid way to assess when plants are under stress (Wang *et al.*, 2018). The current study found that Fv/Fm significantly decreased under MD and further decreased under SD compared with the control (Table 2).

Because CO₂ fixation in "Calvin cycle" is stress-sensitive (Murata *et al.*, 2007), possibly limits the photosynthesis and faba bean leaves absorb more light energy than the energy that can be consumed by photosynthetic CO₂ fixation. Reduction in photosynthetic CO₂ fixation decrea-

ses the use of NADPH, reducing NADP⁺ level (Murata & Takahashi, 2008). Because NADP⁺ is the primary electron acceptor in PSI, NADP⁺ depletion hastens the transport of electrons from PSI to O₂, generating H₂O₂ through O₂⁻ (Asada, 1999). Plants possess some protecting mechanisms [*e.g.*, Mehler reaction (Asada, 1999), photorespiration (Cornic & Fresneau, 2002), and non-photochemical quenching (Nabe *et al.*, 2007)] that can dissipate excess energy but the energy squandered by these mechanisms remains minimal. When drought intensity

exceeds the tolerance in plants, excessive energy will result in excessive generation of ROS, including H_2O_2 and $O_2^{\cdot-}$ (Wang *et al.*, 2018). Similarly to this study, Merwad *et al.* (2018) found that during stress, many low molecular weight antioxidants are increased in the plant. Additionally, a series of antioxidative enzymes are developed to scavenge different ROS. SOD removes $O_2^{\cdot-}$ (Bowler *et al.*, 1992), which is directly followed by the decomposition of H_2O_2 into O and H_2O by CAT (Garg & Manchanda, 2009) via "ascorbate (AsA)-glutathione (GSH) cycle" and antioxidant enzymes (Blokhina *et al.*, 2003). In addition, APX involved in the "AsA-GSH cycle" utilizes AsA as a donor of electrons to remove H_2O_2 . In this study, the activities of antioxidative enzymes (*e.g.*, SOD, POD, CAT, APX, and GR) were increased with aggravating IWD stress (Fig. 1). These results suggest a role of the "AsA-GSH cycle" in IWD-stressed faba bean plant in the removal of H_2O_2 . The steady increase in the enzymes activity, in this study, with aggravating IWD stress indicates an increase in ROS accumulation, and reductions in the attributes of growth and outcomes (*e.g.*, average height of plants, pods number per plant, seeds number per pod, weight of 100 seeds, and total yield of seeds and above-ground biomass per hectare; Fig. 2) occurred due to IWD stress indicates insufficient protective mechanisms in faba bean plants to remove excessive ROS under IWD stress. This excess of ROS accumulation damages plant proteins, lipids, cell membranes, chlorophylls, carbohydrates, and DNA, resulting in irreversible damages and eventually cell death (Gill & Tuteja, 2010). Thus, it is crucial to explore effective techniques to enhance plant growth and yield performances under the adverse conditions of drought stress, particularly under climate change. Of these techniques, applying the reinforcing substances such as antioxidants and osmoprotectants to enhance the tolerance to drought in field crops and reduce the negative effects of drought to produce acceptable yields in water-limited environments. Exogenously-applied antioxidants and/or osmoprotectants have been shown to alleviate drought stress impacts on morphological, physiological, and biochemical processes in the plant, and thus improving its growth, productivity and WUE_s and WUE_{ab} (Zhang *et al.*, 2017; Alzahrani *et al.*, 2018; Merwad *et al.*, 2018). One of these substances is AsA, which is a critical antioxidant in the plant's antioxidant defense system. It is implicated in many types of the biological activities (*e.g.*, antioxidant, enzymatic co-factor, and a donor/receptor in the electron transport either in chloroplasts or at plasma membranes) in the plant, which are related to the plant's ability to withstand the effects of oxidative stress (Conklin, 2001; Rady & Hemida, 2016). In chloroplasts, "Halliwell-Asada pathway" displays that APX utilizes AsA for oxidizing monodehydro-ascorbate to elevate dehydro-ascorbate (DHA). This step is followed by a decrease in both DHA and monodehydro-ascorbate to regenerate AsA pool. This

type of scavenging can be observed near PSI to diminish the hazards of ROS escaping in addition to reducing the ROS reactions with each other (Foyer, 2005). Additionally, the application of Si considerably enhances plant growth criteria and yield characteristics under the adverse conditions of drought stress (Merwad *et al.*, 2018). Furthermore, Alzahrani *et al.* (2018) reported that exogenous Si increases wheat yield under different stress conditions, including drought. This result is due to Si's ability to regulate the metabolism of cell walls by promoting the extensibility of tissues and enhancing the activities of cellular physiological and biochemical processes. In addition, elevating the rigidity of leaves by making them rougher in texture is another potential mechanism of Si (Ouzounidou *et al.*, 2016).

Accordingly, foliar application of AsA or Si promotes scavenging activities of metal ion chelating and ROS, which forms a remarkable part of abiotic stress responses in plant cells. Plants possessing AsA and Si are able to develop a complex defense system as a strategy for cellular defense against the effects of oxidative stress caused by drought. As a result, damage from overproduction of ROS will be mitigated and repaired (Merwad *et al.*, 2018). The results of this work display that AsA or Si treatment showed significant different responses for all investigated attributes of all faba bean cultivars compared to those of untreated ones under normal or stressed conditions. Mukhtar *et al.* (2016) reported that foliar-applied AsA is effective in improving plant growth due to AsA-induced decreases in the oxidative stress biomarker H_2O_2 and membrane permeability and increase in chlorophylls contents under IWD conditions. In addition, Si elevates the leaf area to receive more light and increases leaf blade erectness and turgidity by holding more horizontally to maintain higher leaf RWC (Merwad *et al.*, 2018) and use it effectively (WUE , Table 4). These results lead to allow light to penetrate smoothly, delay leaf senescence, increase enzymatic activity and chlorophylls contents may by prohibiting chlorophyll destruction, and increase photosynthesis to supply assimilates to growing tissues, which are associated with mitigating stress damage (Gong *et al.*, 2003; Ming *et al.*, 2012; Ouzounidou *et al.*, 2016).

In the current study, IWD stress considerably elevated the contents of osmoprotectants (*i.e.*, proline and soluble sugars) in all faba bean cultivars compared to WW condition. In addition, SD showed higher contents of these attributes than MD. However, the application of AsA or Si resulted in further elevations in proline and soluble sugar contents compared to those of untreated control. These accumulations in osmoprotectants were more pronounced with AsA treatment than with Si treatment. Normally, plants under stress create and accumulate many osmolytes/osmoprotectants and compatible solutes within them as an effective mechanism to protect against stress effects (Rios *et al.*, 2017). Under the adverse conditions

of stress, proline and soluble sugar accumulations occur in plant cells to balance the osmotic strengths of vacuole and cytosol with the osmotic strength of external environment (Gadallah, 1999). Proline accumulation occurs as a response to osmotic stress because it is a major osmolyte, which contributes to cellular osmotic modification (Zhang *et al.*, 2017). Furthermore, Siripornadulsil *et al.* (2002) notified that proline may react directly with hydroxyl radicals or physically quench singlet oxygen (1O_2). These reactions diminish the damages caused by ROS (low levels of MDA, Table 3) with a high level of antioxidants (Table 1, Fig. 1). As an effective role, proline maintains the integrity of membranes and subcellular structures, stabilizing proteins, and protecting cellular functions by ROS scavenging under stress conditions (Kavi Kishor *et al.*, 2005). Like proline, soluble sugars are one of the major solutes, which contribute in osmotic modification in stressed glycophytic plants (El-Bassiouny & Sadak, 2015).

The components of antioxidant system are the master controller of the ROS level in plant cells (Schutzendubel & Polle, 2002). Hence, antioxidant enzyme levels are expected to increase by exposing faba bean plants to drought stress. This study showed, under IWD stress, that the application of AsA or Si to faba bean plants led to significant improvements in the antioxidant enzymes activities to control various ROS in association with non-enzymatic antioxidants. CAT, POD, APX, SOD, and GR activities, as well as soluble sugars and proline contents, were elevated under the oxidative stress induced by drought and were further increased with the application of AsA or Si. In this context, Latif *et al.* (2016) notified that AsA treatment mitigates drought stress effects by detoxification of ROS and increased endogenous content of AsA that are accompanied with the raised activities of SOD and CAT in *Brassica oleracea* plants. In addition, Merwad *et al.* (2018) reported that Si treatment ameliorates IWD stress influences in *Vigna unguiculata* plants by increasing the endogenous contents of Si, which helps improve plant water status and (non-enzymatic and enzymatic) antioxidants activities, including Si, free proline, SOD, CAT, and POD. These findings are in harmony with the data obtained in this study (Table 1, Fig. 1). From these results, the application of Si leads to a decrease in the overproduced ROS and an increment in the scavenging of ROS by enzymatic antioxidants and non-enzymatic ones (Rios *et al.*, 2017). Therefore, at the cellular level, AsA or Si may relieve oxidative stress induced by drought stress due to the use of metabolic pathways more effectively in scavenging ROS, leading to enhance the integrity of cellular membranes. In this study, tolerance to drought stress in faba bean cultivars was enhanced by addition of AsA or Si as foliar spray treatments due to the increased activities of antioxidant system components.

Since it is a highly sensitive attribute of various stress factors, including drought, Fv/Fm ratio is typically an indicator of photo-inhibition and/or other impairments as a consequence of PSII complexes (Ranjbarfordoei *et al.*, 2006). This attribute was considerably raised, in the current study (Table 2), by AsA or Si treatment under IWD stress. The activity of cellular physiological processes, particularly photosynthesis, has been shown to be low under drought due to osmotic and oxidative stresses, and nutritional imbalances (Rios *et al.*, 2017). As needful bases for photosynthesis, drought-stressed leaf gas exchange (*e.g.*, g_s , Pn, and Tr) showed effective retrieval by AsA or Si application, leading to a higher increase in the effectiveness of photosynthesis in the current study (Table 2). In this respect, Semida *et al.* (2018) noted that AsA treatment raised the ratio of Fv/Fm and Pn of stressed cucumber transplants. Moreover, Mateos-Naranjo *et al.* (2013) noted that the negative influences of high salt stress on leaf gas exchange and Fv/Fm are recovered by Si supplementation to a halophytic grass.

The balance of water in plant leaves can be assessed by estimating the value of RWC under IWD (Merwad *et al.*, 2018). RWC evaluates the ratio of water existing in the leaves of the plant as a fraction of the overall volumetric water, which the leaf can keep at its complete turgor. Having a high RWC in the tissues awards the metabolic activity to continue by osmotic modifications and other acclimations to stress conditions (Slabbert & Krüger, 2014). The current study results display that IWD stress led to a decrease in RWC, but the application of AsA or Si alleviated the deleterious effects of IWD stress by raising the RWC, which positively reflected in MSI and stability of cell membranes due to the decrease of EL from plant cells (Table 3) (Kabir *et al.*, 2016). In addition, AsA or Si treatment depresses MDA content (Table 3), helping conserve cell plasma membrane integrity and optimize EL (Coskun *et al.*, 2016; Semida *et al.*, 2018). This positive result may be due to the increase in the antioxidant activities that can become auxiliary mechanism, and AsA or Si prohibits the deterioration of cell membranes in stressful plants and enhances their structures and functions, thereby promoting plant growth and productivity (Agarie *et al.*, 1998; Semida *et al.*, 2018). The improvements occurred for all attributes tested in this study were more pronounced with AsA than Si.

Faba bean is a sensitive pulse crop to drought stress, and therefore, identifying drought-tolerant genotypes is crucial to alleviate the destructive effects associated with drought stress in arid regions, particularly in light of recent climate changes. In the current study, physiological and agro-morphological characters have been used to evaluate the response of five faba bean cultivars to drought stress. The evaluated cultivars performed differently under drought stress conditions. In this context, Siddiqui *et*

al. (2015) and Abid *et al.* (2017) noted statistically significant differences among faba bean genotypes in their response to IWD stress, and more tolerant genotypes showed an increase in photosynthetic pigment contents, RWC, proline content, and enzymes activities, which are associated with higher seed yield compared to sensitive ones. Similarly, in general, 'Nubaria-2' collected highest antioxidant (enzymatic and non-enzymatic) activities, osmoprotectant contents, gas exchange, RWC, MSI, Fv/Fm, and chlorophylls contents, and gained less EL and MDA, which positively reflected in highest growth, seed yield and WUE_s and WUE_{ab} under IWD conditions. Accordingly, this cultivar is considered the most tolerant to drought stress followed by 'Giza-843' and 'Sakha-3', while 'Giza-716' and 'Sakha-4' were less tolerant. Furthermore, the tolerant cultivars exhibited highest response to foliar application by AsA or Si of all evaluated traits more than sensitive ones and assists in improving faba bean production under IWD conditions.

Obviously, estimating the degree of association between physiological and agronomic traits is valuable and provides useful information. Biplot of principal components is an appropriate statistical approach for visualizing the interrelationships among traits, which is determined by angle size of traits vectors. The obtained results proved that agronomic traits positively associated with photosynthetic pigments (chlorophyll a and b), gs, Pn, Tr, Fv/Fm, RWC, and MSI. Additionally, lower association with soluble sugar, proline, antioxidant enzymes activities (CAT, POD, SOD, GR, and APX), and EL was shown. Hence, high values of the evaluated physiological traits provide more agronomic trait and seed yield. Accordingly, enhancing these physiological traits would improve seed yield, which is the major target in agricultural production. Furthermore, these traits could be considered as suitable physiological traits for indirect evaluation for agronomic traits and seed yield under irrigation treatments.

Economically, the benefits that producers get from applying AsA or Si to increase faba bean yield under drought stress (especially MD) will be satisfactory. The increase in faba bean yield obtained by applying AsA or Si under stress will give benefits (average 1100 USD ha⁻¹) more than the order costs of applications (average 100 USD ha⁻¹). In addition, WUE by plants under IWD was higher than under WW conditions, because plants used water more efficiently and reduce water loss under IWD conditions.

It could be concluded that the combination between IWD-tolerant faba bean cultivars with foliar application using AsA or Si resulted in improving drought tolerance and enhancing faba bean productions under IWD, particularly under MD stress conditions; the shortage in irrigation water by 25% (from 400 to 300 mm water ha⁻¹). The physiological traits; photosynthetic pigments (chlorophy-

ll a and b), gs, Pn, Tr, Fv/Fm, RWC, and MSI exhibited high positive association with agronomic traits and seed yield obtained under MD stress.

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