

Effect of vermicompost fertilizer on photosynthetic characteristics of chickpea (*Cicer arietinum* L.) under drought stress

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Abstract

Water availability is an important factor for plant growth in arid environments. In recent decades, vermicompost (VC) fertilizer has been used in agriculture as a safe and effective fertilizer with high water-holding capacity. The aim of the present study was to characterize effects of VC fertilizer on photosynthetic activity of chickpea (*Cicer arietinum* L. cv. Karaj) under drought conditions at three different growth stages. Tests were carried out with four volumetric ratios of VC to soil, *i.e.*, 0:100, 10:90, 20:80, and 30:70, and three levels of drought stress, *i.e.*, no stress (NS), moderate drought (MS), and severe drought (SS) (100, 75, and 25% of field capacity, respectively). Evaluations were performed at the seedling, flowering, and podding stage. We found that the VC treatment under NS conditions significantly increased total chlorophyll content [Chl (*a+b*)], intercellular CO₂ concentration (*C_i*), net photosynthetic rate (*P_N*), transpiration rate (*E*), and maximal quantum yield of PSII photochemistry (*F_v/F_m*) at all three stages. The VC addition of 10 and 20% significantly enhanced the Chl content and *F_v/F_m* under MS and *F_v/F_m*, *C_i*, and *P_N* under SS at the flowering stage. In conclusion, our results proved a positive effect of the VC fertilizer on photosynthesis of chickpea under NS conditions, but it was not found under MS and SS.

Additional key words: chlorophyll *a* fluorescence; gas exchange; organic fertilizer; stomatal limitation; water stress.

Introduction

Chickpea (*Cicer arietinum* L.) is a good source of proteins, carbohydrates, as well as minerals, vitamins and unsaturated fatty acids; it plays an important role in the human diet and is cultivated worldwide (Ganjeali *et al.* 2011). Legumes are highly sensitive to water deficiency and drought stress is one of the most important abiotic factors that can limit photosynthesis, yield, and affect plant distribution (Hosseinzadeh *et al.* 2014). Drought stress and high irradiation increase the rate of light-caused inhibition (Ismaili *et al.* 2015, Sikder *et al.* 2015). In conditions of reduced soil water content, a decline in photochemical efficiency of PSII and electron transport occurs (Hosseinzadeh *et al.* 2014). An initial response of plants to water stress is activation of the mechanism for stomata closure to prevent further water loss (Flexas and Medrano 2002). Stomata closure decreases internal CO₂ concentration in the leaf and inhibits Rubisco enzyme

activity, which ultimately lowers net photosynthesis (Rahbarian *et al.* 2011). Chickpea is a relatively drought-resistant plant but research has focused more on strategies to alleviate the effects of drought in order to maximize performance (Ganjeali *et al.* 2011). Some species of earthworms consume decaying organic material and are able to convert this material to nutrient-rich compounds that form an environment in the soil supporting plant growth (Atik 2013). VC is produced by the decomposition of organic material using nonthermal interaction between earthworms and microorganisms (Sallaku *et al.* 2009). Previous studies have shown that VC improves the status of mineral nutrients and biological properties of soil (Pant *et al.* 2011). VC is highly porous, allows high ventilation, good drainage, and has a high water-storage capacity (Atiyeh *et al.* 2002). Marinari *et al.* (2000) showed that the application of VC increased production of CO₂ and

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Abbreviations: *C_i* – intercellular CO₂ concentration; *E* – transpiration rate; *F₀* – initial fluorescence of dark-adapted leaf; *F_m* – maximal fluorescence yield of the dark-adapted state; *F_v/F_m* – maximal quantum yield of PSII photochemistry; MS – moderate drought stress; NS – no stress; *P_N* – net photosynthetic rate; ROS – reactive oxygen species; SS – severe drought stress; VC – vermicompost.

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humic compounds, which resulted in an increase in microbial activity. VC is rich in humic compounds and researchers speculate that the hormone-like activities of humic substances play a role in amelioration of drought stress (Atik 2013).

Maximal quantum yield of PSII photochemistry is a major parameter used for estimating drought tolerance in plants (Sikder *et al.* 2015). It is determined as the F_v/F_m

ratio (Liu *et al.* 2015). Studies on water deficit stress have shown that under a decreased soil water content, the F_v/F_m ratio is lower (Kumar and Prasad 2015). The aim of this study was to determine: (1) whether the VC application can improve the negative effect of drought stress in chickpea and (2) what combination ratio of VC to soil is required to maximize photosynthetic traits.

Materials and methods

Experimental details: The experiment was performed at Khatam-Alanbia University of Behbahan in Iran. Tests were carried out as a factorial experiment in a randomized plot design with three replications under greenhouse conditions. We tested four combinations of VC and soil

at the following ratios: control (CK, 100% soil); 10% VC + 90% soil (VC10); 20% VC + 80% soil (VC20); 30% VC + 70% soil (VC30). The chemical properties of the soil and VC were as follows:

Sample	EC [ds m ⁻¹]	pH	C/N	P [%]	Ca [%]	K [%]	Fe [%]	Total N [%]	Mg [%]
Vermicompost	1.5	7.1	15.5	0.9–2.5	4.5–8	0.6–2.5	0.5–2.5	0.9–3	0.5–2.3
Soil	0.4	7.8	18.9	0.01–0.03	0.5–1	0.2–0.4	0.002–0.004	0.8–1.5	0.01–0.05

The conditions of drought stress treatment were following: control (NS, 100% of field capacity), MS (75% of field capacity), and SS (25% of field capacity). Seeds were placed in a solution containing 40% sodium hypochlorite for 30 min for superficial sterilization and washed with purified water to remove any remaining sodium hypochlorite. They were then sown in a standard Petri dish in a germinator chamber. When the seedlings reached a height of 5 cm, they were transplanted at a rate of three seedlings per pot. The pots were placed in a growth chamber. The seedlings were maintained under a 12.5/11.5 h of light/dark period, temperatures of 25°C day/15°C night for the first month and under a 13/11 h light/dark period under temperatures of 27°C day/17°C night during the second month; the conditions were similar to normal field conditions in the chickpea growing region. Photosynthetic parameters were measured three times during the growing season, at the seedling, flowering, and podding stages.

Gas-exchange and Chl measurements: Leaf Chl content was measured with a portable leaf chlorophyll meter (CCM-200 Plus, Opti-Sciences Inc., NH, USA) on the second uppermost leaf from three plants per pot. Chl was determined from averages taken from ten readings per sample. Evaluations for intercellular CO₂ concentration [C_i , $\mu\text{mol (CO}_2\text{) mol}^{-1}$], net photosynthetic rate (P_N , $\mu\text{mol m}^{-2} \text{ s}^{-1}$), and transpiration rate [E , $\text{mmol (H}_2\text{O) m}^{-2} \text{ s}^{-1}$] were taken from measurement of the central sector, from attached young and fully expanded leaves of main shoots. Measurements were taken between 9:00 and 11:00 h using a portable infrared gas analyzer (KR8700 system,

Korea Tech Inc., Suwon, Korea) in conjunction with an automatic leaf chamber with a leaf surface area of 6 cm². Leaf chamber conditions were controlled by the system and PPFD, relative humidity, CO₂ concentration, and leaf temperature were set to equal conditions used for plant growth. Measurement was carried out on the second and third leaves of chickpea plants under uniform conditions for all plants.

Maximal quantum yield of PSII photochemistry: Evaluation of the F_v/F_m ratio was determined for chickpea plants using a portable chlorophyll fluorometer (*Pocket PEA*, Hansatech Instruments Ltd., King's Lynn, Norfolk, England). Measurements were taken using the same leaf used for the gas-exchange determination, illuminated with saturating light [$3,500 \mu\text{mol (photon) m}^{-2} \text{ s}^{-1}$] after 15 min of dark adaptation. F_v/F_m was determined automatically as $(F_m - F_0)/F_m$, where F_m and F_0 were maximum and initial fluorescence yields of the dark-adapted leaves, respectively (Maxwell and Johnson 2000). All photosynthetic measurements used three plants from each treatment.

Statistical analysis: The data are presented as mean values \pm standard deviation (SD) of three replicates. Analysis of variance (ANOVA) was performed to determine the significance of the differences between the responses of chickpea to different combinations of vermicompost and drought stress and the significance of differences between treatments means was checked with Duncan's multiple range test ($p \leq 0.05$). Statistical analysis was performed using MASTAT-C software.

Results

Chl content: At the seedling stage, all VC combinations showed the higher Chl content under NS compared with CK, except for VC30 under NS conditions (Table 1). At the flowering stage, Chl content was influenced significantly by both VC treatment and drought stress. The leaf Chl content decreased under MS and SS compared with NS conditions. VC treatment enhanced the Chl content compared with CK and results were significant for leaves of VC10 in the plants under NS and MS

(Table 2). SS decreased Chl in all VC treatments compared with the NS condition (Table 3). VC treatment determined a significant increase of the Chl content under NS compared with the CK conditions.

F_v/F_m: At the seedling stage, the F_v/F_m ratio increased more under NS than those under the drought conditions (MS and SS). VC treatments enhanced the F_v/F_m ratio under SS and NS more than that of CK (Table 1). MS and

Table 1. Effects of vermicompost (VC) on total chlorophyll content (Chl), maximal quantum yield of PSII photochemistry (F_v/F_m), intercellular CO₂ concentration (C_i), net photosynthetic rate (P_N), and transpiration rate (E) under drought stress at the seedling stage. Data are means ± SD (n = 3). Difference between data of each column followed by the same letter was not statistically significant (p<0.05).

Treatments	Chl [μg cm ⁻²]	F _v /F _m	C _i [μmol(CO ₂)mol ⁻¹]	P _N [μmol m ⁻² s ⁻¹]	E [mmol(H ₂ O)m ⁻² s ⁻¹]
No drought stress (100% of field capacity)					
Control	2.01 ± 0.32 ^{bc}	0.733 ± 0.041 ^b	665.9 ± 15.4 ^{bcd}	6.09 ± 0.78 ^{bc}	147.5 ± 64.8 ^b
VC 10%	3.43 ± 1.13 ^a	0.813 ± 0.005 ^a	772.9 ± 49.9 ^a	7.01 ± 1.25 ^{abc}	225.5 ± 24.1 ^a
VC 20%	3.33 ± 0.55 ^a	0.810 ± 0.001 ^a	768.5 ± 43.8 ^a	12.57 ± 5.06 ^a	256.7 ± 15.6 ^a
VC 30%	2.76 ± 0.94 ^{ab}	0.820 ± 0.002 ^a	726.1 ± 41.6 ^{ab}	11.91 ± 7.92 ^{ab}	258.8 ± 45.8 ^a
Moderate drought stress (75% of field of capacity)					
Control	1.20 ± 0.10 ^c	0.703 ± 0.023 ^{bc}	588.1 ± 14.0 ^{def}	3.49 ± 1.08 ^c	99.1 ± 2.9 ^{cd}
VC 10%	1.66 ± 0.11 ^{bc}	0.733 ± 0.012 ^b	613.7 ± 38.0 ^{def}	6.05 ± 0.87 ^{bc}	122.8 ± 25.4 ^{bc}
VC 20%	1.70 ± 0.26 ^{bc}	0.746 ± 0.041 ^b	660.5 ± 30.3 ^{bcd}	7.08 ± 5.13 ^{abc}	149.0 ± 21.9 ^b
VC 30%	1.53 ± 0.38 ^{bc}	0.743 ± 0.012 ^b	638.1 ± 33.6 ^{cde}	4.77 ± 0.53 ^c	129.6 ± 17.7 ^{bc}
Severe drought stress (25% of field capacity)					
Control	1.03 ± 0.05 ^c	0.673 ± 0.026 ^c	484.1 ± 17.7 ^e	1.80 ± 1.51 ^c	58.0 ± 19.1 ^d
VC 10%	1.53 ± 0.06 ^{bc}	0.736 ± 0.031 ^b	555.8 ± 45.4 ^{efg}	3.03 ± 0.71 ^c	68.2 ± 21.3 ^d
VC 20%	1.13 ± 0.05 ^c	0.730 ± 0.025 ^b	547.2 ± 30.6 ^{fg}	3.78 ± 1.40 ^c	77.4 ± 15.9 ^d
VC 30%	1.76 ± 0.45 ^{bc}	0.733 ± 0.015 ^b	560.3 ± 33.1 ^{efg}	5.22 ± 1.82 ^c	87.8 ± 7.5 ^{cd}

Table 2. Effects of vermicompost (VC) on total chlorophyll content (Chl), maximal quantum yield of PSII photochemistry (F_v/F_m), intercellular CO₂ concentration (C_i), net photosynthetic rate (P_N), and transpiration rate (E) under drought stress at the flowering stage. Data are means ± SD (n = 3). Difference between data of each column followed by the same letter was not statistically significant (p<0.05).

Treatments	Chl [μg cm ⁻²]	F _v /F _m	C _i [μmol(CO ₂)mol ⁻¹]	P _N [μmol m ⁻² s ⁻¹]	E [mmol(H ₂ O)m ⁻² s ⁻¹]
No drought stress (100% of field capacity)					
Control	2.83 ± 0.35 ^{bc}	0.723 ± 0.021 ^{cd}	780.2 ± 10.5 ^b	5.67 ± 1.41 ^{bcd}	125.0 ± 11.5 ^{bc}
VC 10%	3.66 ± 0.45 ^a	0.813 ± 0.054 ^a	824.1 ± 9.4 ^a	8.97 ± 1.62 ^a	155.4 ± 10.2 ^{ab}
VC 20%	3.40 ± 0.43 ^{ab}	0.806 ± 0.056 ^a	839.1 ± 10.4 ^a	7.83 ± 2.10 ^{ab}	145.1 ± 9.5 ^{ab}
VC 30%	3.20 ± 0.25 ^{ab}	0.807 ± 0.012 ^a	835.0 ± 11.4 ^a	7.12 ± 2.05 ^{ab}	172.1 ± 12.8 ^a
Moderate drought stress (75% of field capacity)					
Control	1.50 ± 0.31 ^{ef}	0.663 ± 0.022 ^e	775.3 ± 6.1 ^b	3.75 ± 1.57 ^{cd}	97.1 ± 8.6 ^c
VC 10%	2.30 ± 0.50 ^{cd}	0.766 ± 0.014 ^b	778.5 ± 16.3 ^b	6.71 ± 3.74 ^{abc}	103.9 ± 1.5 ^c
VC 20%	2.16 ± 0.47 ^d	0.726 ± 0.016 ^c	802.4 ± 8.3 ^{ab}	5.68 ± 0.68 ^{bcd}	101.1 ± 5.4 ^c
VC 30%	1.96 ± 0.75 ^{de}	0.761 ± 0.023 ^c	809.2 ± 15.1 ^{ab}	3.69 ± 0.87 ^{cd}	132.5 ± 22.2 ^{bc}
Severe drought stress (25% of field capacity)					
Control	1.13 ± 0.05 ^f	0.646 ± 0.030 ^e	622.6 ± 29.0 ^e	1.18 ± 0.47 ^e	40.8 ± 16.8 ^d
VC 10%	1.33 ± 0.23 ^{ef}	0.710 ± 0.019 ^{cd}	682.2 ± 33.6 ^d	4.87 ± 2.44 ^{bcd}	52.9 ± 3.2 ^d
VC 20%	1.20 ± 0.10 ^f	0.706 ± 0.057 ^d	637.8 ± 10.7 ^d	3.78 ± 1.70 ^{cd}	63.0 ± 11.7 ^d
VC 30%	1.16 ± 0.11 ^f	0.706 ± 0.052 ^d	639.2 ± 10.7 ^d	2.97 ± 1.68 ^d	62.0 ± 4.6 ^d

Table 3. Effects of vermicompost (VC) on total chlorophyll content (Chl), maximal quantum yield of PSII photochemistry (F_v/F_m), intercellular CO_2 concentration (C_i), net photosynthetic rate (P_N), and transpiration rate (E) under drought stress at the podding stage. Data are means \pm SD ($n = 3$). Difference between data of each column followed by the same letter was not statistically significant ($p < 0.05$).

Treatments	Chl [$\mu g\ cm^{-2}$]	F_v/F_m	C_i [$\mu mol\ (CO_2)\ mol^{-1}$]	P_N [$\mu mol\ m^{-2}\ s^{-1}$]	E [$mmol\ (H_2O)\ m^{-2}\ s^{-1}$]
Nodrought stress (100% of field capacity)					
Control	2.23 ± 0.64^{bcd}	0.873 ± 0.011^a	790.9 ± 16.6^{cde}	9.49 ± 3.08^{de}	124.2 ± 7.4^b
VC 10%	3.60 ± 0.61^a	0.896 ± 0.012^a	928.6 ± 22.6^{ab}	15.72 ± 2.88^{bc}	157.9 ± 21.7^a
VC 20%	3.46 ± 0.05^a	0.876 ± 0.015^a	992.7 ± 28.4^a	23.99 ± 3.42^a	174.4 ± 9.5^a
VC 30%	3.16 ± 0.77^a	0.910 ± 0.001^a	953.2 ± 13.3^{ab}	19.89 ± 3.47^{ab}	155.6 ± 11.1^a
Moderate drought stress (75% of field capacity)					
Control	2.33 ± 0.15^{bcd}	0.780 ± 0.050^{bc}	792.4 ± 24.1^{cde}	8.19 ± 2.35^{de}	96.3 ± 21.6^b
VC 10%	2.93 ± 0.26^{abc}	0.806 ± 0.005^{bc}	839.2 ± 21.1^{bcd}	12.80 ± 0.99^{cd}	112.0 ± 11.6^b
VC 20%	2.30 ± 0.15^{bcd}	0.803 ± 0.022^{bc}	837.1 ± 10.4^{bcd}	9.90 ± 3.45^{cd}	110.2 ± 16.7^b
VC 30%	2.16 ± 0.11^{cd}	0.816 ± 0.055^b	765.9 ± 18.0^{def}	10.46 ± 2.53^{cde}	121.4 ± 1.6^b
Severe drought stress (25% of field capacity)					
Control	1.30 ± 0.13^e	0.753 ± 0.011^c	636.5 ± 13.5^f	4.99 ± 2.31^e	52.4 ± 11.1^c
VC 10%	1.83 ± 0.47^{de}	0.790 ± 0.050^{bc}	682.2 ± 26.9^{ef}	9.39 ± 0.92^{de}	64.1 ± 9.3^c
VC 20%	1.63 ± 0.41^{de}	0.796 ± 0.013^{bc}	715.2 ± 26.0^{def}	9.53 ± 2.23^{de}	68.3 ± 12.4^c
VC 30%	1.50 ± 0.52^{de}	0.795 ± 0.012^{bc}	680.1 ± 17.4^{ef}	6.66 ± 1.22^e	65.1 ± 12.2^c

SS showed a significant decline in the F_v/F_m ratio compared with NS conditions. Under all drought treatments, VC addition resulted in a significant increase of the F_v/F_m ratio at the flowering stage (Table 2). Drought stress (MS and SS) lowered the F_v/F_m ratio, regardless of VC treatment. However, VC did not significantly affect the F_v/F_m ratio at the podding stage (Table 3).

C_i : At the seedling stage, C_i decreased significantly under MS and SS compared with NS. At this stage, under NS conditions, C_i increased in the plants treated with VC10 and VC20 (Table 1). At the flowering stage, C_i decreased significantly in response to SS. A significant increase was found after VC treatment under NS and SS compared with CK (Table 2). At the podding stage, C_i was reduced by SS in all VC treatments. VC elevated C_i under NS compared with CK (Table 3).

P_N : In comparison with VC treatments at the seedling stage, VC10 stimulated P_N by 51% under NS conditions. At this stage, different VC treatments did not affect P_N

significantly under MS and SS (Table 1). At the flowering stage, SS induced the 80% decrease in P_N compared with NS conditions. VC ameliorated the negative effects of SS conditions on P_N (Table 2). At the podding stage, P_N in leaves decreased under SS and MS conditions compared with NS. The presence of VC induced a significant increase in P_N under NS (Table 3).

E : At the seedling stage, E decreased significantly in response to MS and SS conditions. A significant increase was found after VC treatments under NS and VC20 under MS compared with CK (Table 1). At the flowering stage, under MS and SS, E was significantly reduced compared with the NS treatment. E in response to VC30 showed an increase of 27% compared with CK under NS conditions (Table 2). A significant decrease in E was observed at MS and SS in comparison with NS. At the podding stage, VC showed higher E compared with CK under NS conditions, but under MS and SS, there was no significant difference between VC and CK (Table 3).

Discussion

Chl: The Chl content is known as an index of tolerance to water stress in plants; a high stability index has no effect on stress in a plant and increases access to Chl (Anyia and Herzog 2004). Drought stress induces ROS production in leaves and it stimulates Chl degradation (Flexas and Medrano 2002). It appears that VC fertilizer contains micronutrients, *e.g.*, iron, acting as a prosthetic group of hemoproteins, such as catalase, peroxidase, and superoxide dismutase (Samiran *et al.* 2010), enzymes

consuming ROS in plants. VC fertilizer contains higher amounts of nutrients, such as nitrogen, phosphorus, potassium, calcium, magnesium, and micronutrients, such as iron, zinc, copper, and manganese in comparison to other organic fertilizers (Atik 2013). The decline in Chl in response to drought stress may influence the nitrogen metabolism for biosynthesis of nitrogenous compounds, such as proline, during osmotic regulation (Chen *et al.* 2012). An increase in the proline content inhibits

glutamate, which is a precursor of Chl and proline; it may decrease the Chl synthesis (Chen *et al.* 2012). It appears that VC maintains water availability and nutrients, such as potassium and nitrogen, which are involved in regulation of osmotic pressure.

F_v/F_m : Chl *a* fluorescence measurement has been often used to determine the physiological condition of plants and to evaluate damage to photosynthetic apparatus (Liu *et al.* 2015). The F_v/F_m ratio is generally accepted as an efficiency indicator of PSII (Giorio 2011). Lowering the ratio of F_v/F_m under water stress indicates a decrease in the efficiency of PSII caused by a decline in the transfer of electrons from PSII to PSI (Sikder *et al.* 2015). It has been reported that drought stress has a detrimental effect on D1 protein of PSII (Zlatev and Yordanov 2004). Desirable properties of VC, such as high capacity for water holding and cation exchange, increased absorption of nutrients and other beneficial physical, chemical, and biological properties, which can improve stability of the photosynthetic apparatus in plants (Lakhdar *et al.* 2009). Our results showed that the application of the VC organic fertilizer reduced negative effects of drought stress in chickpea.

C_i : Lower CO_2 concentration and assimilation found under MS occurred due to stomata closure, but under SS, it was caused by inhibition of biochemical reactions (Johnson *et al.* 2002). Stomata closure during drought stress lowers water loss but decreases C_i and P_N (Karimi *et al.* 2015). VC exhibits also other features, including abundant soil microbial populations, such as nitrogen stabilizers, actinomycetes, fungal spores, and mycorrhizal symbionts for plant roots (Pant *et al.* 2011). Microbial populations of mycorrhizal fungi require plant carbohydrates for their metabolism and probably accumulate sugars in the roots. Sugars are compatible osmolytes and regulate osmotic pressure in roots; it improves tolerance to drought stress (Oliva *et al.* 2008). Besides, application of VC increases CO_2 production in the soil surrounding a plant and increases microbial activity by contributing organic material to the soil (Marinari *et al.* 2000).

P_N : Several studies have shown that drought stress lowers P_N (Rahbarian *et al.* 2011, Hosseinzadeh *et al.* 2014). There are two sets of factors that limit photosynthesis

under water stress. One factor is related to stomata, as the mechanism of stomata closure decreases CO_2 concentration in the leaf and in chloroplasts; it has a detrimental effect on photosynthesis (Pagter *et al.* 2005). The second set of factors is not related to stomata, but it includes Chl content, a reduction of Rubisco content and activity, inhibition of the synthesis of ribulose-1,5-bisphosphate, and a decline in transport of electrons to the PSII (Pagter *et al.* 2005). VC is rich in humic compounds and researchers have speculated that the hormone-like activities of humic substances play a role in amelioration of water deficit stress (Matos and Arruda 2003). Humic substances in VC have high capacity for absorption of metal due to the presence of negatively charged groups, such as carboxylic acid and phenolic acids (Matos and Arruda 2003). VC is highly porous and shows a high capacity for ventilation, proper drainage, and water storage, which limit stomata closure and increase the supply of CO_2 necessary for photosynthesis (Arancon *et al.* 2004).

E : Reduction of E under drought stress may be a mechanism to protect plants against water loss (Gates 1968). Under conditions of drought stress, plants close their stomata, a mechanism which inhibits movement of atmospheric CO_2 into the leaf (Matos *et al.* 1998, Sicher *et al.* 2015). Increased E in the presence of VC at the seedling, flowering, and podding stages may occur due to physical, chemical, and biological structures of VC. VC increases the amount of water entering roots due to its good water-holding capacity (Atik 2013) and its microorganism content including mycorrhizal fungi (Oliva *et al.* 2008). Therefore, the rise of E went along with the increase in plant water availability.

Conclusion: We demonstrated that under NS condition, different ratios of VC could improve photosynthetic parameters at all three stages. In the flowering stage, 10 and 20% of VC significantly increased Chl content and F_v/F_m under MS conditions. At this stage, VC treatments significantly increased values of F_v/F_m , C_i , and P_N compared with the control under SS. This study confirmed that drought stress significantly lowered all photosynthetic traits. Nevertheless, the application of VC did not improve photosynthetic response under MS and SS conditions at the seedling and podding stages.

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