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Response of okra (*Abelmoschus esculentus* L.) to soil and foliar application of L-methionine

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Abstract

L-Methionine (L-MET) is an established precursor of ethylene (C_2H_4) which is known to induce early flowering and ripening of fruits. Therefore, exogenous application of L-MET may induce early flowering in okra. Impact of soil and foliar applied L-MET on growth and performance of okra was investigated in a pot experiment. Different levels of L-MET were applied to the soil (0, 20, 40 and 80 mg kg^{-1}) and okra (0, 5, 10 and 20 mg L^{-1}) in two splits (15 and 30 days after sowing). For foliar application, 0.1% Tween 20 was used as a binding agent. Results revealed that L-MET applied at 10 mg L^{-1} (foliar application) and 40 mg kg^{-1} soil had a significant effect on the growth, yield and physiological parameters of okra as compared to untreated control. Foliar application of L-MET increased the number of flowers, number of fruits, root length, shoots fresh and dry weight, photosynthetic rate, chlorophyll contents and fruit yield up to 77, 96, 71, 64, 65, 71, 60 and 64%, respectively. In the case of soil applied L-MET, up to 97, 85, 96, 77, 82, 92, 54 and 81% increase in growth and yield parameters, respectively, was observed compared to untreated control. These results imply that soil and foliar application of L-MET could be effective in inducing more flowering and promoting growth and yield of okra.

Keywords: L-methionine, soil application, foliar application, flowering, okra

Introduction

Plant growth regulators (PGRs) are the organic molecules other than the vitamins and essential plant nutrients, which play role in the development and growth of plants even at very low concentration (Raddadi *et al.*, 2008). PGRs are grouped into auxins, cytokinins, ethylene, gibberellins, abscisic acids, salicylic acids and jasmonic acids (Kaufman *et al.*, 1994; Khalid *et al.*, 2006). These are synthesized within plant bodies and are also released by microbes living in the rhizosphere (Ali *et al.*, 2010). Around the globe, application of these PGRs is considered as an essential practice and the use of these PGRs has been increased rapidly over the period of last few years (Khalid *et al.*, 2001, 2006).

It has been studied that production of phytohormones can be enhanced many times by the provision of suitable precursors to the microbes residing in soil. Microbes present in the rhizosphere utilize these precursors and convert them into phytohormones and thus provide a continuous source of these active substances for the uptake of plants (Arshad and Frankenberger, 1990a). Another positive impact of these hormones on the plant growth is the close contact between the microbes and the plant roots (Arshad and Frankenberger, 1998). However, the production of phytohormones by the microorganisms depends on the availability of specific precursors which serve as main source for microbial secretions of these PGRs. It has been clearly found that exogenous application of precursors enhance the production of PGRs many fold in soil and culture (Arshad and Frankenberger, 1991).

Ethylene (C_2H_4) is an important gaseous plant hormone that affects almost all stages of growth and developmental processes (Arshad and Frankenberger, 2002). It plays a vital role in embryogenesis, germination, senescence, leaf abscission, flowers induction and fruit maturity (Abeles *et al.*, 1992). It also promotes the transcription and translation of ripening related genes involved in cell wall breakdown and carotenoid biosynthesis (Gray *et al*., 1994). Concentration of C_2H_4 in the soil depends upon soil properties, substrate nature and native microbiota (Nazli *et al.*, 2003; Arshad *et al.*, 2004).

L-MET is an established precursor of C_2H_4 which is known to induce early onset of flowering and ripening of fruits in different vegetables and agronomic crops (Arshad and Frankenberger, 2002; Akhtar *et al.*, 2005; Khalid *et al*., 2006). L-MET applied to soil not only increases growth but also increases the level of C_2H_4 in the soil

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(Khalid *et al*., 2001; Shaharoona *et al*., 2008). Exogenous application of L-MET induces early flowering in different vegetables and agronomic crops (Matilla *et al*., 2005). C_2H_4 accumulation was increased in the soil atmosphere by the addition of L-MET (Frankenberger and Phelan, 1985; Arshad and Frankenberger, 1990a). C_2H_4 production in soil is highly dependent on availability of substrate (Frankenberger and Arshad, 1985). The addition of L-MET in soil stimulates C_2H_4 production and a significant growth and yield response in soybean was noticed by the application of L-MET to soil (Arshad and Frankenberger, 2002).

Okra (*Abelmoschus esculentus* L.), an annual herbaceous plant, belongs to the family *Malvaceae* and is more prevalent in the Indo-Pak subcontinent (Emuh *et al.*, 2006). It has high nutritive value as it contains fats, carbohydrates, minerals, vitamin A, vitamin C and potassium so plays very important role in human diet (Benchasri, 2012; Ijoyah and Dzer, 2012). In spite of having good nutritional value, its per hectare yield is very low, which might be due to low nutrition and environmental stress. The present study was designed to evaluate the effect of different levels of L-MET on growth, physiology and yield of okra.

Materials and Methods

A pot trial was conducted in a net house of the Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad-Pakistan to evaluate the effect of soil and foliar applied L-MET on growth, physiology and yield of okra (*Abelmoschus esculentus* L. cultivar Sabz pari). Soil used in the pots was analysed for various physico-chemical characteristics: texture, clay loam (sand, 47.98%; silt, 29.52%; clay, 22.50%); pH, 7.79; EC, 2.5; organic matter, 0.75%; total nitrogen, 0.05%, available phosphorus, 9.3 mg kg^{-1} and extractable potassium 113 mg kg^{-1}. Each pot was filled with 10 kg soil. Three seedlings were maintained per pot and treatments were arranged using completely randomized design (CRD). Four levels of L-MET (0, 20, 40 and 80 mg kg^{-1}) were applied to soil in solution form. Similarly four levels of L-MET (0, 5, 10 and 20 mg L^{-1}) was applied with 0.1% Tween 20 (V/V). The L-MET were applied in two splits i.e., 15 and 30 days after in solution form. The experiment was performed with sowing three replication.

Basal doses of N, P and K were applied at 25, 12.5 and 12.5 mg kg^{-1} in the form of urea, diammonium phosphate and sulphate of potash, respectively. After one month of sowing, second dose of urea (50 mg kg^{-1} soil) was applied. Tap water was used for irrigation when required for plant growth.

Okra fruits were picked at 50, 70 and 90 days after sowing. Plants were harvested 90 days after sowing and the data regarding plant growth and fruit yield parameters were recorded.

Physiological parameters i.e., photosynthetic rate (A), transpiration rate (E), stomatal conductance (g_s) and sub-stomatal CO_2 concentration were measured 45 days after sowing by using CIRAS-3 (PP System, Amesbury, MA, USA) with PLC 3 universal leaf cuvette, measuring both sides of the top third, fully developed leaf of each plant at the ambient light. Cuvette was provided light via light emitting diodes (LED) and with a photon flux of 1000 µmol m^{-2} s^{-1}, ambient leaf temperature and 390 µmol mol^{-1} CO_2. Chlorophyll contents were measured and recorded by using chlorophyll meter (Spad-502, Konica Minolta, Japan).

Plant growth parameters i.e. plant height, number of flowers, number of floral bud dropped, fruit length, fruit diameter, root length, number of leaves, number of branches, inter-nodal distance and fresh and dry weights of root were recorded. Number of days taken to flowering and fruiting was observed on visual basis. Fruit number and weight were recorded after harvesting. Root sample was collected from each pot. Both shoot and root samples were thoroughly washed with distilled water and then blotted dry with tissue papers before sun drying. Sun dried plants were placed in an oven at 72 $^{\circ}$C for 72 h and dry mass were recorded.

Statistical analysis

The data recorded were subjected to analysis of variance using computer software Statistix v. 8.1 (Analytical Software, USA). The treatment means were compared by least significant difference test (Steel *et al*., 1997).

Results

Growth parameters

Soil and foliar application of L-MET significantly improved the growth parameters of okra. Minimum number of days were taken to flowering and fruiting of okra where L-MET was applied as soil and foliar at 40 mg kg^{-1} and 10 mg L^{-1}, respectively as compared to control (Figure 1 and 2). The soil application decreased the flowering and fruiting time by 21 and 23% while it was 15, 16% less in case of the application of L-MET as foliar to the crop plants as compared to untreated control. Plant height differed non-significantly in both applications of L-MET over control while floral bud drop were reduced significantly compared to control by the application of L-MET as soil and foliar at 40 mg kg^{-1} and 10 mg L^{-1}, respectively (Table 1). Similarly, L-MET applied as soil (40 mg kg^{-1}) and foliar (10 mg L^{-1})

had a significant effect on number of leaves, inter-nodal distance and fruit diameter which were increased upto 72, 77 and 63%, respectively, with respect to control (Table 2). mg L^{-1} while these were 97, 142, 73 and 96%, respectively, higher in case of soil applied L-MET at 40 mg kg^{-1} compared to untreated control.

Table 1: Effect of soil and foliar applied L-methionine on growth parameters of okra

Treatment (L-MET)	Plant height (cm)	No. of branches $plant^{-1}$	No. of leaves $plant^{-1}$	Root length (cm)
	Foliar application (mg L^{-1})			
0 (control)	26.43^{NS}	5.66 ^{d}	7.33 ^{c}	17.31 ^{d}
5	24.17	7.76 ^{c} (37)	10.07 ^{b} (37)	21.63 ^{c} (25)
10	25.47	12.44 ^{a} (119.78)	12.66 ^{a} (72)	29.57 ^{a} (71)
20	27.90	9.00 ^{b} (59)	10.65 ^{b} (45)	24.16 ^{b} (39)
	Soil application (mg kg^{-1})			
0 (control)	29.37^{NS}	6.33 ^{c}	7.67 ^{d}	18.27 ^{d}
20	28.23	7.00 ^{c} (10.58)	13 .00 ^{c} (69)	24.56 ^{c} (34)
40	30.60	15.33 ^{a} (142.18)	17.00 ^{a} (121.64)	35.87 ^{a} (96)
80	29.23	10.00 ^{b} (58)	13.00 ^{c} (69)	29.40 ^{b} (60.9)

^{NS} – non significant; () = % increase from the control; Values sharing the same letter(s) are statistically non-significant at $p < 0.05$

Table 2: Effect of soil and foliar applied L-methionine on growth parameters of okra

Treatment (L-MET)	Inter-nodal distance cm)	Fruit length (cm)	Fruit diameter (cm)	No. of floral buds dropped pot^{-1}
	Foliar application (mg L^{-1})			
0 (control)	3.66 ^{d}	4.15 ^{d}	3.53 ^{c}	8.76 ^{a}
5	4.53 ^{c} (24)	4.82 ^{c} (16)	4.58 ^{b} (29)	5.29 ^{b} (-39)
10	6.50 ^{a} (77)	7.33 ^{a} (77)	5.77 ^{a} (63)	2.13 ^{c} (-76)
20	5.40 ^{b} (47)	5.93 ^{b} (43)	4.80 ^{b} (36)	3.80 ^{b} (-56)
	Soil application (mg kg^{-1})			
0 (control)	3.12 ^{d}	4.67 ^{c}	3.48 ^{c}	8.27 ^{a}
20	3.27 ^{c} (4.80)	6.17 ^{b} (32)	4.41 ^{b} (27)	4.45 ^{b} (-46)
40	7.10 ^{a} (127.56)	8.08 ^{a} (73)	6.19 ^{a} (78)	2.01 ^{c} (-75)
80	4.60 ^{b} (47.43)	6.53 ^{b} (40)	4.70 ^{b} (35)	3.06 ^{b} (-62)

() = % increase from the control; (-) = % decrease from the control; Values sharing the same letter(s) are statistically non-significant at $p < 0.05$

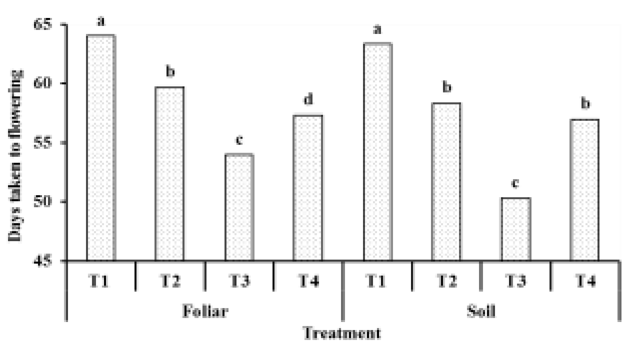

Figure 1: Effect of soil and foliar applied L-methionine on days taken to flowering

Data regarding number of flowers (Figure 3), number of branches (Table 1), fruit length (Table 2) and root length (Table 1) showed a significant increase up to 76, 119, 77 and 71%, respectively, by foliar application of L-MET at 10

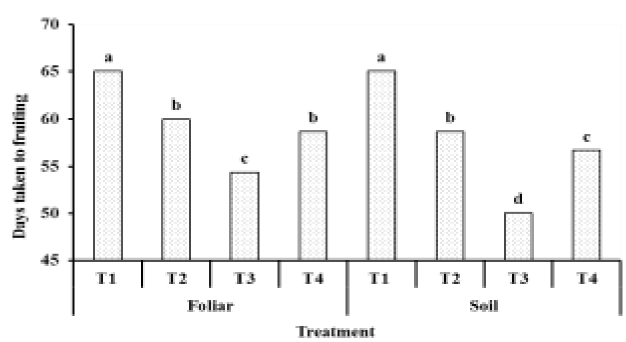

Figure 2: Effect of soil and foliar applied L-methionine on days taken to fruiting

Physiological parameters

Maximum chlorophyll contents (Figure 4) and photosynthetic rate was observed with the application of L-MET at 40 mg kg^{-1} soil and it caused an increase of 54 and

101%, respectively, compared to control. In case of foliar applied L-MET at 10 mg L^{-1}, the increase in chlorophyll contents and photosynthetic rate was 60, 101%, respectively, compared to control. Contrary to the above, transpiration rate, stomatal conductance and sub-stomatal CO_2 concentration were reduced by 53, 56 and 22%, respectively compared to control, with the application of L-MET as foliar at 10 mg L^{-1} while in case of soil applied L-MET at 40 mg kg^{-1}, the decrease was 56, 60 and 24%, respectively, compared to the control (Table 3).

of L-MET at 10 mg L^{-1} compared to control. Similar trend was observed in case of maximum number of fruits and it showed an increase of 77.42 and 97.33% with the application of soil and foliar applied L-MET at 40 mg kg^{-1} and 10 mg L^{-1}, respectively, compared to control (Table 5).

In case of fruit fresh and dry weight, the foliar application of L-MET at 10 mg L^{-1} showed an increase of 139 and 180%, respectively, while this increase was 117 and 143% when L-MET was applied to soil at 40 mg kg^{-1} compared to control (Table 5). Total fruit yield was also significantly enhanced by soil and foliar applied L-MET at 40 mg kg^{-1} and 10 mg L^{-1}, respectively and it showed an increase of 134 and 64%, respectively, compared to control (Table 5).

Table 3: Effect of soil and foliar applied L-methionine on physiological parameters of okra

Treatment (L-MET)	Photosynthetic rate (µmol CO_2 m^{-2} s^{-1})	Transpiration rate (µmol H_2O m^{-2} s^{-1})	Stomatal conductance (µmol m^{-2} s^{-1})	Sub-stomatal CO_2 Conc. (µmol mol^{-1})
		Foliar application (mg L^{-1})		
0 (control)	9.50 ^{d}	8.79 ^{a}	483.33 ^{a}	268.00 ^{d}
5	12.37 ^{c} (30)	6.38 ^{b} (-27)	367.67 ^{b} (-24)	250.67 ^{c} (-27)
10	19.13 ^{a} (101)	4.11 ^{c} (-53)	214.33 ^{d} (-56)	209.67 ^{a} (-22)
20	15.63 ^{b} (64)	5.72 ^{b} (-35)	349.00 ^{c} (-28)	241.67 ^{b} (-10)
		Soil application (mg kg^{-1})		
0 (control)	10.5 ^{d}	9.50 ^{a}	554.33 ^{a}	275.67 ^{d}
20	12.57 ^{c} (20)	7.25 ^{b} (-24)	380.00 ^{b} (-31)	256.33 ^{c} (-7)
40	22.35 ^{a} (111)	4.13 ^{c} (-56)	218.00 ^{d} (-60)	208.33 ^{a} (-24)
80	16.68 ^{b} (58)	6.09 ^{b} (-36)	360.67 ^{c} (-34)	247.33 ^{b} (-10)

() = % increase from the control; (-) = % decrease from the control; Values sharing the same letter(s) are statistically non-significant at $p < 0.05$

Table 4: Effect of soil and foliar applied L-methionine on root-shoot biomass of okra

Treatment (L-MET)	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)
		Foliar application		
0 (control)	15.94 ^{d}	14.41 ^{c}	4.76 ^{c}	3.68 ^{d}
5 mg L^{-1}	20.71 ^{c} (30)	18.63 ^{b} (29)	6.28 ^{c} (32)	4.83 ^{c} (31)
10 mg L^{-1}	27.76 ^{a} (74)	25.87 ^{a} (79)	10.83 ^{a} (127)	9.11 ^{a} (147)
20 mg L^{-1}	22.49 ^{b} (41)	20.06 ^{b} (39)	6.39 ^{b} (34)	5.05 ^{b} (37)
		Soil application		
0 (control)	16.42 ^{d}	14.52 ^{d}	5.24 ^{d}	4.19 ^{d}
20	21.94 ^{c} (34)	20.08 ^{c} (38)	6.89 ^{c} (31)	5.61 ^{c} (34)
40	32.61 ^{a} (98)	31.06 ^{a} (114)	13.38 ^{a} (155)	12.12 ^{a} (189)
80	26.54 ^{b} (22)	24.91 ^{b} (71)	8.78 ^{b} (67)	7.18 ^{b} (71)

() = % increase from the control; Values sharing the same letters are statistically non-significant at $p < 0.05$

Biomass and yield parameters

Data in Table 4 showed a significant increase in biomass parameters like shoot and root fresh and dry weights by soil and foliar applied L-MET at 40 mg kg^{-1} and 10 mg L^{-1}, respectively. The increase in case of shoot fresh and dry weights and root fresh and dry weights was 98, 114, 155 and 189%, respectively, with the application of soil applied L-MET at 40 mg kg^{-1} while this increase was 74, 79, 127 and 142%, respectively, with foliar application

Discussion

In the present study, L-MET, a precursor of ethylene, was used and its effect on the induction of early flowering and ultimately on the growth and yield parameters of okra was investigated through soil and foliar application. A

Table 5: Effect of soil and foliar applied L-methionine on yield parameters of okra

Treatment (L-MET)	Fruit fresh weight pot^{-1} (g)	Fruit dry weight pot^{-1} (g)	Total fruit yield pot^{-1} (g)	No. of fruits pot^{-1}
	Foliar application (mg L^{-1})			
0 (control)	7.71 ^{c}	6.32 ^{c}	24.28 ^{c}	4.40 ^{c}
5 mg L^{-1}	10.33 ^{c} (34)	9.26 ^{c} (46)	28.04 ^{c} (15)	5.20 ^{c} (18)
10 mg L^{-1}	18.48 ^{a} (139)	17.71 ^{a} (180)	39.80 ^{a} (64)	10.17 ^{a} (131)
20 mg L^{-1}	13.27 ^{b} (72)	12.17 ^{b} (92)	31.58 ^{b} (30)	7.10 ^{b} (61)
	Soil application (mg kg^{-1})			
0 (control)	11.19 ^{d}	9.59 ^{d}	20.69 ^{d}	3.67 ^{c}
20 mg kg^{-1}	15.02 ^{c} (34)	14.02 ^{c} (46)	29.70 ^{c} (43)	7.00 ^{b} (91)
40 mg kg^{-1}	24.35 ^{a} (117)	23.30 ^{a} (143)	48.41 ^{a} (134)	13.63 ^{a} (271)
80 mg kg^{-1}	19.38 ^{b} (73)	19.04 ^{b} (98)	38.83 ^{b} (88)	8.00 ^{b} (119)

() = % increase from the control; Values sharing the same letters are statistically non-significant at $p < 0.05$

significant improvement in the number of flowers and reduction in the number of days taken to flowering and fruiting was noted by the application of L-MET in the present study (Figure 1 and 2). This might be due to C_2H_4 role in improving the growth and development processes of plants by stimulating seed germination, shoot and root growth and early crop maturity (Arshad and Frankenberger, 2002). Siddiq *et al*. (2009) reported that C_2H_4 application to tomato accelerates the flower induction and fruit formation as compared to control. Devlin and Kay (2000) found flower induction between 25-45 days in *Arabidopsis* by the application of ethylene. Similar results were also reported by Arshad *et al.* (1993) in *Albizia lebbeck* L by using L-MET under pot conditions.

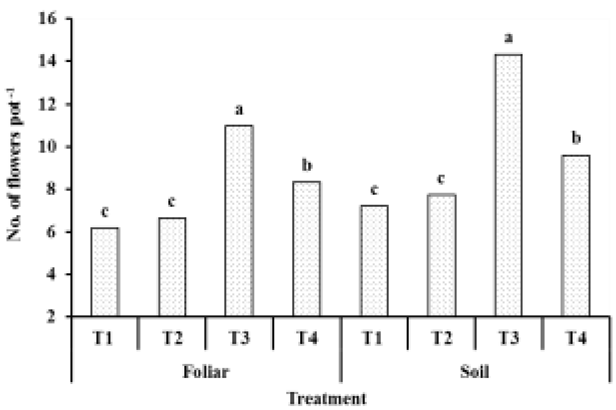

Figure 3: Effect of soil and foliar applied L-methionine on number of flowers pot^{-1}

T1: Control, T2: 5 mg L^{-1}, T3: 10 mg L^{-1}, T4: 20 mg L^{-1} foliar applied L-MET; T1: Control, T2: 20, T3: 40 and T4: 80 mg kg^{-1} soil applied L-MET. Bars showing the same letters are statistically non-significant at $p < 0.05$.

Significant improvement in the growth and physiological parameters was noted in the present study with the application of L-MET at 40 mg kg^{-1} as soil and 10

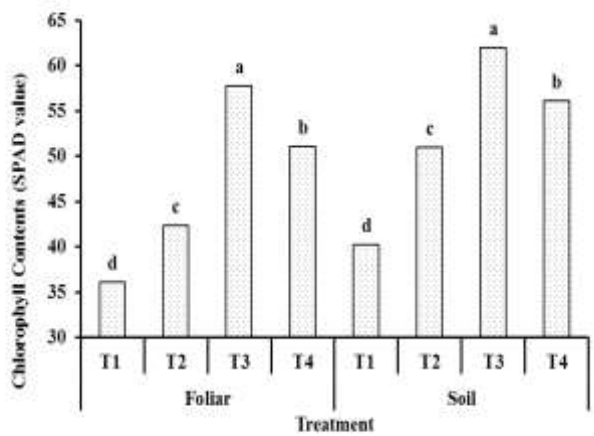

Figure 4: Effect of soil and foliar applied L-methionine on chlorophyll contents (mg g^{-1})

T1: Control, T2: 5, T3: 10, T4: 20 mg L^{-1} foliar applied L-MET, T1: Control, T2: 20, T3: 40 and T4: 80 mg kg^{-1} soil applied L-MET. Bars showing the same letters are statistically non-significant at $p < 0.05$.

mg L^{-1} as foliar. These yield contributing parameters ultimately improved the yield of okra in the form of higher number of fruits and total biomass which might be due to enhancement of endogenous C_2H_4 level (Arshad *et al.,* 1993). Similarly, C_2H_4 has a key role in almost all phases of growth and development of crop plants (Abeles *et al*., 1992; Reid, 1995; Arshad and Frankenberger, 2002). It might also be due to the role of C_2H_4 in stimulation of flower and fruit formation or due to increase in the root primordial to explore more volume of soil to acquire more nutrients

(Ahmad *et al*., 2004). Soil application of L-MET at 0.0185 to 1.85 mg kg^{-1} soil significantly improved the vegetative growth (shoot height, stem diameter, shoot fresh and dry weights) and reduced lodging in corn (Arshad and Frankenberger, 1990a). Similarly, significant increase in growth and yield was caused by the application of L-MET in soybean (Arshad *et al*., 1994). Muromtsev *et al*. (1990) reported that soil application of L-MET (2×10^{-4} to 20 mg kg^{-1}) significantly improved growth and yield parameters of soybean. Similar results were observed by Zahir and Arshad (1998) on sarsoon (*Brassica carinata*) and lentil (*Lens culinaris*).

Regarding physiological parameters, a significant improvement in chlorophyll contents and photosynthetic rate of okra was noted in the present study. Earlier, Khan (2004) reported a significant improvement in photosynthetic rate with exogenous application of L-MET upto 1.5 mM. However, application of higher concentration up to 3 mM significantly reduced the photosynthetic rate. In case of transpiration rate, stomatal conductance and sub-stomatal CO_2 concentration, a significant reduction was noted in the present study which might be due to enhancement of endogenous levels of ethylene. Earlier, Ahmad *et al.* (2009) found a significant reduction in transpiration rate with exogenous application of C_2H_4. Other researchers have also reported improved growth and physiology by application of C_2H_4 (Brown and Early, 1973; Keerthisinghe *et al.*, 1993; Seneweera *et al.*, 2003; Abbasi *et al.*, 2009).

Conclusion

Application of L-MET is effective in inducing early flowering and fruit formation and improving growth and yield of okra compared to the control. Application of L-MET as soil and foliar spray at 40 mg kg^{-1} and 10 mg L^{-1}, respectively, was found to be optimum in improving the growth, physiology and yield of okra compared to the other L-MET levels and untreated control. However, multisite field trials should be performed to warrant successful performance of L-MET application under field condition.

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