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# Mycorrhizal impact on *Ocimum basilicum* grown under drought stress

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## Abstract

**Background:** *Ocimum basilicum* was grown under three levels of drought stress (100% Field capacity, 70% Field capacity, and 40% Field capacity). Half of the plants were inoculated with Arbuscular mycorrhiza and the other half was not inoculated. Arbuscular mycorrhizal fungi (AMF) were applied to improve plant growth and to alleviate drought stress on sweet basil.

**Results:** Drought Cause inhibition in the colonization of Arbuscular mycorrhiza, reduction in plant growth, decrease stomatal size increase stomatal density, a decline in soluble carbohydrates, accumulation of amino acids, proline, and glycine betaine, and reduction in some minerals such as P, K, and Na.

**Conclusions:** The effect of drought was alleviated by the application of inoculation with Arbuscular mycorrhiza.

**Keywords:** Stomata, Osmoregulating, Proline, Glycine betaine, Amino acids, Chlorophyll, Minerals

## 1 Background

The world faces many global challenges like scarcity of water resources that enforced scientists to solve [1]. Many efforts must be done to cope with this environmental stress in both economic and environmentally sustainable ways [2]. Drought is defined as the deficiency of available soil moisture which may be sufficient to cause a reduction in growth, [3]. Plants require water for photosynthesis, nutrient uptake, and translocation transportation as well as cooling [4]. Drought stress is a major and serious threat to agriculture and crop production in arid and semiarid regions. Approximately, 70% of the global available water is used in agriculture to produce about 40% of the world's food [5]. Drought causes retardation in plant growth leading finally to a reduction in their yields. It disturbs plant metabolism causing inhibitions in total carbohydrates, total nitrogen contents, and total lipids [6, 7]. Several studied concerned with the negative effect of drought on plants, for example, it was observed a

dramatic reduction in the grain yield of *Zea mays* plants grown under drought stress [8, 9].

Many trials were done to overcome the drought problem; it was reported that the application of organic fertilizers and biofertilizers reduced the drought stress effect and improve plant growth [10]. In this context, arbuscular mycorrhizal fungi (AMF) are used as biofertilizers to improve plant growth, production, and plant water status [11]. AMF are associated with the roots of over 80% of terrestrial plant species [12]. Broad bean plants grown under salinity stress show better growth when inoculated with mycorrhiza than non-inoculated plants, under stress or non-stress condition [13]. Inoculation with AMF succeeded in the alleviation of the negative effects of drought stress on tomato plants, and this alleviation was more pronounced in the moderated stress than severe stress [14]. AMF stimulated the growth and photosynthesis of sweet basil plants confirming the role of mycorrhizal symbiosis in plant defense against biotic and abiotic stress. They also noted that symbiosis leads to an improvement in the growth and yield regardless of the water status of the plant (stressed or none) [15]. The main advantage of mycorrhiza for host plants may be the extension of the penetration zone of the root fungus system

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which increases active uptake and transport of nutrients especially immobile minerals like P, Zn, and Cu [16].

Sweet basil (*Ocimum basilicum* L.) is a member of the family Lamiaceae, which is recorded as one of the most widespread species in the world. It is widely used in food and oral care products and its essential oil is also used as perfumery [17, 18]. The leaves and flowering of sweet basil have been used in traditional medicine for many ailments like headaches, coughs, and diarrhea and it is generally recognized as a safe rich source of phenolic antioxidant and flavonoid compounds [19]. It also possesses other various medicinal applications like antiseptic, carminative, antimicrobial, and antioxidative agents [20, 21].

This work aimed to study the interaction effect of AMF inoculation and drought stress, on the growth, yield, and biochemical parameters of sweet basil. Moreover, the possibility to use AMF to alleviate drought stress on sweet basil.

## 2 Methods

### 2.1 Seeds and Mycorrhiza propagules

Sweet basil (*Ocimum basilicum*) seeds were provided from Agricultural Research Center (ARC), Seds Station, Beni-Suef Governorate, Egypt, but the propaguled arbuscular mycorrhiza (*Glomus versiform*) was provided from Soil, Water and Environment Research Institute (SWERI), Agricultural Research Center (ARC), Giza, Egypt.

### 2.2 Soil

The used soil was a garden soil that was collected from the surface layer (0-30 cm depth) from arable fields at Somasta Center, Beni-Suef Governorate, Egypt. The soil pH was 7.83 with EC ( $\text{dSm}^{-1}$ ) 4.21, and its texture was silty clay (1:2.5). It contains 201, 12.88, and 0.37 mg/g soil as N, P, and CO<sub>3</sub>, respectively. The soil was air-dried and then was sieved through a 2-mm sieve. Sieved soil was autoclaved for sterilization and 3 kilograms of this prepared soil was dispensed into black plastic pots (40-cm diameter × 50-cm Height) to be ready for cultivation.

Two experiments were done; first for calculating soil (FC) percentage to identify the water holding state of the used soil, while the second experiment was to estimate FC levels which will be applied to the main experiment.

#### 2.2.1 Determination of field capacity (FC%)

Field capacity (FC%) was measured according to the method described by [22] with the formula:

$$\text{FC (\%)} = [(a - b)/b] \times 100,$$

where (*a*) is the weight of the wet soil, while (*b*) is the weight of the oven-dried soil. The obtained results showed that FC was equal to 31.25%.

#### 2.2.2 Determination of maximum drought stress

To determine the maximum drought stress under which basil can be grown, seeds were germinated in serial FCs. In this experiment, 9-groups were irrigated with serial FCs (from 100% down to 20%), each group was of 5-replicates. After 5-weeks, the percentage of germination was calculated. It was observed that basil persisted growth down to FC 40%. According to these results, three FCs levels were selected; 100% (served as a control), 70% (as moderate water stress), and 40% (as severe water stress).

### 2.3 The main experiment

It was an open-field randomized pot experiment carried out in April 2019 within temperature range ( $26.76 \pm 4.32$  °C, and  $15.50 \pm 1.94$  °C, maximum and minimum respectively), in the garden of the Department of Botany and Microbiology, Faculty of Science, Beni-Suef University. The experiment was divided into two main groups. The first group was inoculated by 10 gm of mycorrhiza (0.003% W/W) [23], while the second group was not inoculated. Each group was subdivided into 3 subgroups; the 1st subgroup was irrigated up to 100% FC, the 2nd subgroup was irrigated at 70% FC and the last subgroup was irrigated at 40% FC. Each subgroup consisted of 20 pots and each pot with 30 seeds. Five harvested were carried out at the seedling stage, vegetative stage, flowering stage, fruiting stage, and yield finally. Growth criteria of each harvest were recorded then plants were subjected to several different physiological and biochemical investigations.

#### 2.3.1 Estimation of colonization percentage of arbuscular mycorrhizal fungi

After 30-days of inoculation, colonization percentage was estimated by staining the digested squashed root slices with 0.05% trypan blue stain [24].

#### 2.3.2 Determination of stomatal criteria

Stomatal criteria included stomatal density (number per  $\text{mm}^2$ ), stomatal size (guard cell length and stomatal pore width), and stomatal spacing, by using optical micrometers (linear and square). For measuring these criteria, two well-expanded leaves of each plant were selected, and both of their lower surface (abaxial side) and the upper surface (adaxial side) were stucked by nail varnish technique [25].

#### 2.3.3 Physiological and biochemical investigations

Soluble carbohydrate was determined by arsenomolybdate reagent [26]. Glycine-betaine (GB) estimation was done by cold KI/I<sub>2</sub> reagent as the method described by

[27]. Proline was determined 3% sulphosalicylic acid due to [28]. Extraction and determination of amino acids were carried out ninhydrin reagent [29]. Total soluble nitrogen in plant samples was measured by using Kjeldahl digestion method [30]. Determination of photosynthetic pigments (chlorophyll a, b, and carotenoids) was measure spectrophotometrically by [31]. Phosphorus (P) was estimated by using ammonium molybdate-sulphuric acid reagent [32]. The potassium and sodium content was extracted with 2% trichloroacetic acid (TCA) then elements were measured by flame

photometry by using Venema automation b.v., Groningen, Holland "Venema", automatic laboratory system at sugar factory of Beni-Suef, Egypt. All obtained results were subjected to different statistical analyses by using the SPSS program.

**2.4 Statistical analyses**

Kolmogorov-Smirnov and Levene's tests were used to check the normality and homogeneity, respectively, of the data obtained from pot experiment. All data exhibited normality and homogeneity. They analyzed by using the independent sample T test, then one-way analysis of variance (ANOVA) was applied and the significant differences among values were analyzed with Duncan's new multiple range tests at  $p \leq 0.05$  level of significance. To illustrate the effect of inoculation with mycorrhiza, drought and the interaction among the mentioned factors, two-way ANOVA analysis was conducted. Data were analyzed by using SPSS V20.

**Table 1** One-way ANOVA of the effects of drought on colonization % of *Ocimum basilicum*

Field capacity %	Colonization %
100	83.33 ± 2.64 c
70	65.0 ± 4.86 b
40	30.0 ± 4.25 a

Data showed in the table represent mean ± SD superscripted with small letter

**Table 2** One-way ANOVA of growth criteria of *Ocimum basilicum* under drought and mycorrhiza

Parameter	Growth stage	Soil field capacity		
		100%	70%	40%
Root length (cm)	Seedling	6.11 ± 0.13 <sup>c</sup> (7.17 ± 0.17 <sup>c</sup> )*	4.23 ± 0.08 <sup>b</sup> (4.94 ± 0.06 <sup>b</sup> )*	2.24 ± 0.14 <sup>a</sup> (3.44 ± 0.14 <sup>a</sup> )*
	Vegetative	8.26 ± .10 <sup>c</sup> (9.79 ± 0.18 <sup>c</sup> )*	5.99 ± 0.14 <sup>b</sup> (7.16 ± 0.11 <sup>b</sup> )*	3.77 ± 0.07 <sup>a</sup> (4.54 ± 0.12 <sup>b</sup> )*
	Flowering	9.49 ± 0.18 <sup>c</sup> (10.97 ± 0.19 <sup>c</sup> )*	7.21 ± 0.11 <sup>b</sup> (8.23 ± 0.12 <sup>b</sup> )*	4.99 ± 0.11 <sup>a</sup> (5.81 ± 0.10 <sup>a</sup> )*
	Fruiting	11.35 ± 0.48 <sup>c</sup> (13.71 ± 0.37 <sup>c</sup> )*	8.55 ± 0.37 <sup>b</sup> (10.00 ± 0.34 <sup>d</sup> )*	5.81 ± 0.40 <sup>a</sup> (7.14 ± 0.29 <sup>a</sup> )*
Root fresh weight (g)	Seedling	0.06 ± 0.00 <sup>c</sup> (0.13 ± 0.01 <sup>c</sup> )*	0.03 ± 0.00 <sup>ab</sup> (0.04 ± 0.00 <sup>b</sup> )*	0.01 ± 0.00 <sup>a</sup> (0.02 ± 0.00 <sup>a</sup> )*
	Vegetative	0.16 ± 0.00 <sup>c</sup> (.29 ± 0.01 <sup>c</sup> )*	0.09 ± 0.00 <sup>b</sup> (0.13 ± 0.00 <sup>b</sup> )*	0.03 ± 0.00 <sup>a</sup> (0.07 ± 0.00 <sup>a</sup> )*
	Flowering	0.32 ± 0.01 <sup>c</sup> (0.45 ± 0.01 <sup>c</sup> )*	0.16 ± 0.00 <sup>b</sup> (.22 ± 0.01 <sup>b</sup> )*	0.07 ± 0.01 <sup>a</sup> (0.12 ± 0.01 <sup>a</sup> )*
	Fruiting	0.49 ± 0.04 <sup>c</sup> (0.75 ± 0.08 <sup>c</sup> )*	0.19 ± 0.02 <sup>b</sup> (0.31 ± 0.05 <sup>b</sup> )*	0.09 ± 0.02 <sup>a</sup> (0.13 ± 0.01 <sup>a</sup> )*
Root dry weight (g)	Seedling	0.04 ± 0.00 <sup>c</sup> (0.06 ± 0.00 <sup>b</sup> )*	0.02 ± .00 <sup>b</sup> (03 ± .00 <sup>a</sup> )*	0.01 ± 0.00 <sup>a</sup> (0.03 ± 0.00 <sup>a</sup> )ns
	Vegetative	0.07 ± 0.0031 <sup>c</sup> (0.09 ± 0.003 <sup>b</sup> )*	0.05 ± 0.0009 <sup>b</sup> (.06 ± 0.0016 <sup>a</sup> )*	0.02 ± .0008 <sup>a</sup> (0.03 ± 0.0008 <sup>a</sup> )*
	Flowering	0.33 ± 0.012 <sup>b</sup> (0.48 ± 0.021 <sup>b</sup> )*	0.10 ± 0.005 <sup>ab</sup> (0.38 ± 0.226 <sup>b</sup> )*	0.04 ± 0.001 <sup>a</sup> (0.06 ± 0.004 <sup>a</sup> )*
	Fruiting	0.42 ± 0.013 <sup>c</sup> (0.61 ± 0.014 <sup>b</sup> )*	0.15 ± 0.006 <sup>b</sup> (0.24 ± 0.011 <sup>b</sup> )*	0.07 ± 0.003 <sup>a</sup> (0.11 ± 0.005 <sup>a</sup> )*
Shoot length (cm)	Seedling	11.82 ± 0.24 <sup>c</sup> (14.43 ± 0.13 <sup>c</sup> )*	9.28 ± 0.15 <sup>b</sup> (10.48 ± 0.11 <sup>b</sup> )*	6.38 ± 0.26 <sup>a</sup> (8.03 ± 0.09 <sup>a</sup> )*
	Vegetative	20.81 ± 0.99 <sup>c</sup> (24.07 ± 0.28 <sup>c</sup> )*	16.9 ± 0.24 <sup>b</sup> (18.80 ± 0.18 <sup>b</sup> )*	11.76 ± 0.21 <sup>a</sup> (14.63 ± 0.16 <sup>a</sup> )ns
	Flowering	24.97 ± 0.38 <sup>c</sup> (27.14 ± 0.51 <sup>c</sup> )*	18.36 ± 0.18 <sup>b</sup> (20.57 ± 0.23 <sup>b</sup> )*	13.86 ± 0.4 <sup>a</sup> (16.39 ± 0.15 <sup>a</sup> )*
	Fruiting	27.81 ± 0.99 <sup>c</sup> (34.12 ± 1.34 <sup>c</sup> )*	20.71 ± 0.93 <sup>b</sup> (25.3 ± 0.63 <sup>b</sup> )*	15.17 ± 0.52 <sup>a</sup> (18.31 ± 0.37 <sup>a</sup> )*
Shoot fresh weight (g)	Seedling	0.7 ± 0.02 <sup>c</sup> (1.38 ± 0.08 <sup>c</sup> )*	0.4 ± 0.01 <sup>b</sup> (0.54 ± 0.02 <sup>b</sup> )*	0.27 ± 0.01 <sup>a</sup> (0.33 ± 0.01 <sup>a</sup> )*
	Vegetative	3.98 ± .19 <sup>c</sup> (5.35 ± .10 <sup>c</sup> )*	1.74 ± .04 <sup>b</sup> (2.69 ± .04 <sup>b</sup> )*	0.53 ± 0.03 <sup>a</sup> (0.98 ± 0.03 <sup>a</sup> )*
	Flowering	4.51 ± 0.08 <sup>c</sup> (6.73 ± 0.10 <sup>c</sup> )*	2.62 ± 0.08 <sup>b</sup> (3.49 ± 0.12 <sup>b</sup> )*	1.20 ± 0.09 <sup>a</sup> (1.76 ± 0.08 <sup>a</sup> )*
	Fruiting	5.44 ± 0.60 <sup>c</sup> (8.89 ± 1.14 <sup>c</sup> )*	2.66 ± .20 <sup>b</sup> (4.00 ± 0.33 <sup>b</sup> )*	1.43 ± 0.12 <sup>a</sup> (1.89 ± 0.13 <sup>a</sup> )*
Shoot dry weight (g)	Seedling	0.34 ± 0.01 <sup>c</sup> (0.41 ± 0.01 <sup>c</sup> )*	0.20 ± 0.00 <sup>b</sup> (0.26 ± 0.01 <sup>b</sup> )*	0.09 ± 0.00 <sup>a</sup> (0.14 ± 0.01 <sup>a</sup> )*
	Vegetative	1.64 ± 0.04 <sup>c</sup> (2.09 ± 0.07 <sup>c</sup> )*	0.98 ± 0.03 <sup>b</sup> (1.34 ± 0.03 <sup>b</sup> )*	0.37 ± 0.002 <sup>a</sup> (0.02 ± 0.0008 <sup>a</sup> )*
	Flowering	2.84 ± 0.15 <sup>c</sup> (4.07 ± 0.08 <sup>c</sup> )*	1.31 ± 0.05 <sup>b</sup> (1.86 ± 0.08 <sup>b</sup> )*	0.6 ± 0.01 <sup>a</sup> (0.92 ± 0.03 <sup>a</sup> )*
	Fruiting	3.94 ± 0.13 <sup>c</sup> (5.02 ± 0.11 <sup>c</sup> )*	2.25 ± 0.06 <sup>b</sup> (3.02 ± 0.14 <sup>b</sup> )*	1.20 ± 0.06 <sup>a</sup> (1.83 ± 0.08 <sup>a</sup> )*

Values of parameters in parentheses correspond to the application of mycorrhiza. Values in a row with different superscript letters are significantly different at  $P < 0.05$ . \*Significant effect of mycorrhiza application at each soil field capacity at  $P < 0.05$  according to  $t$ -test. ns: non-significant effect of mycorrhiza application at each soil field capacity at  $P < 0.05$  according to  $t$ -test

**Table 3** Two- way ANOVA of Growth criteria of *Ocimum basilicum* under Drought and mycorrhiza; Error, 36

Parameter	Growth stage	Mycorrhiza	Drought	Mycorrhiza × Drought
Root length	Seedling	91.15**	448.21**	1.93ns
	Vegetative	129.02**	760.00**	4.61*
	Flowering	94.91**	599.48**	2.95ns
	Fruiting	190.04**	842.84**	5.78**
Root fresh weight	Seedling	44.52**	125.58**	20.88**
	Vegetative	266.64**	604.53**	58.74**
	Flowering	134.45**	607.32**	12.40**
	Fruiting	113.19**	552.76**	22.58**
Root dry weight	Seedling	15.13**	28.70**	0.64ns
	Vegetative	118.10**	499.62**	4.97*
	Flowering	4.05ns	7.34**	1.00ns
	Fruiting	172.68**	1035.25**	35.01**
Shoot length (cm)	Seedling	159.34**	567.50**	8.26**
	Vegetative	106.32**	1036.54**	10.48**
	Flowering	122.43**	667.82**	1.13ns
	Fruiting	312.02**	1122.48**	17.56**
Shoot fresh weight (g)	Seedling	116.29**	276.99**	54.06**
	Vegetative	143.60**	880.00**	11.98**
	Flowering	265.68**	1042.34**	46.41**
	Fruiting	98.41**	347.24**	27.82**
Shoot dry weight (g)	Seedling	150.09**	877.51**	2.86ns
	Vegetative	150.69**	639.02**	1.71ns
	Flowering	121.21**	629.60**	18.41**
	Fruiting	93.66**	408.45**	2.42ns

\*\* $P < 0.01$ ; \* $P < 0.05$ ; ns, non- significant

### 3 Results

The effect of drought on colonization percentage was negatively affecting as shown in Table 1. The reduction of colonization was at its maximum inhibition (72%) at 40% field capacity. This significant inhibition was obvious in all investigated parameters later.

The response of the investigated growth criteria is tabulated in Tables 2 and 3. In all growth stages, all investigated parameters were significantly affected with treatment with mycorrhiza and drought, while the interaction between them was significantly varied. Particularly, drought stress inhibited root (length, fresh and dry weights), and shoot (length, fresh weight, and dry weight). For all parameters, the treatment with mycorrhiza alleviated the stress impact. The integration of all data showed that root length in all growth stages under the different FCs has significantly differed in both inoculated and non-inoculated plants. This pattern is similar in root fresh weight and root dry weight except in inoculated plants under 40% FC which gave a non-significant difference.

The response of the stomatal structure to the applied stress and mycorrhizal application was tabulated in

Tables 4 and 5. In non-inoculated plants, under 100 FC and 40% FC, the length of guard cell (adaxial and abaxial) increased from growth stage to other, but under 70% FC there was no significant response. Stomatal guard cell length and stomatal pore width were decreased under drought stress especially in 40% of non-inoculated plants. Stomatal spacing decrease under drought stress, while stomatal density increases under stress especially in 40% of non-inoculated plants. Inoculation under all FCs gave no significance.

Tables 6 and 7 showing the effect of drought and mycorrhiza on some soluble metabolites (soluble carbohydrate, total amino acids, glycine betaine, proline, and total nitrogenous fractions). The concentration of soluble carbohydrates inhibited due to drought stress especially under the higher level of drought (40% non-inoculated). The maximum retardation due to drought was 71.45% at the seedling stage. AM fungus application improves significantly soluble carbohydrates content in both control samples and plants subjected to a combination between drought and AM treatment.

Plants subjected to drought stress showed accumulations of total amino acids especially proline, glycine

**Table 4** One-way ANOVA of Stomatal Criteria of *Ocimum basilicum* under Drought and mycorrhiza

Parameter	Growth stage	Soil field capacity		
		100%	70%	40%
Guard cell length (mm) (Adaxial side of lamina)	Seedling	0.0298 ± 0.0002 <sup>b</sup> (0.0300 ± 0.0000 <sup>b</sup> )ns	0.0275 ± 0.0000 <sup>b</sup> (0.0279 ± 0.0004 <sup>b</sup> )ns	0.0210 ± 0.0017 <sup>a</sup> (0.0240 ± 0.0008 <sup>a</sup> )ns
	Vegetative	0.0317 ± 0.0004 <sup>c</sup> (0.0328 ± 0.0000)*	0.0290 ± 0.0000 <sup>b</sup> (0.0296 ± 0.0000 <sup>a</sup> )ns	0.0246 ± 0.0004 <sup>a</sup> (0.0250 ± 0.0000 <sup>a</sup> )ns
	Flowering	0.0335 ± 0.0000 <sup>a</sup> (0.0346 ± 0.0004 <sup>c</sup> )*	0.0306 ± 0.0000 <sup>a</sup> (0.0312 ± 0.0000 <sup>b</sup> )ns	0.0253 ± 0.0000 <sup>a</sup> (0.0253 ± 0.0000 <sup>a</sup> )ns
	Fruiting	0.0354 ± 0.0004 <sup>c</sup> (0.0383 ± 0.0008 <sup>c</sup> )*	0.0321 ± 0.0002 <sup>b</sup> (0.0329 ± 0.0004 <sup>b</sup> )ns	0.0279 ± 0.0004 <sup>a</sup> (0.0302 ± 0.00017 <sup>a</sup> )*
Guard cell length (mm) (Abaxial side of lamina)	Seedling	0.0283 ± 0.0008 <sup>b</sup> (0.0300 ± 0.0000 <sup>c</sup> )ns	0.0267 ± 0.0008 <sup>ab</sup> (0.0275 ± 0.000 <sup>b</sup> )ns	0.0230 ± 0.0008 <sup>a</sup> (0.0240 ± 0.0008 <sup>a</sup> )ns
	Vegetative	0.0294 ± 0.0005 <sup>c</sup> (0.0348 ± 0.0000 <sup>c</sup> )ns	0.0294 ± 0.0000 <sup>b</sup> (0.0308 ± 0.0008 <sup>b</sup> )ns	0.0255 ± 0.0008 <sup>a</sup> (0.0269 ± 0.0000 <sup>a</sup> )ns
	Flowering	0.0321 ± 0.00017 <sup>c</sup> (0.0375 ± 0.00017 <sup>c</sup> )*	(0.0321 ± 0.0000 <sup>b</sup> ) (0.0323 ± 0.0000 <sup>b</sup> )ns	0.0281 ± 0.0008 <sup>a</sup> (0.0298 ± 0.0000 <sup>a</sup> )ns
	Fruiting	0.0384 ± 0.0003 <sup>c</sup> (0.0408 ± 0.0005 <sup>c</sup> )*	0.0348 ± 0.0001 <sup>b</sup> (0.0362 ± 0.0005 <sup>b</sup> )ns	0.0305 ± 0.00029 <sup>a</sup> (0.0327 ± 0.00017 <sup>a</sup> )*
Stomatal pore width (mm) (Adaxial side of lamina)	Seedling	0.0075 ± 0.0000 <sup>b</sup> (0.0075 ± 0.0000 <sup>b</sup> )ns	0.0054 ± 0.0004 <sup>a</sup> (0.0063 ± 0.0000 <sup>b</sup> )ns	0.0048 ± 0.0002 <sup>a</sup> (0.0050 ± 0.0000 <sup>a</sup> )ns
	Vegetative	0.0075 ± 0.0000 <sup>b</sup> (0.0075 ± 0.0000 <sup>a</sup> )ns	0.005 ± 0.0000 <sup>a</sup> (0.0063 ± 0.0000 <sup>a</sup> )ns	0.0048 ± 0.00017 <sup>a</sup> (0.0050 ± 0.0000 <sup>a</sup> )ns
	Flowering	0.0075 ± 0.0000 <sup>c</sup> (0.0075 ± 0.0000 <sup>a</sup> )ns	0.0057 ± 0.0001 <sup>b</sup> (0.0063 ± 0.0000 <sup>a</sup> )*	0.0050 ± 0.0000 <sup>a</sup> (0.0052 ± 0.0000 <sup>a</sup> )ns
	Fruiting	0.0079 ± 0.0004 <sup>b</sup> (0.0095 ± 0.0004 <sup>c</sup> )*	0.0068 ± 0.0002 <sup>b</sup> (0.0075 ± 0.0000 <sup>b</sup> )ns	0.0051 ± 0.0000 <sup>a</sup> (0.0061 ± 0.0002 <sup>a</sup> )*
Stomatal pore width (mm) (Abaxial side of lamina)	Seedling	0.0075 ± 0.0000 <sup>a</sup> (0.0075 ± 0.0000 <sup>b</sup> )ns	0.0050 ± 0.0000 <sup>a</sup> (0.0067 ± 0.0004 <sup>b</sup> )ns	0.0050 ± 0.0000 <sup>a</sup> (0.0050 ± 0.0000 <sup>a</sup> )ns
	Vegetative	0.0075 ± 0.0000 <sup>a</sup> (0.0075 ± 0.0000 <sup>b</sup> )ns	0.0063 ± 0.0000 <sup>a</sup> (0.0067 ± 0.0004 <sup>b</sup> )ns	0.0050 ± 0.0000 <sup>a</sup> (0.0050 ± 0.0000 <sup>a</sup> )ns
	Flowering	0.0075 ± 0.0000 <sup>c</sup> (0.0100 ± 0.0000 <sup>a</sup> )ns	0.0066 ± 0.0003 <sup>b</sup> (0.0075 ± 0.0000 <sup>a</sup> )*	0.0051 ± 0.0000 <sup>a</sup> (0.0052 ± 0.0000 <sup>a</sup> )ns
	Fruiting	0.0098 ± 0.0002 <sup>c</sup> (0.0100 ± 0.0000 <sup>c</sup> )ns	0.0075 ± 0.0000 <sup>b</sup> (0.0085 ± 0.0003 <sup>b</sup> )ns	0.0051 ± 0.0000 <sup>a</sup> (0.0058 ± 0.0004 <sup>a</sup> )ns
Stomatal spacing (mm) (Adaxial side of lamina)	Seedling	0.10938 ± 0.00322 <sup>a</sup> (0.0912 ± 0.0022 <sup>a</sup> )*	0.0786 ± 0.00182 <sup>b</sup> (0.0658 ± 0.00167 <sup>b</sup> )*	0.0593 ± 0.00044 <sup>c</sup> (0.0475 ± 0.00144 <sup>c</sup> )ns
	Vegetative	0.0942 ± 0.00417 <sup>a</sup> (0.0733 ± 0.00083 <sup>a</sup> )*	0.0731 ± 0.00098 <sup>b</sup> (0.0617 ± 0.00083 <sup>b</sup> )*	0.0534 ± 0.00175 <sup>c</sup> (0.0471 ± 0.0011 <sup>c</sup> )*
	Flowering	0.0914 ± 0.00074 <sup>a</sup> (0.0842 ± 0.00083 <sup>a</sup> )*	0.0697 ± 0.00148 <sup>b</sup> (0.0599 ± 0.00152 <sup>b</sup> )*	0.0503 ± 0.00148 <sup>c</sup> (0.0417 ± 0.00083 <sup>c</sup> )*
	Fruiting	0.09 ± 0.00144 <sup>a</sup> (0.0725 ± 0.00144 <sup>a</sup> )*	0.0671 ± 0.00182 <sup>b</sup> (0.0587 ± 0.00073 <sup>b</sup> )*	0.0498 ± 0.0013 <sup>c</sup> (0.0367 ± 0.0022 <sup>c</sup> )*
Stomatal spacing (mm) (Abaxial side of lamina)	Seedling	0.0939 ± 0.00145 <sup>a</sup> (0.0828 ± 0.00148 <sup>a</sup> )*	0.0695 ± 0.00153 <sup>b</sup> (0.0579 ± 0.0011 <sup>b</sup> )*	0.0485 ± 0.00176 <sup>c</sup> (0.0383 ± 0.00083 <sup>c</sup> )*
	Vegetative	0.0896 ± 0.0015 <sup>a</sup> (0.0738 ± 0.0026 <sup>a</sup> )*	0.0633 ± 0.00159 <sup>b</sup> (0.0570 ± 0.0012 <sup>b</sup> )*	0.0458 ± 0.0022 <sup>a</sup> (0.0343 ± 0.00167 <sup>c</sup> )*
	Flowering	0.0857 ± 0.00205 <sup>a</sup> (0.0632 ± 0.00093 <sup>a</sup> )*	0.0599 ± 0.0022 <sup>b</sup> (0.0527 ± 0.00098 <sup>b</sup> )ns	0.0456 ± 0.00156 <sup>c</sup> (0.0332 ± 0.0022 <sup>c</sup> )*
	Fruiting	0.0717 ± 0.00093 <sup>a</sup> (0.0639 ± 0.00074 <sup>a</sup> )*	0.0542 ± 0.0022 <sup>b</sup> (0.0481 ± 0.00098 <sup>b</sup> )ns	0.0404 ± 0.0015 <sup>c</sup> (0.0303 ± 0.00157 <sup>c</sup> )*
Stomatal density (number per mm <sup>2</sup> ) (Adaxial side of lamina)	Seedling	19.6296 ± 0.93208 <sup>a</sup> (23.3827 ± 1.09731 <sup>a</sup> )*	28.3951 ± 1.23457 <sup>b</sup> (35.8025 ± 1.23457 <sup>b</sup> )*	39.5062 ± 1.23457 <sup>c</sup> (46.9136 ± 1.23457 <sup>c</sup> )*
	Vegetative	25.2469 ± 0.61728 <sup>a</sup> (27.3333 ± 0.0000 <sup>a</sup> )*	33.0247 ± 0.98765 <sup>b</sup> (40.1235 ± 0.61728 <sup>b</sup> )ns	47.5309 ± 0.61728 <sup>c</sup> (55.9259 ± 0.37037 <sup>c</sup> )*
	Flowering	29.284 ± 1.23457 <sup>a</sup> (30.7901 ± 0.53814 <sup>a</sup> )*	38.5802 ± 0.24691 <sup>b</sup> (43.8889 ± 1.06917 <sup>b</sup> )*	52.267 ± 1.9931 <sup>c</sup> (60.4321 ± 0.53814 <sup>c</sup> )*

**Table 4** (continued)

Parameter	Growth stage	Soil field capacity		
		100%	70%	40%
Stomatal density (number per mm <sup>2</sup> ) (Abaxial side of lamina)	Fruiting	33.5432 ± 0.32664a (32.7531 ± 0.12346a)*	43.4691 ± 0.44513b (49.6667 ± 0.21383b)*	59.1221 ± 1.58395c (68.963 ± 0.42767c)*
	Seedling	16.7901 ± 1.07627a (23.0864 ± 0.53814a)*	27.1605 ± 1.23457b (41.9753 ± 1.23457b)*	45.6790 ± 1.23457c (54.321 ± 1.23457c)*
	Vegetative	22.4444 ± 0.64150a (26.2593 ± 0.85533a)*	34.9383 ± 0.49383b (47.7531 ± 0.96423b)*	52.716 ± 0.53814c (58.5185 ± 0.42767c)*
	Flowering	28.4691 ± 0.24691a (31.0741 ± 1.1315a)*	49.6296 ± 0.21383b (54.9753 ± 0.65327b)*	81.5272 ± 1.9931c (92.0123 ± 0.53814c)*
	Fruiting	30.2469 ± 0.32664a (34.0741 ± 0.42767a)*	54.4074 ± 0.21383b (59.2469 ± 0.32664b)*	90.5235 ± 0.91449c (99.6914 ± 0.2469c)*

Values of parameters in parentheses correspond to the application of mycorrhiza. Values in a row with different superscript letters are significantly different at  $P < 0.05$ . \*Significant effect of mycorrhiza application at each soil field capacity at  $P < 0.05$  according to  $t$ -test. ns: non-significant effect of mycorrhiza application at each soil field capacity at  $P < 0.05$  according to  $t$ -test

**Table 5** Two-way ANOVA of stomatal criteria of *Ocimum basilicum* under Drought and mycorrhiza, Error 12

Parameter	Growth stage	Mycorrhiza		Drought		Mycorrhiza × Drought	
		Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial
Guard cell length	Seedling	4ns	4.176ns	32.25**	47.37**	0.25ns	2.53ns
	Vegetative	7.27*	72**	196.51**	730.5**	0.35ns	10.5**
	Flowering	53.48**	121**	367.59**	1267**	0.7ns	31**
	Fruiting	28.33**	46.68**	145.51**	256.08**	2.67ns	0.95ns
stomatal pore width	Seedling	16**	4.97*	112**	102.21**	16**	2.9ns
	Vegetative	1ns	72.25**	109**	769.75**	1ns	49.75**
	Flowering	49**	256**	2749**	676**	49**	76**
	Fruiting	15.16**	29.63**	231.9**	80.24**	2.21ns	1.69ns
stomatal spacing	Seedling	97.866**	37.119**	334.333**	174.292**	2.454ns	2.42ns
	Vegetative	64.918**	51.6**	385.583**	243.868**	0.092ns	0.318ns
	Flowering	41.389**	147.49**	200.621**	470.899**	2.247ns	7.6**
	Fruiting	57.578**	53.877**	261.33**	389.6**	1.715ns	2.322ns
stomatal density	Seedling	117.335**	73.882**	359.432**	129.979**	7.699**	0.674ns
	Vegetative	85.383**	81.757**	380.525**	541.48**	0.35ns	15.25**
	Flowering	147.007**	109.20**	493.166**	418.709**	32.903**	6.782*
	Fruiting	109.441**	168.02**	850.353**	806.535**	13.176**	1.698ns

\*\* $P < 0.01$ ; \* $P < 0.05$ ; ns, non-significant

betaine. The vegetative stage showed maximum accumulation as it recorded 69.5%, 65.8%, and 11.8%, respectively. AM fungus application improved the concentration of proline, glycine betaine, and total free amino acids in control plant samples. Interaction between drought and AM decreased the accumulation of all three parameters, in relation to, the stressed non-treated samples.

Under normal growth conditions, total soluble nitrogen fractions recorded the most increment during the vegetative growth stage, while the lowest concentration was recorded in the fruiting stage. Drought caused an

accumulation of total nitrogen soluble fractions and the degree of accumulation was concentration-dependent. The application of AM enhanced total soluble nitrogen metabolism which was reflected as an increment of their contents. AM also alleviated the drought inhibitory effect.

As shown in Tables 8 and 9, photosynthetic pigments (chlorophyll a, chlorophyll b, and Carotenoids) concentration recorded their maximum concentration in the vegetative growth stage, and application of AM upgraded these concentrations. On the other hand, drought stress induced a large decline in these

**Table 6** One-way ANOVA of some soluble metabolites of *Ocimum basilicum* under Drought and mycorrhiza

Parameter	Growth stage	Soil field capacity		
		100%	70%	40%
Soluble carbohydrate (mgg <sup>-1</sup> DWt)	Seedling	12.75 ± 0.22 <sup>c</sup> (14.36 ± 0.19 <sup>c</sup> )*	5.20 ± 0.09 <sup>b</sup> (7.85 ± 0.18 <sup>b</sup> )*	3.64 ± 0.15 <sup>a</sup> (4.90 ± 0.44 <sup>a</sup> )*
	Vegetative	14.04 ± 0.46 <sup>c</sup> (16.13 ± 0.16 <sup>c</sup> )*	10.80 ± 0.31 <sup>b</sup> (12.45 ± 0.22 <sup>b</sup> )*	9.78 ± 0.14 <sup>a</sup> (10.63 ± 0.13 <sup>a</sup> )*
	Flowering	13.73 ± 0.13 <sup>c</sup> (14.68 ± 0.22 <sup>c</sup> )*	9.34 ± 0.28 <sup>b</sup> (10.32 ± 0.03 <sup>b</sup> )*	7.57 ± 0.10 <sup>a</sup> (8.48 ± 0.26 <sup>a</sup> )*
	Fruiting	12.25 ± 0.31 <sup>c</sup> (14.26 ± 0.23 <sup>c</sup> )*	8.88 ± 0.27 <sup>b</sup> (9.42 ± 0.04 <sup>b</sup> )*	6.46 ± 0.27 <sup>a</sup> (7.79 ± 0.31 <sup>a</sup> )*
Amino acids (mgg <sup>-1</sup> DWt)	Seedling	250.27 ± 4.78 <sup>a</sup> (271.87 ± 10.01 <sup>a</sup> )*	451.20 ± 3.49 <sup>b</sup> (311.47 ± 2.31 <sup>b</sup> )*	535.73 ± 3.24 <sup>c</sup> (512.80 ± 9.23 <sup>c</sup> )ns
	Vegetative	671.20 ± 4.33 <sup>a</sup> (691.47 ± 3.53 <sup>a</sup> )*	726.40 ± 3.67 <sup>b</sup> (697.33 ± 4.13 <sup>b</sup> )*	760.93 ± 4.42 <sup>c</sup> (736.40 ± 7.47 <sup>c</sup> )*
	Flowering	359.60 ± 6.38 <sup>a</sup> (478.80 ± 4.21 <sup>a</sup> )*	613.90 ± 3.32 <sup>b</sup> (527.83 ± 5.09 <sup>b</sup> )*	742.80 ± 5.56 <sup>c</sup> (687.87 ± 2.67 <sup>c</sup> )*
	Fruiting	109.47 ± 8.75 <sup>a</sup> (114.13 ± 4.73 <sup>a</sup> )ns	148.53 ± 4.56 <sup>b</sup> (135.73 ± 2.70 <sup>b</sup> )ns	206.53 ± 5.43 <sup>c</sup> (154.93 ± 13.09 <sup>b</sup> )*
Glycine betaine (mgg <sup>-1</sup> DWt)	Seedling	1.36 ± 0.05 <sup>a</sup> (1.46 ± 0.08 <sup>a</sup> )ns	1.82 ± 0.03 (1.58 ± 0.01)*	2.77 ± 0.06 (2.29 ± 0.08)*
	Vegetative	2.23 ± 0.10 <sup>a</sup> (2.77 ± 0.08 <sup>a</sup> )*	4.07 ± 0.05 <sup>b</sup> (2.53 ± 0.00 <sup>b</sup> )*	6.53 ± 0.33 <sup>c</sup> (4.88 ± 0.17 <sup>c</sup> )*
	Flowering	2.07 ± 0.04 <sup>a</sup> (2.64 ± 0.13 <sup>a</sup> )*	3.56 ± 0.19 <sup>b</sup> (2.36 ± .06 <sup>b</sup> )*	6.18 ± 0.34 <sup>c</sup> (4.64 ± 0.17 <sup>c</sup> )*
	Fruiting	1.03 ± 0.00 <sup>a</sup> (1.84 ± 0.07 <sup>a</sup> )ns	1.63 ± 0.02 <sup>b</sup> (1.37 ± 0.07 <sup>b</sup> )*	2.09 ± 0.04 <sup>c</sup> (1.81 ± 0.03 <sup>c</sup> )*
Proline (mgg <sup>-1</sup> DWt)	Seedling	3.67 ± 0.12 <sup>a</sup> (3.44 ± 0.09 <sup>a</sup> )*	4.74 ± 0.28 <sup>b</sup> (2.88 ± 0.05 <sup>b</sup> )*	5.39 ± 0.09 <sup>c</sup> (7.42 ± 0.30 <sup>c</sup> )*
	Vegetative	7.02 ± 0.59 <sup>a</sup> (7.11 ± 0.37 <sup>b</sup> )*	12.30 ± 0.24 <sup>b</sup> (10.91 ± 0.45 <sup>b</sup> )*	23.03 ± 1.46 <sup>c</sup> (16.41 ± 0.05 <sup>c</sup> )*
	Flowering	4.37 ± 0.12 <sup>a</sup> (4.67 ± 0.17 <sup>a</sup> )*	10.95 ± 0.82 <sup>b</sup> (3.70 ± 0.18 <sup>a</sup> )*	14.32 ± 0.67 <sup>c</sup> (9.53 ± 0.12 <sup>c</sup> )*
	Fruiting	1.39 ± 0.21 <sup>a</sup> (1.49 ± 0.14 <sup>a</sup> )*	7.05 ± 0.46 <sup>b</sup> (2.51 ± 0.29 <sup>b</sup> )*	12.88 ± 1.43 <sup>c</sup> (2.84 ± 0.55 <sup>c</sup> )*
Total soluble nitrogen (mgg <sup>-1</sup> DWt)	Seedling	1.32 ± 0.09 <sup>a</sup> (2.37 ± 0.15 <sup>a</sup> )ns	2.63 ± 0.04 <sup>b</sup> (2.90 ± 0.01 <sup>ab</sup> )*	3.41 ± 0.03 <sup>b</sup> (3.50 ± 0.03 <sup>c</sup> )*
	Vegetative	2.84 ± 0.03 <sup>a</sup> (3.98 ± 0.03 <sup>b</sup> )*	3.46 ± 0.10 <sup>b</sup> (4.37 ± 0.06 <sup>b</sup> )*	5.01 ± 0.15 <sup>c</sup> (8.13 ± 0.15 <sup>c</sup> )*
	Flowering	2.16 ± 0.7 <sup>a</sup> (3.06 ± 0.01 <sup>a</sup> )*	3.44 ± 0.24 <sup>b</sup> (3.78 ± 0.04 <sup>b</sup> )*	4.53 ± 0.24 <sup>c</sup> (5.40 ± 0.09 <sup>c</sup> )*
	Fruiting	0.85 ± 0.04 <sup>a</sup> (1.61 ± 0.20 <sup>a</sup> )*	2.25 ± 0.01 <sup>b</sup> (2.45 ± 0.03 <sup>b</sup> )*	2.16 ± 0.07 <sup>c</sup> (2.86 ± 0.08 <sup>a</sup> )*

Values of parameters in parentheses correspond to the application of mycorrhiza. Values in a row with different superscript letters are significantly different at  $P < 0.05$

\*Significant effect of mycorrhiza application at each soil field capacity at  $P < 0.05$  according to *t*-test

ns: non-significant effect of mycorrhiza application at each soil field capacity at  $P < 0.05$  according to *t*-test

photosynthetic pigments, at all FC levels. The highest decline percentage was recorded in vegetative growth stages and was positively dependent on the level of the applied stress. AM fungus application enhanced all estimated pigments under the different drought levels at all growth stages.

Data of minerals fraction recorded in Tables 10 and 11 cleared that, K, Na, and P uptake and their concentration were elevated due to water deficit. AM application significantly helped plants to accumulate more minerals, which was more obvious in non-stressed plants, as well as, helped the stressed plant to overcome the bad drought stress. The pattern of accumulation of all minerals was to some extent parallel to each other.

#### 4 Discussion

Drought reduces the growth of mycorrhiza and retards its colonization percentage. In this study, the obtained retardation was concomitant with many similar results obtained from Mays which suffered retardation of the colonization percentage [33] and similar retardation was observed in *Leymus chinensis* and *Hemarhria altissima*

grasses [34]. The retardation in the percentage of colonization due to drought stress may be attributed to the consumption of a part of the energy to adjust the osmotic potential rather than being used for fungal growth [35]. The reduction of colonization may be also considered as a result due to an inhibition that occurred in hydrolyzing enzymes responsible for fungal growth and/or synthesizing enzymes especially those of protein synthases to keep amino acids free to raise cell osmotic potential [14, 15]. The inhibition of AM colonization may be a result of the inhibition that occurred in the growth of the host plant via strigolactones which are plant growth stimulating and enhancing proliferation of cell AM, as a shortage of strigolactones causes less proliferation and colonization retardation [36].

The negative response of basil growth to drought was recognized in several studies [16, 37, 38]. The reduction that occurred in plant growth due to drought stress may be attributed to the inhibition that occurred in the cell division [39]. Drought also may inhibit the growth due to the inhibition that occurred in anabolic activity due to consumption of more energy to maintain cell water

**Table 7** Two- way ANOVA of soluble metabolites of *Ocimum basilicum* under Drought and mycorrhiza; Error, 12

Parameter	Growth stage	Mycorrhiza	Drought	Mycorrhiza × Drought
Soluble carbohydrate	Seedling	124.45**	1235.90**	2.41ns
	Vegetative	89.43**	753.40**	10.34**
	Flowering	48.27**	329.70**	7.04**
	Fruiting	49.88**	173.75**	3.17ns
Amino acid	Seedling	14.83**	50.04**	5.65*
	Vegetative	3.56ns	1972.46**	275.20**
	Flowering	85.71**	897.32**	89.70**
	Fruiting	71.53**	375.94**	38.92**
Glycine betaine	Seedling	42.97**	248.57**	0.61ns
	Vegetative	40.64**	240.80**	3.19ns
	Flowering	57.95**	224.13**	8.35*
	Fruiting	71.49**	228.85**	7.26**
Proline	Seedling	16.92**	39.88**	49.70**
	Vegetative	18.78**	268.65**	60.70**
	Flowering	49.90**	183.06**	3.49ns
	Fruiting	32.45**	107.81**	6.14*
Total Soluble Nitrogen	Seedling	0.838ns	8.56**	0.3ns
	Vegetative	30.41**	188.48**	6.35*
	Flowering	30.41**	188.48**	6.35*
	Fruiting	24.94**	159.93**	4.50*

\*\* $P < 0.01$ ; \* $P < 0.05$ ; ns, non- significant

**Table 8** One-way ANOVA of pigment fractions of *Ocimum basilicum* under Drought and mycorrhiza

Parameter	Growth stage	Soil field capacity		
		100%	70%	40%
Chlorophyll a (mgg <sup>-1</sup> FWt)	Seedling	4.56 ± 0.001 <sup>a</sup> (5.34 ± 0.001 <sup>a</sup> )*	3.20 ± 0.001 <sup>b</sup> (3.98 ± 0.001 <sup>b</sup> )*	1.64 ± 0.001 <sup>c</sup> (2.42 ± 0.001 <sup>c</sup> )*
	Vegetative	4.96 ± 0.001 <sup>a</sup> (5.74 ± 0.001 <sup>a</sup> )*	3.40 ± 0.001 <sup>b</sup> (4.18 ± 0.001 <sup>b</sup> )*	1.84 ± 0.001 <sup>c</sup> (2.62 ± 0.001 <sup>c</sup> )*
	Flowering	4.76 ± 0.001 <sup>a</sup> (5.64 ± 0.002 <sup>a</sup> )*	3.01 ± 0.001 <sup>b</sup> (3.97 ± 0.001 <sup>b</sup> )*	1.45 ± 0.001 <sup>c</sup> (2.23 ± 0.001 <sup>c</sup> )*
	Fruiting	3.37 ± 0.001 <sup>a</sup> (4.15 ± 0.001 <sup>a</sup> )*	2.04 ± 0.001 <sup>b</sup> (3.04 ± 0.001 <sup>b</sup> )*	1.01 ± 0.001 <sup>c</sup> (1.25 ± 0.001 <sup>c</sup> )*
Chlorophyll b (mgg <sup>-1</sup> FWt)	Seedling	1.56 ± 0.001 <sup>a</sup> (2.34 ± 0.001 <sup>a</sup> )*	1.20 ± 0.001 <sup>b</sup> (1.98 ± 0.001 <sup>b</sup> )*	0.64 ± 0.001 <sup>c</sup> (1.42 ± 0.001 <sup>c</sup> )*
	Vegetative	1.96 ± 0.001 <sup>a</sup> (3.04 ± 0.001 <sup>a</sup> )*	1.42 ± 0.001 <sup>b</sup> (2.18 ± 0.001 <sup>b</sup> )*	1.04 ± 0.001 <sup>c</sup> (1.62 ± 0.001 <sup>c</sup> )*
	Flowering	1.76 ± 0.001 <sup>a</sup> (2.54 ± 0.002 <sup>a</sup> )*	1.01 ± 0.001 <sup>b</sup> (1.97 ± 0.001 <sup>b</sup> )*	0.89 ± 0.001 <sup>c</sup> (1.23 ± 0.001 <sup>c</sup> )*
	Fruiting	1.37 ± 0.001 <sup>a</sup> (2.15 ± 0.001 <sup>a</sup> )*	0.91 ± 0.001 <sup>b</sup> (1.14 ± 0.001 <sup>b</sup> )*	0.72 ± 0.001 <sup>c</sup> (1.25 ± 0.001 <sup>c</sup> )*
Carotenoids (mgg <sup>-1</sup> FWt)	Seedling	1.40 ± 0.001 <sup>a</sup> (2.08 ± 0.001 <sup>a</sup> )*	1.06 ± 0.001 <sup>b</sup> (1.73 ± 0.001 <sup>b</sup> )*	0.54 ± 0.001 <sup>c</sup> (1.21 ± 0.001 <sup>c</sup> )*
	Vegetative	1.74 ± 0.001 <sup>a</sup> (2.71 ± 0.001 <sup>a</sup> )*	1.24 ± 0.001 <sup>b</sup> (1.91 ± 0.001 <sup>b</sup> )*	0.89 ± 0.001 <sup>c</sup> (1.36 ± 0.001 <sup>c</sup> )*
	Flowering	1.56 ± 0.001 <sup>a</sup> (2.26 ± 0.002 <sup>a</sup> )*	0.89 ± 0.001 <sup>b</sup> (1.73 ± 0.001 <sup>b</sup> )*	0.75 ± 0.001 <sup>c</sup> (1.03 ± 0.001 <sup>c</sup> )*
	Fruiting	1.22 ± 0.001 <sup>a</sup> (1.91 ± 0.001 <sup>a</sup> )*	0.81 ± 0.001 <sup>b</sup> (1.01 ± 0.001 <sup>b</sup> )*	0.62 ± 0.001 <sup>c</sup> (0.91 ± 0.001 <sup>c</sup> )*

Values of parameters in parentheses correspond to the application of mycorrhiza. Values in a row with different superscript letters are significantly different at  $P < 0.05$

\* Significant effect of mycorrhiza application at each soil field capacity at  $P < 0.05$  according to  $t$ -test

ns: non-significant effect of mycorrhiza application at each soil field capacity at  $P < 0.05$  according to  $t$ -test

potential [14, 40]. The inhibition that occurred in photosynthesis may have participated in the general growth inhibition that occurred [41].

The enhancement impact obtained by AM on plant growth can be interpreted due to a direct improvement of water absorption from the soil into the roots, by the

expanding surface of mycorrhizal roots which means more water uptake efficiency than more plant growth [42]. The improvement in water content due to AM inoculation can be also discussed as a result of fungal hyphae exploitation of water which finally caused more water content that enhanced more plant growth [16].

**Table 9** Two-way ANOVA of pigment fractions of *Ocimum basilicum* under Drought and mycorrhiza; Error, 12

Parameter	Growth stage	Mycorrhiza	Drought	Mycorrhiza × Drought
Chlorophyll a	Seedling	217.38**	562.60**	65.78**
	Vegetative	54.20**	122.18**	8.10**
	Flowering	2.98**	7.25**	1.00**
	Fruiting	39.38**	308.16**	2.71**
Chlorophyll b	Seedling	12.68**	53.24**	1.39**
	Vegetative	29.09**	141.76**	2.27**
	Flowering	7.48*	20.94**	1.05**
	Fruiting	44.41**	129.17**	17.38**
Carotenoids	Seedling	3.97ns	9.56**	1.11**
	Vegetative	27.21**	80.09**	0.64**
	Flowering	13.33**	81.35**	1.03**
	Fruiting	10.99**	20.33**	3.3**

\*\* $P < 0.01$ ; \* $P < 0.05$ ; ns, non-significant

**Table 10** One-way ANOVA of elements of *Ocimum basilicum* under Drought and mycorrhiza

Parameter	Growth stage	Soil field capacity		
		100%	70%	40%
Potassium ( $\mu\text{g}^{-1}$ DWt)	Seedling	2641.53 $\pm$ 1.34 <sup>a</sup> (4020.03 $\pm$ 0.85 <sup>a</sup> )*	4620.50 $\pm$ 2.14 <sup>b</sup> (4750.37 $\pm$ 2.00 <sup>b</sup> )*	5751.97 $\pm$ 1.11 <sup>c</sup> (6254.93 $\pm$ 1.25 <sup>c</sup> )*
	Vegetative	3610.00 $\pm$ 0.60 <sup>a</sup> (4670.87 $\pm$ 1.60 <sup>a</sup> )*	5529.07 $\pm$ 0.90 <sup>b</sup> (5947.10 $\pm$ 2.14 <sup>b</sup> )*	7625.07 $\pm$ 1.77 <sup>c</sup> (8130.77 $\pm$ 2.53 <sup>c</sup> )*
	Flowering	2973.90 $\pm$ 1.72 <sup>a</sup> (4410.83 $\pm$ 0.75 <sup>a</sup> )*	5322.10 $\pm$ 2.07 <sup>b</sup> (5647.40 $\pm$ 2.05 <sup>b</sup> )*	5966.47 $\pm$ 0.98 <sup>c</sup> (7239.50 $\pm$ 0.85 <sup>c</sup> )*
	Fruiting	2305.10 $\pm$ 0.76 <sup>a</sup> (3120.50 $\pm$ 1.23 <sup>a</sup> )*	4114.03 $\pm$ 0.64 <sup>b</sup> (5114.70 $\pm$ 2.40 <sup>b</sup> )*	5108.77 $\pm$ 1.37 <sup>c</sup> (6312.30 $\pm$ 1.55 <sup>c</sup> )*
Sodium ( $\mu\text{g}^{-1}$ DWt)	Seedling	1076.61 $\pm$ 1.35 <sup>a</sup> (1608.01 $\pm$ 0.86 <sup>a</sup> )*	1848.22 $\pm$ 2.15 <sup>b</sup> (1900.14 $\pm$ 2.01 <sup>b</sup> )*	2300.79 $\pm$ 1.11 <sup>c</sup> (2501.11 $\pm$ 1.25 <sup>c</sup> )*
	Vegetative	1444.25 $\pm$ 0.61 <sup>a</sup> (1868.13 $\pm$ 1.62 <sup>a</sup> )*	2211.62 $\pm$ 0.91 <sup>b</sup> (2378.85 $\pm$ 2.15 <sup>b</sup> )*	3050.18 $\pm$ 1.77 <sup>c</sup> (3252.66 $\pm$ 2.53 <sup>c</sup> )*
	Flowering	1169.62 $\pm$ 1.73 <sup>a</sup> (1764.32 $\pm$ 0.76 <sup>a</sup> )*	2118.52 $\pm$ 2.08 <sup>b</sup> (2258.84 $\pm$ 2.06 <sup>b</sup> )*	2368.25 $\pm$ 0.98 <sup>c</sup> (2895.14 $\pm$ 0.85 <sup>c</sup> )*
	Fruiting	922.85 $\pm$ 0.77 <sup>a</sup> (1248.54 $\pm$ 1.24 <sup>a</sup> )*	1645.16 $\pm$ 0.66 <sup>b</sup> (2045.66 $\pm$ 2.42 <sup>b</sup> )*	2043.77 $\pm$ 1.37 <sup>c</sup> (2584.11 $\pm$ 1.55 <sup>c</sup> )*
Phosphorus ( $\mu\text{g}^{-1}$ DWt)	Seedling	3758.58 $\pm$ 1.30 <sup>a</sup> (5628.24 $\pm$ 0.81 <sup>a</sup> )*	4686.57 $\pm$ 2.10 <sup>b</sup> (6651.21 $\pm$ 1.95 <sup>b</sup> )*	8051.14 $\pm$ 1.18 <sup>c</sup> (5756.32 $\pm$ 1.15 <sup>c</sup> )*
	Vegetative	5054.27 $\pm$ 0.56 <sup>a</sup> (6538.91 $\pm$ 1.54 <sup>a</sup> )*	7740.61 $\pm$ 0.86 <sup>b</sup> (8525.32 $\pm$ 2.09 <sup>b</sup> )*	10,815.24 $\pm$ 1.71 <sup>c</sup> (11,382.56 $\pm$ 2.43 <sup>c</sup> )*
	Flowering	4042.61 $\pm$ 1.68 <sup>a</sup> (6174.87 $\pm$ 0.71 <sup>a</sup> )*	7450.22 $\pm$ 2.02 <sup>b</sup> (7905.47 $\pm$ 2.00 <sup>b</sup> )*	8352.54 $\pm$ 0.99 <sup>c</sup> (10,134.67 $\pm$ 0.86 <sup>c</sup> )*
	Fruiting	3227.81 $\pm$ 0.72 <sup>a</sup> (4368.24 $\pm$ 1.19 <sup>a</sup> )*	5759.47 $\pm$ 0.58 <sup>b</sup> (7159.66 $\pm$ 2.36 <sup>b</sup> )*	7151.15 $\pm$ 1.31 <sup>c</sup> (8836.62 $\pm$ 1.49 <sup>c</sup> )*

Values of parameters in parentheses correspond to the application of mycorrhiza. Values in a row with different superscript letters are significantly different at  $P < 0.05$ . \*Significant effect of mycorrhiza application at each soil field capacity at  $P < 0.05$  according to  $t$ -test. ns: non-significant effect of mycorrhiza application at each soil field capacity at  $P < 0.05$  according to  $t$ -test

The improving effect of AM fungi on the estimated growth criteria may be also explained as a result of an anabolic acceleration of photosynthetic activity [14]. Such improvement can be also understood through the improvement of nutrient status which means more enzymatic activation as well as more raising of plant osmotic potential [38].

Stomata regulate and control water vapor fluxes through stomatal conductance and stomatal resistance via transpiration. The movement of the stomata is controlled by the guard cell which is harmonized by its turgor pressure. Turgor pressure of guard cell is affected by many regulatory factors including plant water status

[43] which depends on and correlates with the degree of soil hydration [44]. The change of guard cell length is a mechanical mechanism of stomatal working to control its conductance, its resistance, and its pore width to overcome water shortage as a morphological adaptation [45]. Underwater shortage, stomata decrease their conductance and pore width [46] to hold more water content [47]. Stomatal density is another adaptive anatomical mechanism to acclimatize and resist drought to reduce the transpiration rate by increasing stomatal dimension [48] through specific gene system transcription [49]. In a recent study [50] it was shown that reduced soil water content significantly stimulated stomatal generation in

**Table 11** Two-way ANOVA of Elements of *Ocimum basilicum* under Drought and mycorrhiza; Error, 12

Parameter	Growth stage	Mycorrhiza	Drought	Mycorrhiza × Drought
Phosphorus	Seedling	8.49*	40.29**	2.08ns
	Vegetative	70.36**	493.07**	8.87**
	Flowering	9.39*	120.13**	0.96ns
	Fruiting	38.17**	139.35**	4.77*
Potassium	Seedling	2787.80**	21,222.55**	666.35**
	Vegetative	16,637.22**	43,480.93**	2960.90**
	Flowering	5790.54**	43,999.99**	1402.18**
	Fruiting	3116.52**	6710.98**	574.11**
Sodium	Seedling	17,683.45**	12,508.79**	32,541.01**
	Vegetative	4671.44**	92,737.91**	52,632.96**
	Flowering	24.50**	32,566.65**	23,856.49**
	Fruiting	183,948.84**	256,713.15**	101,912.29**

\*\* $P < 0.01$ ; \* $P < 0.05$ ; ns, non-significant

maize, resulting in a significant increase in stomatal density but a decrease in stomatal size and aperture. In this research, it was also reported that stomatal density was significantly negatively correlated with both net photosynthetic rate and transpiration rate, but more strongly with the latter, so leaf instantaneous water use efficiency was correlated positively with stomatal density in *Platanus acerifolia* [51] and in *Ziziphus jujuba* which showed a negative correlation between stomatal density and stomatal length under different irrigation regimes [52].

The observed positive effect of mycorrhiza on stomata was estimated in maize [32], in walnut [23], and *Leymus chinensis* and *Hemarthria altissima* grasses [33]. This improvement may be attributed to an enhancement occurred in leaf water potential by the accumulation of osmoprotectant and solutes such as proline and total soluble sugars [53]. Also, AM accumulated phenolics which may improve leaf water potential [16]. The development of stomatal morphology may be accredited to increasing water and mineral uptake by fungal hyphae [54, 55]. The progress of stomatal structure and function may be ascribed finally to the excreted plant hormones by fungal cell especially abscisic acid (ABA) which regulate stomatal working [23].

Plants accumulate osmotically active soluble molecules as a major mechanism for increasing plant cell osmotic activity. In this study, *Ocimum* accumulated free amino acids (especially proline and glycine betaine) and total nitrogen soluble fractions. Amino acids and nitrogenous fractions may play a signaling role in plant tolerance besides their roles of osmotic adjustment, protection of cellular macromolecules, storage of nitrogen, maintenance of cellular pH, detoxification of the cells, and scavenging of free radicals [56]. Such accumulation may be due to activation that occurred in their biosynthesis

and/or inhibition that occurred in their polymerization [57]. Accumulation of proline means an enhancement occurred in its anabolism and/or retardation occurred in oxidation, particularly, through those controlling enzymes; glutamate synthase, glutamate dehydrogenase, and glutamate decarboxylase [58]. Proline rises cellular osmolarity, regulates redox buffering and energy transfer to regulate redox potentials and a hydroxyl radical scavenger to alleviate oxidative stress, and programs cell death [33]. In parallel, glycine betaine acts as an osmoregulator, osmo-protector, and stomatal work controller [57, 59].

On the other hand, drought inhibited soluble carbohydrate fractions, and this may be attributed to stomatal closure, the reduction of chlorophyll concentration, and/or photosynthesis inhibition [60], and this obtained reduction may point to the trouble that occurred in their biosynthesis which is a gen dependent response [61].

The alleviating impact of mycorrhiza may be attributed to the more water absorbed by fungal hyphae [62]. The improvement of these soluble fractions due to mycorrhizal symbiosis may be ascribed to the activation occurred in their anabolic activity due to more mycorrhizal absorbed minerals acting as specific enzyme activator [63]. Mycorrhiza also secretes some hormones like ABA which regulate stomatal working which means more photosynthetic products acting as a precursor of these osmolytes [64]. Mycorrhiza also supplies plant roots with necessary N, K, P, and especially Mg elements and more anabolized molecules used in the biosynthesis of such osmoregulatory and osmo-protecting molecules [65].

Retardation in photosynthetic pigments due to drought stress is a commonly observed phenomenon. In the current study, this retardation is coincident with decrement occurred insoluble carbohydrate. The inhibition of

photosynthetic pigment may be attributed to the general anabolic inhibition due to drought stress [14]. The inhibition of Mg concentration may be a direct factor of chlorophyll inhibition [66].

On the other hand, the assuaging impact of mycorrhiza may be accredited on the improvement of plant water status helped stomata for more opening and efficiency [67]. More absorbed Mg, by mycorrhizal hyphae, may participate in more chlorophyll biosynthesis [65]. Water use efficiency may explain such improvement as it influences the balance between carbon gain in photosynthesis and water loss via transpiration [68].

Accumulation of minerals is a known response mechanism against drought stress to raise cell osmotic potential which consumes more energy leading to deprivation of cells from more anabolic reactions [13]. Besides the participation of osmotic potential raising, some of these minerals are engaged to activate some osmoregulating and osmoprotectant molecules [64]. The improvement of mineral uptake due to AM treatment may be attributed to the absorbing capacity of fungal hyphae especially the P element [12].

Conversely, Mycorrhiza improved mineral uptake and this improvement can be denoted to hyphae uptake itself [69] and/or more root uptake by the saved energy instead of its consumption for raising cell osmotic potential [62].

## 5 Conclusions

Generally, the application of arbuscular mycorrhiza improved the growth of basil (*Ocimum basilicum*) grown either under normal conditions or under drought stress, and this result can advise by using this application to improve the growth and quality of this plant under normal or drought stress conditions.

### List of abbreviations

AM: Arbuscular mycorrhiza; AMF: Arbuscular mycorrhizal fungi; EC: Electrical conductivity; FC: Field capacity; GB: Glycine-betaine.

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### Authors' contributions

NH and AM have drafted the review. SE and NH prepared different tables and figures required for the manuscript. BA provided guidance during the development of the idea and wrote and revised the manuscript. All authors read and approved the final manuscript.

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