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Arbuscular Mycorrhizal Fungal Symbiosis with saffron (*Crocus sativus* L.) Plant

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ABSTRACT

Mycorrhizae are fungal symbionts forming mutualistic relationship with plant roots. Arbuscular mycorrhizal fungal symbiosis have remarkable role in sustainable growth and development of plants as they help the land plants to acclimatize the biotic and abiotic conditions for their better survival, growth and development. In the present study surface sterilized corms of saffron were sown in earthen pots filled with sterile soil. Half the pots were inoculated with AMF spores of the *Glomus* species and 10 grams of maize root inoculated with the species of genus *Glomus.* Another half represented controls with no AMF inoculation.The results show a promising potential of AM fungi in influencing the growth and development parameters of plant. It was observed that corm despite being the modified stem shows AMF colonization The Chlorophyll content besides morphological growth parameters and fresh and dry weight content of plant are shown to present in higher level in the mycorrhiza inoculated as compared to the non-inoculated ones.

Key Words: Saffron Plant, AMF, Scale colonization, growth parameters, chlorophyll.

INTRODUCTION

Mycorrhizae or *mycorrhiza*, a symbiotic association between a fungus and the roots of a plant (Kirk *et al*., 2001). Despite only a small proportion of angiospermic species having been examined, mycorrhizae form a mutualistic relationship with the roots of nearly eighty percent of such plant species (Wang & Qiu 2006). AM fungi and plant roots, improve water and nutrient uptake like phosphorus, nitrogen and micronutrients and thus enhance plant growth (Goussous & Mohammad 2009, Lone et al. 2015). Most of the research effort is concerned with mycorrhiza as a mutualistic association between the underground root of the host plant and soil fungi. However, there are reports that besides roots, these fungi can also associate mutualistically with underground modifications of stem like rhizomes and other associated structures. Taber and Trape (1982) reported for the first time, the presence of AM fungi in the vascular system of rhizomatous tissue and the scale like leaves of *Zingiber officinale* L. Later Nasim (1990) reviewed the presence of AM fungi associated with the portions other than roots in twenty one angiosperms and some non-angiosperm species. Incidence of AM fungal colonisation has been reported in scale leaves and leaf bases of *Curcuma longa* L. (Sampath & Sullia 1992), corms of *Amorphophallus commutatus* Engler (Rodrigues 1995) and tubers of *Pueraria tuberosa* (Willd.) DC (Rodrigues, 1996). On further perusal the availability of literature on stem modifications and AM fungi associations is scanty because of dominance of studies on root-fungi associations. Present study is therefore, based on a simple premise whether or not the AM fungi have any

constitutive association with saffron underground stem propagules which constitute the prime propagule for vegetative propagation and also being the part of commercial utility and importance and thereby assessing a substantive role of AMF associations in the growth and development of the saffron plant.

MATERIALS AND METHODS

Corms of *Crocus sativus* (saffron) were brought from the fields of Pampore, Kashmir. Before sowing the corms, the scales were removed from the saffron corms and surface sterilized by dipping those in 0.01% cetrimide solution for 3-5 minutes first and then dips again in sodium hypochlorite for 3-5 minutes after taken out and washed 3-4 times with sterile distilled water. Corms of uniform size were then sown in pots with equal dimension each filled with sterile soil autoclaved twice at 15 lbs pressure and at a temperature of 120ºC for 45-60 minutes. The physical properties of experiment soil used in pot culture show reddish brown soil and clay loamy texture. Soils are slightly alkaline with pH 7.12. The soil shows electric conductivity of 0.24 dsm^{-1}, organic carbon 0.39 percent, water holding capacity 2.32 percent and density of 1.21 mgm^{-3}. The chemical properties of soil show that soil contains nitrogen 160 kg. ha^{-1}, phosphorous 14.60 kg. ha^{-1} and potassium 230.42 $kg.ha^{-1}$. The micronutrient present in the experiment soils is iron 5.40, zinc 1.23, manganese 6.20 and copper 1.66 ppm. Half the pots were inoculated with 50 AMF spores of the species of *Glomus intraradices* amd *Glomus mosseae* and also 10 grams of maize root inoculated with the species of *Glomus intraradices* amd *Glomus mosseae*. Half the pots represented controls which had no AMF inoculation but were provided with 10 grams of non-inoculated maize root fragments. Inoculation was done twice 3 days before sowing the corms and after sowing of corms to ensure proper colonisation. Pots were watered regularly. The saffron corms were sown in the pots in the month of September when the corms had already germinated by showing bud enlargement. The plants were sampled after 30, 60, 90 and 120 days after seedling emergence, along with their controls at all stages of growth. Plant height, leaf number, neck diameter, corm diameter, corm circumference and root length were recorded. Roots, scales and corms were collected and placed in a 4% isopropanol solution for analysis of AMF colonisation at a later date. Leaves and roots were separated from corms. Plant height was measured as the top most height of the leaf. The leaf number was recorded as the mean value of all the leaves of each plant of all pots divided by the number of all the plant corms. Neck diameter was measured above 3 cms from the surface of the soil. After the plants were uprooted corm diameter and corm circumfences were recorded using a thread after every 30 days interval after planting upto 120 days. Roots were thoroughly washed with tap water and root length recoded as the mean value of the longest roots of each plant from the corm downwards. After uprooting the plant, fresh and dry weights were determined for each plant after separating in leaves and roots from the corm. After weighing fresh leaves, corms and roots, were dried for 48 hours in forced draught oven. The fresh corms after weighing were however, dried for 72 hours and reweighted.

The roots were stained with 0.05% tryphan blue stain using the method suggested by Phillips and Hayman (1970). Root colonization estimation was carried out using Biermann and Linderman (1981) method. Percent root colonization was calculated using following relationship:

$$\text{Percent colonization} = \frac{\text{Total number of colonized root, scale or corm pieces}}{\text{Total number of root, scale or corm pieces examined}} \times 100$$

For chlorophyll a, chlorophyll b and total chlorophyll the method of Arnon (1949) and Witham *et al*. (1971) was employed. Calculation of the amount of chlorophyll present in the extract as mg chlorophyll per gram green tissue using the following equations for each fraction;

For chlorophyll a

$$\text{mg chlorophyll a per gm tissue} = 12.7(A663) - 269(A645) \times \frac{V}{1000 \times W}$$

For chlorophyll b

$$\text{mg chlorophyll b per gm tissue} = 12.7(A645) - 269(A663) \times \frac{V}{1000 \times W}$$

Total chlorophyll

$$\text{mg total chlorophyll} = 20.2(A645) + 8.02(A663) \times \frac{V}{1000 \times W}$$

Where
A= absorbance at specific wavelength
V= final volume of chlorophyll extract in 80% acetone
W= fresh weight of tissue extracted

All the data were statistically analyzed by analysis of variance with the MSTATC PROGRAM (Mich. University, East Lasing Mich., USA) Significant differences at $P<0.0\ 5$ were tested using Duncan's multiple range tests.

RESULTS

Saffron corms are known to belong to the uniform stock because of their having vegetative propagation only. All the pots were sown with the corms of uniform size and these performed 100 per cent uniform emergence and subsequent growth under pot cultures. From observations during various stages of saffron plant growth monitored at

30 days intervals of 30, 60, 90 and 120 days after bud emergence, it is evident that comparative visible effects between the non-inoculated and those inoculated with AMF was observed with clarity even under pot culture conditions.

After corm seedling emergence, at every 30 days interval, mycorrhizal colonisation was observed in all AMF inoculated treatment pots. This is based on the presence of arbuscules, hyphe and vesicles in the AMF inoculated roots. These structures were absent from the uninoculated control pots, however, colonisation is absent in the roots or even scales (Fig. 1). Scales of underground modified propagules in AMF inoculated pots shows AMF colonisation only after 60 days plant growth. A substantial increase in root colonisation was shown at every 30 days after rooting. Starting from lowest of 14.84 percent root colonisation after showing inoculated plants with old corm gradually increases with the age of plant maximising into root colonisation percentage at 120 days showing 88.96 percent (Table 1). Scales of saffron corms of inoculated plants show AMF colonisation after 60 days of bud emergence (Table 1). The absence of AMF structures in roots of uninoculated plants implied that the conditions of soil sterility were adequately met to present the specific responses of plants to AMF from the pots which were inoculated with AMF (Table 1).

Table 1: Comparative colonisation percentages of saffron root and scale at various stages of growth when infused with a mixture of species of genus *Glomus* under pot culture.

Days/ Colonisation	Root colonisation		Scale colonisation	
	-AMF	+AMF	-AMF	+AMF
30	0.00 ±0.00^{e}	14.84 ±1.24^{d}	0.00 ±0.00^{d}	0.00 ±0.00^{d}
60	0.00 ±.00^{e}	32.68 ±1.86^{c}	0.00 ±0.00^{d}	12.32 ±0.62^{c}
90	0.00 ±0.00^{e}	68.28 ±3..16^{b}	0.00 ±0.00^{d}	34.32 ±2.14^{b}
120	0.00 ±0.00^{e}	88.96 ±2.36^{a}	0.00 ±0.00^{d}	58.86 ±3.21^{a}

Different superscripted letters over standard error denotes significance in difference (factorial ANNOVA followed by Duncan Multiple Range test P<0.05).

AMF treated saffron plants don't show significant increases in plant height till 90 days growth however, at 120 days increase is significant in inoculated plants than control. Plant width follows the same trend as that of plant height *viz*. insignificant till 90 days and then significant increased till 120 days. Number of leaves per plant showed slightly insignificant increase in AMF inoculated plants than control till 90 days and then showed significant increase in number after 120 days of plant growth (Table 2). Leaf length follows the same trend as that of plant root length both show slightly significant increase even at 30 days plant growth which gradually becomes significant with passage of time in AMF presence. In inoculated saffron plants corm initiation occurs at 60 days after bud and leaf emergence, however, in control corm initiation is delayed till 90 days of plant growth. There is an increase in the cormlet number in AMF inoculated plants when compared with those without AMF. Cormlet size however, significantly increases in AMF inoculated plants than control till 120 days of plant growth (Table 2). In saffron plants AMF seems to cause insignificant increase in fresh weight till 90 days of plant growth, which however, is significant at and after 120 days. There is a significant increase in fresh weight in inoculated plants than control. Dry weight of shoot is however, influenced by AMF inoculation by showing slight but significant increase at 60 days plant growth and later becomes more significant at 120 days. Though root fresh and dry weight increaseed in AMF inoculated plants than control with the age of plant, the increase seems not significant at any stage of plant growth. Once the corm initiation occurs there is a significant difference in both fresh and dry weights of old and newly initiated corm at all stages of growth. The total plant fresh weight does not show any significant increase at 30 days plant growth. The total dry weight of plant showed slight significant increase even at 30 days plant growth and then increased significantly with the age of plant in AMF inoculated plants (Table 3).

After the emergence of leaves the chlorophyll content shows no change or negligible increase at 30 days plant growth. However from 60 days onwards there is a continuous substantial increase in chlorophylls a, b and total contents in inoculated than non-inoculated plants, also that AMF presence shows more contents at all stages further on (Fig.2). The chlorophyll b content at all stages is much lower than the chl. a fraction. The analysis shows that these increases due to AMF may be statistically insignificant (Fig. 2).

The saffron plant (*Crocus sativus* L.) belongs to the Iris family (Iridaceae). Plant is propagated vegetatively by an underground modification- a 'Corm'. It produces mauve coloured flowers which emerge out of the soil within bud sheaths.

DISCUSSION

The present study has been undertaken with a sole view that work on the saffron plants growth and development *vis a vis* mycorrhizal relationship is at the most scattered. As far as AMF symbiosis is concerned, the metabolite mobilization and metabolism in crops and other plants has been a subject of interest for many workers (Van der Heijden *et al*., 2006; Smith and Smith, 2011; Smith

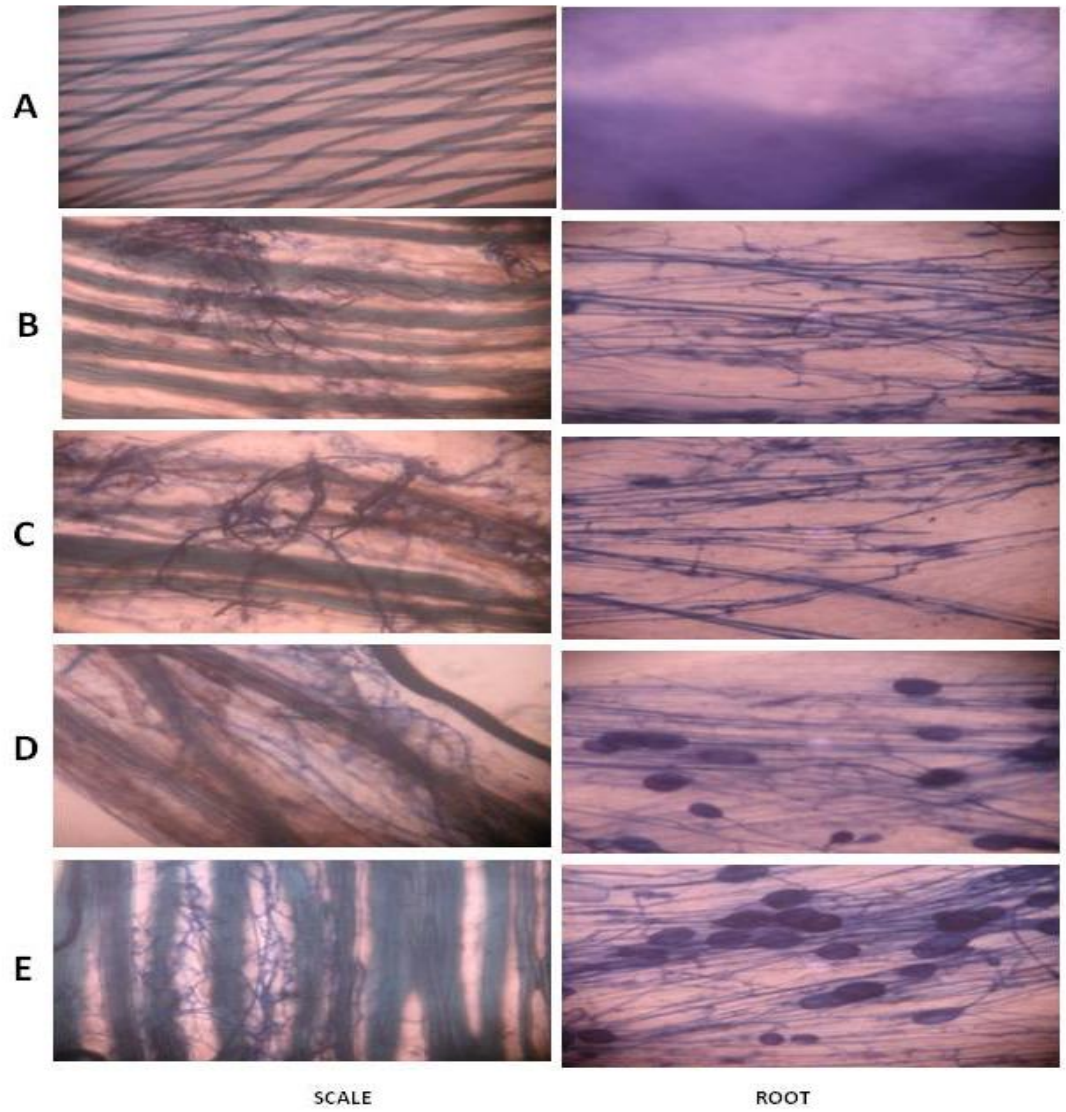

Fig. 1. Scale and root colonisation in saffron plant under pot culture conditions at 30, 60, 90 and 120 days growth after seedling emergence. A, represents control and B, C, D & E represents at 30, 60, 90 and 120 days colonisation respectively.

and Smith, 2012; Lehmann *et al*., 2014; Rillig *et al*., 2014). Though there is sizable amount of referral material available on various such studies yet there seems to be less available on the modified or storage underground stem systems. Moreover, it is felt that the overall work available pertains predominantly to the shoot or above ground parts of commercial importance dependent on shoot *vis a vis* their relationship with the mycorrhizal presence or absence in their underground root part. Therefore, there seems a meager attempt made to study the growth and development changes of the underground stem itself, more so, when a modified stem system becomes mycorrhizic. Although, AMF increased the number of corms produced significantly, it increased shoot fresh weight and root dry weight. Furthermore, inoculation with AMF produced higher number of corms and root fresh and dry weights than those not inoculated with AMF. These results suggest a kind of compatibility between plant and AM fungi. Such compatibility between AMF fungi and host plant was previously observed in other plants also such as onion (Shuab *et al*., 2014: Lone *et al*., 2015a), Potato (Lone *et al*., 2015b) and soybean and maize cultivars. AMF colonisation in scales of corms was observed. This has been earlier reported that underground Saffron corm sacle show AMF colonisation (Lone 2014). Taber and Trape (1982) reported for the first time the presence of AMF in the vascular system of rhizomatous tissue and scale like leaves of ginger (*Zingiber officinale*) and later was reported in the tubers of *Colocasia esculanta* by Bhat and Kaveriappa (1997), the tubers of *Gloriosa superba* by Khade and Rodrigues (2003). The inoculation of AMF significantly initiated and

Table 2: Various growth and developmental parameters of saffron plants as affected by the presence of AMF

Days/ Parameter	Height (cm)		Width (cm)		No of leaves		Leaf length (cm)		Root length (cm)		No of corms		New corm size (cm)	
	-AMF	+AMF	-AMF	+AMF	-AMF	+AMF	-AMF	+AMF	-AMF	+AMF	-AMF	+AMF	-AMF	+AMF
30	5.24 ±0.22^{e}	5.25 ±0.23^{e}	0.78 ±0.02^{e}	0.78 ±0.4^{e}	4.96 ±0.21^{f}	5.02 ±0.26^{ef}	5.02 ±0.14^{e}	5.02 ±0.23^{e}	1.86 ±0.62^{g}	2.00 ±0.12^{fg}	0.00 ±0.00^{e}	0.00 ±0.00^{e}	0.00 ±0.00^{f}	0.00 ±0.00f
60	8.28 ±0.43^{d}	9.06 ±0.14^{d}	1.72 ±0.12^{d}	1.76 ±0.12^{d}	5.37 ±0.32^{de}	5.81 ±0.32^{d}	7.24 ±0.16^{d}	7.42 ±0.42^{d}	2.72 ±0.12^{f}	3.69 ±0.22^{e}	0.00 ±0.00^{e}	1.14 ±0.11^{d}	0.00 ±0.00^{f}	3.14 ±0.14^{e}
90	13.76 ±0.62^{c}	14.26 ±0.62^{c}	2.14 ±0.13^{c}	2.36 ±0.12^{c}	7.22 ±0.32^{c}	7.86 ±0.42^{c}	9.86 ±0.42^{c}	11.64 ±0.62^{c}	5.07 ±0.16^{d}	8.92 ±0.32^{b}	1.98 ±0.10^{c}	2.68 ±0.08^{b}	4.86 ±0.24^{d}	5.98 ±0.16^{c}
120	21.86 ±1.20^{b}	23.67 ±1.13^{a}	2.58 ±0.16^{b}	2.86 ±0.15^{a}	8.86 ±0.26^{b}	9.76 ±0.53^{a}	16.92 ±0.86^{b}	18.92 ±1.32^{a}	7.64 ±0.26^{c}	10.47 ±0.52^{a}	2.86 ±0.11^{b}	3.78 ±0.14^{a}	6.76 ±0.22^{b}	8.52 ±0.42^{a}
LSD Value	1.615		0.1731		0.7199		1.979		0.6945		0.5501		0.5609	

Different superscripted letters over standard error denotes significance in difference (factorial ANNOVA followed by Duncan Multiple Range test P<0.05)

Table 3: Fresh and dry matter content with and without AMF in the plant and corms of saffron plant

Days/ Parameter gm/plant	Shoot fresh weight		Shoot dry weight		Root fresh weight		Root dry weight		Corm fresh weight		Corm dry weight		Total fresh weight		Total dry weight		Total plant fresh weight/ Total plant dry weight	
	-AMF	+AMF	-AMF	+AMF	-AMF	+AMF	-AMF	+AMF	-AMF	+AMF	-AMF	+AMF	-AMF	+AMF	-AMF	+AMF	-AMF	+AMF
30	3.74 ±0.12^{d}	3.83 ±0.14^{d}	0.41 ±0.02^{e}	0.39 ±0.02^{e}	2.02 ±0.14^{d}	2.08 ±0.10^{d}	0.22 ±0.01^{d}	0.24 ±0.01^{d}	0.00 ±0.00^{f}	0.00 ±0.00^{f}	0.00 ±0.00^{d}	0.00 ±0.00^{d}	5.76 ±0.32^{g}	5.91 ±0.48^{g}	0.63 ±0.04^{fg}	0.63 ±0.02^{g}	10.93 ±0.64	10.65 ±0.84
60	4.16 ±0.14^{d}	4.52 ±0.32^{d}	0.56 ±0.04^{de}	0.64 ±0.03^{d}	2.86 ±0.12^{c}	2.92 ±0.12^{c}	0.36 ±0.02^{c}	0.39 ±0.02^{c}	0.00 ±0.00^{f}	3.16 ±0.14^{e}	0.00 ±0.00^{d}	0.46 ±0.02^{c}	7.02 ±0.56^{f}	9.60 ±0.63^{e}	0.92 ±0.06^{f}	1.49 ±0.12^{e}	13.10 ±1.04	15.52 ±1.22
90	5.98 ±0.23^{c}	6.54 ±0.24^{c}	0.88 ±0.02^{c}	1.02 ±0.04c	3.94 ±0.16^{b}	4.13 ±0.20^{b}	0.58 ±0.03^{b}	0.66 ±0.04^{b}	5.39 ±0.26^{d}	6.14 ±0.20^{c}	0.89 ±0.03^{b}	1.06 ±0.04^{b}	15.31 ±1.06^{d}	16.81 ±1.36^{c}	2.35 ±0.24^{d}	2.74 ±0.14^{c}	15.34 ±0.98	16.29 ±1.08
120	8.38 ±0.26^{b}	9.32 ±0.62^{a}	1.34 ±0.10^{b}	1.68 ±0.12a	5.64 ±0.32^{a}	5.98 ±0.32^{a}	0.88 ±0.04^{a}	1.06 ±0.10^{a}	7.26 ±0.42^{b}	8.62 ±0.36^{a}	1.53 ±0.04^{b}	1.99 ±0.10^{a}	21.28 ±0.18^{b}	23.92 ±1.64^{a}	3.75 ±0.36^{b}	4.67 ±0.24^{a}	17.62 ±1.32	19.52 ± 1.28
LSD Value	0.8319		0.1148		0.4447		0.3009		0.3238		0.1448		0.6815		0.2448		0.632	

Different superscripted letters over standard error denotes significance in difference (factorial ANNOVA followed by Duncan Multiple Range test P<0.05)

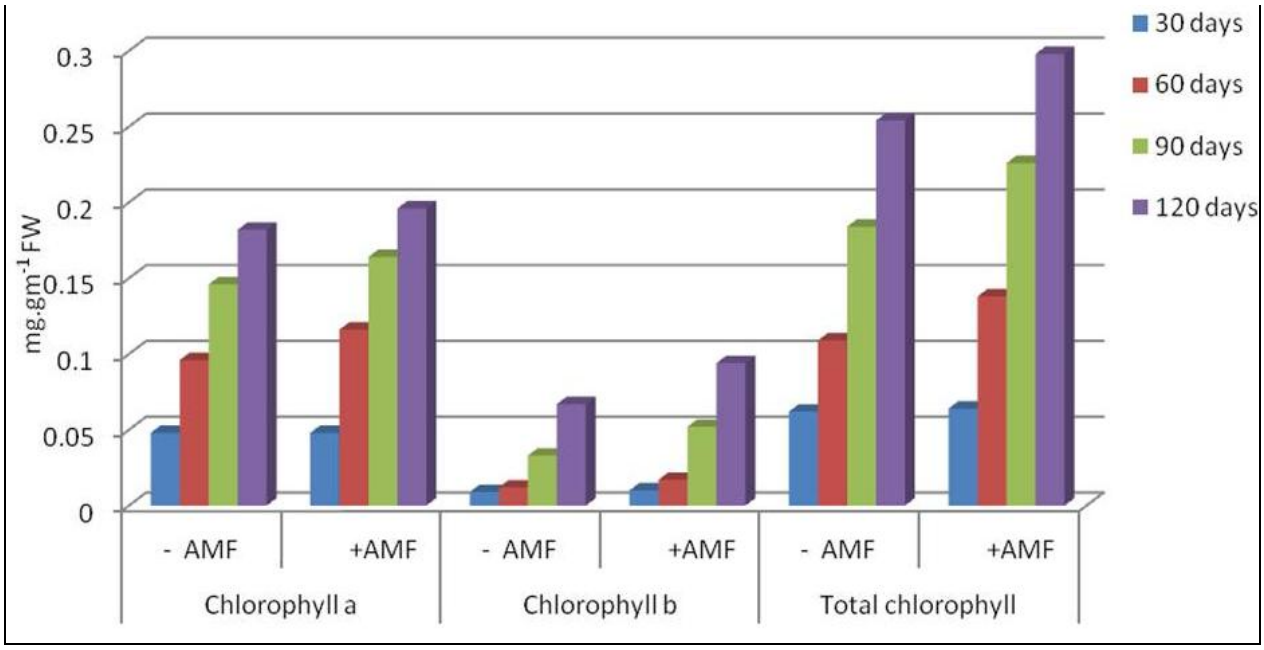

Fig. 2. Changes in chlorophyll contents in the saffron plant at 30, 60, 90 and 120 days growth with AMF and without AMF.

stimulated production of new corms. Such promoting effect of AMF on initiating the new underground stem propoagules was observed previously too (Graham *et al*., 1976; Niemira *et al*., 1995, Shuab *et al.,* 2014, Lone *et al.,* 2015b). Considering that tuber initiation in potato is hormonally mediated (Ewing, 1995), it may be that in present study too AMF affected hormone balance in saffron plant, leading to earlier initiation and production of corms. It will be genuine to consider that the involvement of other mechanisms, such as morpho-physiological or biochemical too may play their roles. Beneficial effects of AMF inoculation on saffron plant growth yield which was observed in this study as high level of root colonisation was observed. Mycorrhizal plants have been reported to have significantly higher root length, projected area, surface area and volume than non mycorrhizal plants (Wu *et al*., 2010, Shuab et al., 2014, Lone et al., 2015b). The observations here are in complete agreement with these reports. In the present study also the saffron plants inoculated with a mixture of the species of *G. intraradices* and *G. mosseae* an AMF, showed elevated growth characteristics of fresh and dry matter and also the saffron root length and root diameter in comparison to those not infected with AMF. Al-Karaki and Clark (1999) indicated that shoot dry matter and root dry matter were higher for mycorrhizal infected wheat plants than non-infected plants which were ascribed to an already established phenomenon of higher Phosphorus (P) uptake by AMF infected roots for the plants.

The AMF presence in the underground broad leaf scales during early development of the new corm. Since new corm development is the result of photosynthates produced by above ground fibrous leaves as source, being translocated to the below ground developing corms as sinks through these leaf sheaths, there seemingly is some relation of the presence of AMF in the sheaths and the new corm development.

Saffron is prone to be photosynthetically an inefficient plant, reason being its live active vegetative growth during extreme climatic conditions when fields are snow/ice covered and under extremes of low temperatures. The results in the present study show a higher total chlorophyll content in the presence of AMF than those when AMF is absent in roots. It is therefore, pertinent to infer reasons for significantly increased mobilization of starch in the corm, thereby these corms having a higher fresh and dry matter content. The controlled field trial for such an effectivity of AMF of saffron should constitute an interesting study.

On concluding it is therefore, worthwhile to remark that AMF and other microorganisms in the rhizosphere can have an additive effect in majority of growth parameters as far as development of saffron plant is considered.

CONCLUSION

The present study pertains that AMF colonization improved positively the overall growth and development of saffron plant. Chlorophyll content

too was found higher in AMF inoculated than control.

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