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Benefits of Inoculation of Arbuscular Mycorrhizal Fungi on Growth and Development of Onion (*Allium cepa*) Plant

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Abstract: Mycorrhizae are fungal symbionts forming mutualistic relationship with plant roots. In the present study surface sterilized seeds of onion were sown in earthen pots filled with sterile soil. Half the pots were inoculated with 30 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. Inoculation was done twice 3 days before sowing the seeds and on the onset of germination. Potted plants were regularly watered. After germination, the inoculated plants along with their controls were sampled at 20, 40, 60 and 80 days of growth. The observed data seems to predict that there is a net increase in the above and below ground growth of the plant with each 20 days interval after germination. The present study seems interesting since it pertains the work on modified stem *vis a vis* mycorrhizal relationship of a modified stem than normal root. The Chlorophyll content besides morphological growth parameters and fresh and dry weight content of onion plant are shown to present in higher levels in the mycorrhiza infected as compared to the non-inoculated ones.

Key words: Growth · Onion (*Allium cepa*) · Plant

INTRODUCTION

Mycorrhizae or *mycorrhiza,* a symbiotic association between a fungus and the roots of a plant [1]. 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 [2]. AM fungi and plant roots, improve water and nutrient uptake like phosphorus, nitrogen and micronutrients and thus enhance plant growth [3]. 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 Trappe [4] 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 Nazim [5] 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. [6], corms of *Amorphophallus commutatus* Engler [7] and tubers of *Pueraria tuberosa* (Willd.) DC [8]. Arbuscular mycorrhizal fungi have been documented in tubers of *Colocasia esculenta* (L.) Schott [9], garlic bulbs [10], tubers of *Gloriosa superba* L. [11] and corms of saffron [12]. 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 onion 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 onion plant.

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MATERIALS AND METHODS

Seeds of *Allium cepa* (Onion Nasik red N-53) were procured from agricultural college, Gwalior. These were soaked in distilled water for one hour and then treated with 0.01% cetrimide solution for 3-5 minutes. These were then washed with distilled water 3-4 times. Soil (3:1 ratio of soil: sand) was autoclaved twice at 15 lbs pressure and 120°C temperature for 30 minutes. Half the pots were inoculated with AMF spores of the species of genus *Glomus* and also 10 grams of maize root inoculated with the species of genus *Glomus*. Half the pots represented controls which had no AMF inoculation. Pot inoculations were done twice, three days before sowing of seeds and on the onset of germination with 10 grams of root fragments of monosporal colonized roots and with 30 spores of *Glomus* species. All the potted plants were watered regularly.. The inoculated plants and controls were sampled after 20, 40, 60 and 80 days after germination. The roots were stained with 0.05% tryphan blue stain using the method suggested by Phillip and Hayman [13]. Root colonization estimation was carried out using Biermann and Lindermann [14] method. Percent root colonization was calculated using following relationship:

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

Morphological parameters including onion plant height, leaf number, neck diameter, bulb diameter, bulb circumference and root length were recorded for every 20 days sampling. Bulbs were separated from leaves and roots. Plant height was measured as the top most height of the main leaf. The leaf number was recorded as the mean value of all the leaves of each plant of all pots sampled divided by the number of total plants. Neck diameter of the plant was measured 3 cm above from the surface of the soil. After uprooting the plant, its bulb diameter and bulb circumfences were recorded at every 20 days interval after germination using a thread. The plant roots were thoroughly washed under tap water and root length presented as the mean value of the longest root/s of each plant from bulb base. Fresh and dry weight determination for onion plants was done after uprooting the plants and separated into shoot, root and bulb. After weighing fresh shoots and roots were dried in an oven at temperature 80±2°C for 48 hours, whereas the bulbs were dried for 72 hours at 80±2°C and dry weights noted.

Estimation of Chlorophyll Content: For chlorophyll a, chlorophyll b and total chlorophyll the method of Arnon [15] and Withman *et al*. [16] was employed. Calculation of the amount of chlorophyll present in the extract as mg chlorophyll per gm 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

RESULTS

The onion plant (*Allium cepa*) is an important vegetable crop of the world. The “onion” of the plant constitutes the underground bulb which is an underground modification of a stem with condensed shoot being covered by fleshy scales compactly. The roots originate superficially from the stem and not from the scales. Seed propagation was tried in the present study. Seeds seem to belong to the uniform stock, since all the pots sown with the seeds showed nearly 90-95 per cent uniform emergence, germination and subsequent seedling growth. The pot culture shows that proper conditions for the growth and overall development are met to delineate effects both with and without the spores of arbuscular mycorrhizal fungi (AMF). After seed germination, at every 20 days interval mycorrhizal colonisation was observed in inoculated seedlings with the presence of arbuscules, hyphae and/or vesicles in the inoculated roots which were absent from the uninoculated rhiza representing controls. Colonization is also absent in the scales of underground modified bulb both in inoculated and uninoculated onion plants. The inoculated plants show substantial increase in root colonisation after every 20 days interval. At 20 days after seedling emergence 23.14 percent colonisation was found

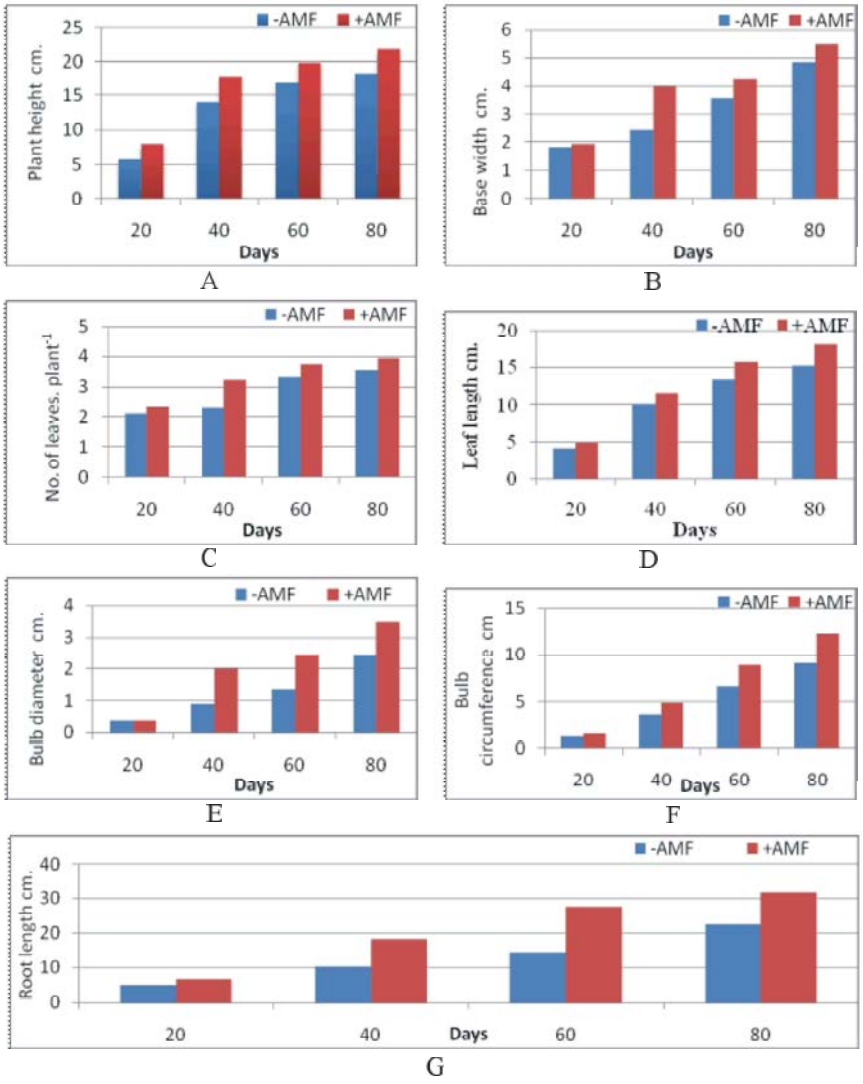

Fig. 1 (A-G): Various growth and developmental parameters of onion plant as affected by the presence of AMF

in inoculated plants which then gradually increased with age of the plant with 32.74 at 40days, 54.16 at 60 days and thus reaching maximum percentage of 75.26 percent colonisation at 80 days growth. The absence of AMF structures in roots of uninoculated plants at all stages implied that the conditions of soil sterility were adequately met to present the specific comparative responses of plants to AMF.

AMF treated onion plants don't show any significant visible morphological increase in plant growth at early days of growth except for the root, the length of which shows significant increase in AMF inoculated plants than control. With increasing days of age plant height and leaf length showed small but significant increase in the presence of AMF. All other parameters however, showed significant increment due to AMF. At 60 days all growth parameters except plant neck shows significant increase in AMF inoculated plants than control and then increase significantly with age of plant at 80 days in AMF inoculated plants than those without AMF plants. Plant height followed by leaf length, root length is affected similarly more with AMF presence. Other growth parameters are also higher in values with AMF inoculation than their respective non-treated ones (Fig. 1 A-G).

In onion plant AMF seems to cause non-significant increase in fresh and dry weight at 20 days of plant growth except shoot dry weight which shows slight increase in AMF inoculated plants than control. Shoot fresh weight, bulb dry weight, total fresh weight and total

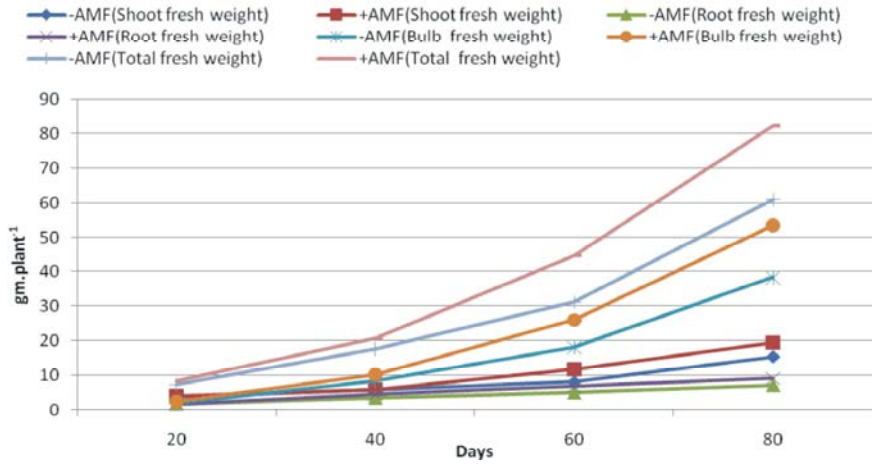

Fig. 2: Fresh matter content of various parts of onion plant with and without AMF at various stages of growth

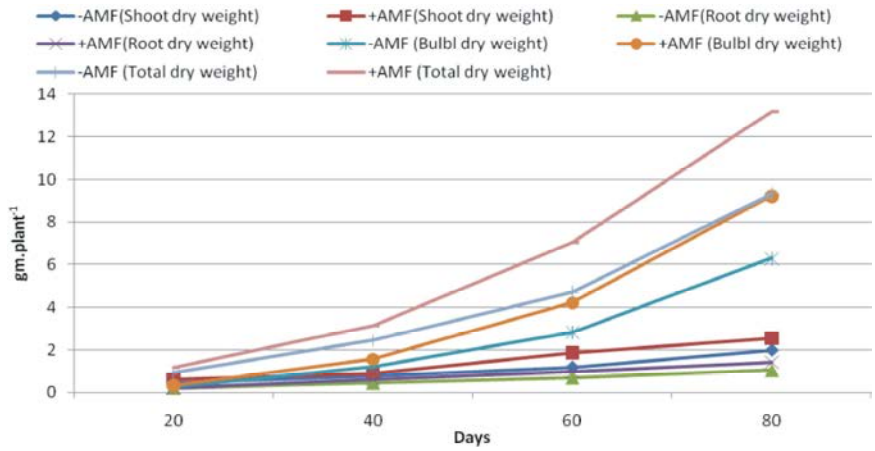

Fig. 3: Dry matter content in the various parts onion plant with and without the presence of AMF at various growth stages

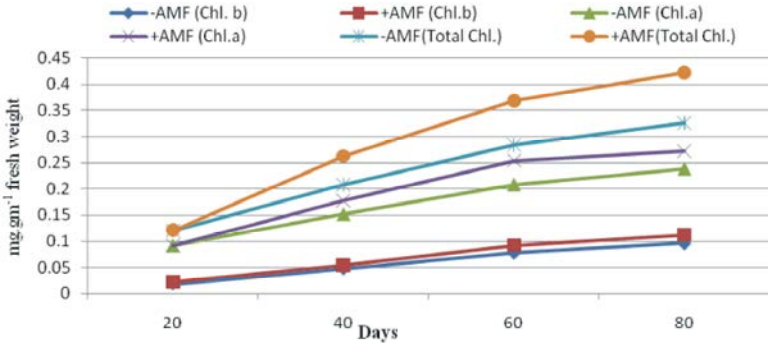

Fig. 4: Changes in chlorophyll a and b and total chlorophyll contents in the onion plant at various growth stages with and without AMF

dry weight increased in AMF inoculated plants than control, however, the increase was not significant. All other fresh and dry weight parameters show significant increase in AMF inoculated plants than control even at 40 days plant growth, while after 60 days plant growth all fresh and dry weight parameters taper with age of the plant and show significant increase in AMF inoculated plants than control at 80 days plant growth. Fresh weight of bulb only or that followed with by shoot fresh weight show an overall significant increase in AMF inoculated plants than in comparison with their respective controls (Fig. 2 and 3).

After the emergence of seedlings the chlorophyll content shows no change or negligible increase at 20 days plant growth. However with subsequent 20 days interval there is a slight increase in the chl a, chl b and total chlorophyll content in AMF inoculated plants than non inoculated ones which continues till 80 days (Fig. 4).

DISCUSSION

The onion plant (*Allium cepa*), is an important vegetable crop of the world. The "onion" of the plant constitutes the underground bulb which is an underground stem modification. Shoot being condensed is covered by fleshy scales compactly. The roots originate superficially from the stem (bulb) and not from the scales. The onion has an insufficient, mostly unbranched, shallow root system with very negligible or even without root hairs. These therefore, cannot maintain adequate uptake of nutrients such as phosphorous which is known to diffuse slowly through the soil solution. This therefore, has a negative effect on the onion yield [17].

Galvan *et al.* [18] reported that *Glomus mosseae*, *G. Coronatum*, *G. caledonium* and *G. geosporum* species complexes were the most abundant AMF for the onion plant. Such plants are often obligate mycorrhizal crops that are unable to complete their life cycle in the absence of AMF because of insufficient P uptake and hence insufficient growth [19; 20]. Mycorrhiza helps plants with such as shallow sparse root system to increase phosphorous uptake. Onions are highly mycorrhizal dependent [21]. The compatibility of the mycorrhizal fungus with onion plants may be of particular importance because they often form unbranched sparse root system [17].

Various techniques are available for harnessing the generation of AMF inoculums under sterile conditions. Those commonly used are through nutrient film technique, aeroponic culture system and root organ culture [22, 23]. However, Chellapan *et al.* [24] recommended a traditional pot culture technique, employing trap plants for large scale production of AMF inoculums, for example multiplication in Cassava peel and tuber [25, 26] and sugar-agar globule with root exudates [27]. However, for experimental purposes, to trace the various parameters as affected by AMF the common pot culture as suggested by Chellapan *et al.* [24] seems appropriate. Previously also AMF mediated growth promoting effects have been shown in pot experiments [28, 29]. In the present investigation too pot culture was standardized to amply substantiating the methodology.

The observed high mycorrhizal responsiveness to mixed inoculums has been reported previously too [30, 31] and is thus often used in commercial agricum plant products. Galvan [18] and Koul *et al*. [32] reported that species of *Glomus* have the highest colonisation potential in *Alliums.,* therefore, the spores of species of *Glomus* were found desirable for the pot experiments here and these as observed proved comparable to others species as far as effective results are concerned. This gives credence to our basic experimentation after standardization.

After seed germination, at every 20 days interval mycorrhizal colonisation was observed in all inoculated treatments. This was shown by the presence of arbuscules, hyphe and vesicles or all these morphoforms in the inoculated roots. The structures were absent in the roots of the plants which were not inoculated and served as controls. Taxa in *Allium* are known to form typical *Arum*-type morphologies [33]. It became evident that AMF encroached only upon the roots of onion did not show any presence in the bulb or scales tissue despite the too being underground and in the direct contact of the soil inoculum. Therefore, AMF presence showed in various morphological structures *viz*. hyphae, vesicles and even arbuscules in the root tissue only. It is well established that during mycorrhiza formation, the AMF undergoes several developmental stages [34]. In a symbiotic stage, spores germinate and AMF show limited hyphal development in the absence of developed rhizal system both morphologically and metabolically. Once the roots mature and produce root exudates with advanced plant growth they switched to presymbiotic growth stage showing extensive hyphal branching. This has vastly being attributed to the exudates having signal molecules as attractants for the AMF symbionts. Subsequently fungus contacts the matured root surface followed by hyphal penetration of the root epidermis and colonisation of the root cortex tissue. This model of Smith and Read [35] may be very close in explaining the root colonisation pattern with age in the present case. Absence of AMF structures in those plant roots where inoculi were not given implies that the conditions of soil sterility were adequately met. This was therefore, appropriate to present specific response of plants to AMF from the pots which were inoculated with the spores of AMF. Soil sterilization decreases non-mycorrhizal onion growth contrary to mycorrhizal ones. Stunted growth of control plants grown in sterilized soils without fungal inoculation has been reported by Charon *et al*. [19] and Sasa *et al*. [36] as was seen in the present study too. Possibly the ecological

equilibrium between microflora and the plant could not be reestablished within the time spare of the experiment. Smith and Smith [37] have earlier mentioned that despite the addition of a microbial filtrate from a natural soil to a sterilized soil, the plethora of microbial flora in broccoli roots was failed to reestablish even in a 28- 40 days period.

The absence of any AMF structure in the bulb and scale tissue, therefore, suggests that in the onion plant too only root provides a congenial symbiotic space to AMF. The bulb and the scale seem not to present a requisite condition both morphologically or physiologically for AMF to adapt despite their equal proximity to the inoculums as the roots. There are however very few reports of AMF presence in the tissue other than the roots. Taber and Trappe [4] reported for the first time the presence of AMF in the vascular system of rhizomatous tissue and scale like leaves of ginger (*Zingiber officinale*), in the tubers of *Colocasia esculanta* by Bhat and Kavrieappa [9], the tubers of *Gloriosa superba* by Khade and Rodrigues [11] and corms of saffron [12]. The most interesting observation is the one reported by Kunwar *et al.,* [10] who have documented the presence of AMF in the garlic bulbs, the plant being a close familial relative of onion. This observation therefore running contrary to the observations in the present study.

The inoculated plants show substantial increase in root colonisation after every 20 days interval. Priyardashini *et al*. [38] reported that shallot roots in conventional agricultural fields had AMF colonisation levels within the range of those reported in other studies for other plants [3; 39]. However, the average AMF colonisation (49.12 percent) here is lower than those reported from onions under conventional cultivation in Fevoland (91 percent) and Zeeland (72 percent) in Netherlands [18].

Bolandnazar *et al*. [40] reported AMF improved onion growth and development in comparison with non- mycorrhizal ones. This improvement resulted from increasing leaf area, plant height and leaf chlorophyll content which led to greater leaf area and probably photosynthetic capacity both leading to greater fresh and dry mass and bulb size. The results here on growth and development parameters are consistent with these findings. Guo *et al*. [41] reported mycorrhizal inoculation resulted in enhanced shoot yield in spring onion plants which is true in the present case too. Increased plant size and yield by mycorrhiza presence have further been reported by Charon *et al*. [19]. Bolandnazar *et al.* [40] reported that mycorrhizal onions had greater bulbing ratio than control plants. Charon *et al.* [19] have further shown that mycorrhizal onions can reach to a marketable size 2 to 3 weeks earlier in comparison to non – mycorrhizal ones.

It is now vastly reported that the mycorrhizal colonisation improves plant growth by facilitating mineral nutrition and progressive water relations which lead to large plant size and higher yield [42]. Bolandnazar [43] reported that mycorrhizal colonisation improved onion seedling survival and establishment and increases its growth and development leading to higher bulb size and bulb yield. Recently, Shinde *et al.,* [44] also reported that leaf length and plant height is significantly higher in AMF inoculated plants than control.

Albrechtora *et al,* [45] reported that onion plant height and bulb size is significantly increased in AMF inoculated plants than non inoculated ones. Bolandnazar and Hakiminia [46] reported that onion plants inoculated with AMF produced the highest bulb yield than non-AMF inoculated. Depending on the individual AMF and soil conditions many plant species do show large positive growth responses to AM colonisation [47]. Onion plant is highly responsive to several AM Fungi which tend to associate with onion roots leading to improved plant growth and nutrient uptake [48]. These workers too have shown that AMF can significantly increase bulb diameter, bulb yield, shoot dry weights and the shoot phosphorous content. These observations are in agreement to the observations presented here.

AMF fungi increased the onion bulb size significantly, but it was also accompanied with an increased shoot fresh weight and root dry weight. The results therefore, suggest a kind of compatibility between onion plants and AM fungi through this system. Such compatibility between AMF and host plant was previously observed in onion by Yao, [49] and cultivars of maize by Khalil *et al.* [50]. Earlier studies, indicated that the onion is highly responsive to mycorrhization resulting in improved plant growth and yield under normal as well as stressed conditions [3, 38, 39, 40]. Species of *Allium* and *Allium* cepa in particular are regarded as highly AMF responsive plants [51]. Shinde *et al*. [42] reported that fresh and dry biomass is more in AMF inoculated plants than non-inoculated plants and corroborate to Hayman and Mosse [52] observation which recorded an eighteen fold increases in the weight of mycorrhizal plants compared to non mycorrhizal ones. AMF colonisation enhances chlorophyll content than non mycorrhizal ones [53].

CONCLUSION

The present study pertains that AMF colonization improved positively the overall growth and development of onion plant. Chlorophyll content too was found higher in AMF inoculated than control. This study shows that AMF can increase the production of onion.

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