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Effects of fluoride toxicity on *Glomus* fungi spore count and their root colonisation in *Ocimum basilicum*

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Abstract

The aim of present study was to assess the effects of different *Glomus* fungi on the growth of *Ocimum basilicum* (Medicinal and Aromatic plant) grown under salinity stress. The fluoride concentration was kept similar for all treatments while different arbuscular mycorrhiza fungi were used. Most fluoride tolerant variety of fungi found was *Glomus mossae*. *G. mossae* showed best stress tolerant results followed by *Glomus fasciculatum, Glomus intraradices* and *Glomus aggregatum.* 50ppm of NaF was used as the treatment given to plants. After 3 months, it was found that the plants in salinity stressed soils when treated with *Glomus mossae* fungi resulted in 15% decrease in spore count and 19% decrease in root colonisation in plants. In sodium fluoride treated plants, *Glomus mossae* showed better stress coping effect in comparison to other *Glomus* fungi resulting better plant productivity even in stressed conditions.

Keywords: medicinal and aromatic plants (MAPs), sodium fluoride (NaF), vesicular arbuscular mycorrhiza (VAM), *Ocimum basilicum*

Introduction

Ocimum basilicum (Sweet basil) is a medicinal and aromatic plant (MAP), belonging to family *Lamiacae* (Grayer *et al.* 1996) [5]. It is an annual herb native to India and other parts of Asia (Klimankova *et al*. 2008) [9]. The use of this plant parts (root, stem and leaves has been maintained traditionally (Leonti *et al*. 2003) [3]. Sweet basil is an economically important plant whose essential oils are stored in trichomes glandular hairs (Sangwan *et al*. 2001) [13]. Plant tolerance can be enhanced by AMF and their mutual relationship with plants help in their growth even in stressed conditions through improved physiological and nutritional stress (Hashem *et al*. 2015, 2016) [6].

Fluorine is the most electronegative atom, and therefore has the ability to make strong hydrogen bonds. It has inhibitory effect on plants and can cause an abnormal change in biochemical and physiological parameters in plants. Higher Na^+ shows antagonistic behavior towards essential elements like potassium at membrane level. AMF inoculation enhances the nutrient uptake and their accumulation and decrease in uptake of Na^+. Growth enhancing by AMF is by means of its positive influence on synthesis of chlorophyll pigment.

Materials and Method

Experimental site

The experimental site, Lucknow (Uttar Pradesh), with a warm humid subtropical climate is situated in the north-eastern part of Uttar Pradesh, India. Latitude: 26°50′21″ N, Longitude: 80°55′23″ E and Elevation above sea level: 126 m = 413 ft. The average annual rainfall of this area is 313 mm, which is evenly distributed from June to October and August is the wettest month of the year. The average temperature ranges from 26°C to 39°C and actual temperature ranges from 29°C to 47°C. The relative humidity fluctuate between 34 % and 92%.

Experimental Set up

O. basilicum seeds were surface sterilized with 10% NaOCl for 5 minutes, rinsed with distilled water for 5 times and sown in the polyethylene bags filled with sterile soil. 15 days old seedlings were then transferred to the earthen pots containing 1kg soil by using *5* g of inoculum each of different AM fungi per seedling respectively placed at *5* cm depth in pots. The inoculum of different *Glomus* fungi was obtained from CSIR-Central institute of medicinal and aromatic plants, Lucknow (U.P). The experimental set up was in completely randomized block design with three replicates of each, i.e. control and treatments in a glass house.

Arbuscular mycorrhizal (AM) inoculation

The seedlings were inoculated with G. *mossae*, G. *aggregatum*, G. *fasciculatum* and G. *intraradices* in nursery by using *5* g of inoculum per seedling placed at *5* cm depth in the pot. After inoculation, a thin layer of soil was added, and the seedlings were planted and covered with the soil. Mycorrhizal inoculated rooted seedlings of tulsi plants were transferred from polyethylene bags to the earthen pots and grown for three months (until they attained pencil-size shoot girth), and in the mean time they were weekly subjected to salinity stress (Fluoride stress).

Spore count and %root colonisation

The spores produced by G. *mossae,* G. *fasciculatum* G. *aggregatum* and G. *intraradices* were counted by following the wet sieving and decanting method (Gerdemann & Nicolson, 1963) [4]. Fifty grams of soil sample was drawn from each pot. It was suspended in a sufficient quantity of water and stirred thoroughly. After the soil was allowed to settle for one minute, each sample was decanted onto the sieves. The suspension was passed through a set of sieves with different mesh size. The spores collected on the sieve were transferred to watch glasses. The spores were counted out using a stereomicroscope (l00x) and were expressed as number of spores per g of soil. Arbuscular Mycorrhizal fungi infection was assessed from randomly selected root material after cutting secondary and tertiary root samples into 1-2 cm pieces. AMF colonization was evaluated from a random sub-sample of approximately 150 root segments per plant. Roots were cleared in 2.5 % KOH (90 °C) for 45 min, acidified with 1 % HCl for 15 min, stained with 0.05 % Trypan Blue in acid glycerol (90 °C) for 45 min, and then stored in acid glycerol (Koske and Gemma, 1989). Randomly selected root fragments were mounted in glycerin on slides without squashing the root pieces. Percentage colonization of roots by arbuscules, vesicles and / or hyphae was recorded at 250x magnification using the magnified intersections' method of McGonigle *et al*. (1990)

Result

Table 1: Effects of NaF stress on Spore count of various species of *Glomus* and % Root colonisation of *Ocimum basilicum*.

S.No.	Treatments	Spore count/gm soil	% Root Colonisation
1.	Gm	4.33a	50.59a
2.	Gf	4.33a	49.04ab
3.	Gi	4.00ab	46.99bc
4.	Ga	3.33ab	44.37c
5.	Gm + F	3.66ab	41.19d
6.	Gf + F	3.66ab	38.67d
7.	Gi + F	3.33ab	34.23e
8.	Ga + F	2.66b	33.03e

*Values denoted by same letter are not significantly different at $P<0.05$ level

Where, NaF: Sodium fluoride, Gm: *Glomus mosseae,* Gf: *G. fasciculatum,* Gi: *G. intraradices,* Ga: *G. aggregatum.*

Fig 1: Arbuscules and vesicles with intraradical hyphae

Discussion

The inoculation of basil seedlings with Arbuscular Mycorrhizal fungi resulted in colonization of roots of *O.basilicum* with the spores of inoculated *Glomus* fungi as per the treatments given. AM fungi (AMF) inoculation with fluoride stress, resulted in decrease in spore count and their root colonization in the herb. Plants inoculated with *G. mossae* and *G. fasciculatum* showed 15% decrease in spore count while *G. intraradices* and *G. aggregatum* showed 16% and 20% decrease respectively. Plants inoculated with *G. mossae* resulted in 18% decrease in root colonization while *G. fasciculatum, G. intraradices* and *G. aggregatum* showed 21%, 27% and 25% decrease respectively. *Glomus mossae* was the best stress coping performer in comparison to other *Glomus* fungi resulting in better plant productivity even in stressed conditions followed by *Gf, Gi, Ga* respectively. So the results were same as obtained in earlier studies.

Conclusion

AMF colonization in the presence of NaF decreases the spore count and percent root colonization. But, *Glomus* species prove to be stress tolerant. As shoots of *O.basilicum* L. are rich in essential oils which are pharmaceutically useful and as a result of increasing interest in natural herbs and medicines, more effort is now needed to develop better quality and quantity of essential oil herbs. In this way, AM fungi can be a promissory note for better yielding of natural herbs in stress conditions in sustainable manner.

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