

## Effect of N fertilizer on formation of Vam Fungi, growth and mineral concentration of Onion

Alok Tripathi<sup>1</sup>, \*N.K. Srivastava<sup>2</sup>, Brijesh Kumar<sup>3</sup> and Shudhanshu Shekhar<sup>4</sup>

<sup>1</sup>Department of Botany,  
S.N.P.G. College,  
AZAMGARH (U.P.)

<sup>2</sup>Department of Botany\*,

<sup>3</sup>Department of Plant Pathology,  
S.D.J.P.G. College Chandeshwar,  
AZAMGARH (U.P.)

<sup>4</sup>Maa Kaushalya Group of Institutes,  
AZAMGARH (U.P.)

\*Corresponding Author

E-mail : naveensrivastava15@yahoo.com

**Received :** 18.07.2019; **Accepted :** 21.08.2019

### ABSTRACT

The effects on N fertilization on growth and root colonization of preinoculated onion (*Allium cepa*) were studied. Onion transplants, inoculated with either *Glomus intraradices*, *Glomus versiforme* or nothing at sowing, were grown under three levels of N in soils which had either been irradiated, Irradiated and amended with non-mycorrhizal microflora, or not irradiated. Interactions between inoculation and soil treatment had a significant effect on dry biomass and final bulb diameter. Control plants cultivated in non-irradiated natural soil grew normally because of the presence of indigenous arbuscular mycorrhizae, but control plants in irradiated soils were stunted. There was no such difference among inoculated plants. In non-irradiated natural soil, bulbs of onions inoculated with *G. intraradices* or *G. versiforme* were significantly firmer than bulb of control plants. Bulb firmness decreased as N fertilization level increased. In non-irradiated natural soil, tissue P concentration of onion plants preinoculated with either fungus was significantly higher than that of control plants. In all soil types, N, P, and Zn concentrations were higher in onion plants colonized by *G. versiforme* than in those colonized by *G. intraradices*. The opposite was true of Mn tissue concentration.

Figure : 00

References : 16

Table : 00

KEY WORDS : *Allium cepa*, Arbuscular mycorrhizae, *Glomus spp.* Irradiated soil, Texture measurement.

### Introduction

Arbuscular mycorrhizal (AM) fungi increase the P intake of inoculated plants and improve their nutrition<sup>10</sup>. However, the influence of N fertilization on root colonization and growth stimulation by arbuscular mycorrhizae of onion (*Allium cepa*) has received little attention.

Earlier workers<sup>1</sup> studied the effect of combined P and N fertilization on chives. In AM plants, N amendments stimulated plant growth irrespective of P levels. Both the sources of N used (NH<sub>4</sub> vs NO<sub>3</sub> ions) and the quantity available to the host plant determined the type of response.

Other workers<sup>8</sup> studied the effects of N, P and K fertilization on the formation of arbuscular mycorrhizae,

growth and mineral concentration of onion. N fertilization stimulated root colonizations and the yield increased because of P fertilization were larger when plants had not been fertilized with N (197%) than when they had been (103%).

The present study was performed to evaluate the influence of N fertilization on the growth of preinoculated onion plants in natural soils that had been irradiated, irradiated and amended with a non-mycorrhizal microflora, or non-irradiated. The non-irradiated soil treatment was designed to assess the influence of indigenous AM fungi and non-mycorrhizal microflora on plant growth.

### Materials and Methods

Soil used in this experiment was a mineral soil (sombric brunison) and sterilized the portion used in the

**ACKNOWLEDGEMENTS :** Authors are thankful to the Principals S.N. (P.G.) College and S.D.J. (P.G.) College Chandeshwar, Azamgarh (U.P.) for providing facilities and encouragements.

natural soil treatments. It was mixed with Silica no. 10 in a 3:1 oil : silica ratio, to avoid compaction.

Onion plants preinoculated with either *G. intraradices* or *G. versiforme* and non-mycorrhizal control were produced<sup>4</sup>. At transplantation 8 weeks after sowing, root colonization in seedlings inoculated with *G. intraradices* or *G. versiforme* had reached >50%. Transplants were placed into 15 cm pots containing the various combinations of N and soil treatments. Over a 24 hour period, half of the pots containing irradiated soil received 2'50 ml pot<sup>-1</sup> of a non-mycorrhizal microflora liquid suspension free of AM fungi. This microbial suspension was obtained from 151 of natural soil mixed with 151 of distilled mixture. The liquid part was then filtered over two layers of triple thickness cheese-cloth separated by a 2 cm layer of cotton. The filtrate was passed through a 38 mm sieve to eliminate all propagules of AM fungi. Samples of the filtrate were observed under a stereoscopic microscope to ascertain the absence of AM Fungus propagules.

Plants were kept in a growth chamber under a 16-n photoperiod, 24/18°C (day/night temperature, SD 1°C), 280 mmol m<sup>-2</sup>s<sup>-1</sup> light intensity, and 65% relative humidity. All onion transplants were grown individually in pots according to N fertilization, soil treatment, inoculum type, and replicate and harvested 12 weeks after transplanting. Starting at the end of the third week after transplanting, until harvest, 'cross-diameters' of all bulbs were measured every 3 weeks<sup>4</sup>.

Bulb firmness (texture, N mm<sup>-1</sup>) was measured 12 hours after harvest with an Instron Computerized Compression Testing System.

Plant tissue of each plants was analyzed for N, P, K, Ca, Mg and micronutrients<sup>4</sup>, except for control plants cultivated in either of the two irradiated soils. These plants did not provide enough tissue for chemicals analyses.

Dry biomass and final bulb diameter required a square root transformation to achieve variance homogeneity<sup>15</sup>. Means were computed on the square-root scale and back transformed for presentation in the text. Difference between these means and SEs of these differences are given on the square root scale.

The square roots of average cross-diameters measured every 3 weeks were analyzed as a separate set of repeated measures. The analysis accounts for the correlation between successive measures on the same plants<sup>5</sup>.

## Results

**Dry biomass and final bulb diameter** : Twelve weeks after transplanting, the interaction between inoculation and soil treatment had a significant effect on

dry biomass and final bulb diameter (P£0.0001). It was caused by the poor growth of control plants cultivated in irradiated soil and irradiated soil amended with a non-mycorrhizal microflora (means, 0.13 g for dry biomass, 5.48 mm for final bulb diameter as compared to their normal growth in natural soil [means, 7.95 g for dry biomass, 43.03 mm for final bulb diameter; difference (square-root scale), -2.46±0.08 mm<sup>1/2</sup> for final bulb diameter. Inoculated plants were not affected by soil irradiation, means, 7.90 g for dry biomass and 43.65 mm for final bulb diameter of inoculated plants in irradiated soils, 7.51 g for dry biomass and 42.90 mm for final bulb diameter of inoculated plants in natural soil; differences (square-root scale), 0.07±0.04 g<sup>1/2</sup> for dry biomass, 0.05±0.06 mm<sup>1/2</sup> for final bulb diameter. There was little or no difference between the dry biomass or final bulb diameter of control plants cultivated in non-irradiated natural soil and those of inoculated ones in any soil type. N fertilization had no apparent effect on dry biomass or final bulb diameter, nor was there any evidence of interactions between N fertilization and the other factors (P>0.06).

Over the 12 weeks period, the diameter of inoculated onions was almost constantly larger than that of the controls (P£0.0001 for both intercept and shape of the growth curves). It was also larger in natural soil than in irradiated soil (P£0.0001 for both intercept and shape). These effects were mainly because of the strong interaction between soil type and inoculation (P£0.0001 for both intercept and shape): control onions grown in irradiated soil were stunted throughout the experiment while onions in other inoculum-soil types combinations grew normally and at similar rates. On average over the 12 week period, bulb diameter increased with N fertilization level (P=0.0027 for the intercept of the growth curves).

**Bulb firmness** : None of the interactions between inoculum, soil treatments, and N fertilization had any effect on bulb firmness (P>0.10 N fertilization had a negative linear effect on bulb firmness (P=0.0004). In non-irradiated natural soil, onion plants inoculated with *G. intraradices* or *G. versiforme* were significantly firmer than control plants (Means, 0.328 N mm<sup>-1</sup> and 0.256 N mm<sup>-1</sup> for inoculated and control plants, respectively : difference, 0.073±0.018 N mm<sup>-1</sup>, P£0.0001). Bulbs of plants inoculated with either AM fungal Species were apparently equally firm in all soil types (P=0.2226 for the contrast between *G. intraradices* and *G. versiforme*). Soil treatments had no apparent effect on bulb firmness (P>0.14).

**Root colonization** : On average over soil treatments, root colonization of onion plants inoculated with *G. intraradices* was more abundant than that of plants

colonized by *G. versiforme* ( $P \leq 0.0001$  means, 72% and 50%, respectively; difference,  $21 \pm 2.3\%$ ). It was also higher on inoculated plants grown in irradiated soil, amended or not than on inoculated plants grown in natural soil ( $P = 0.0003$  means, 65% and 56%, respectively : difference,  $9 \pm 3.0\%$ ). The main indigenous fungi were *Glomus rubiforme* (Gerd. and Trappe) Almeida and Schenck and *G. fasciculatum* (Thaxter). Indigenous fungal species were identified by Dr. Y. Dalpe (Agriculture and Agri-Food Canada, Ottawa).

**Plant tissue mineral concentration :** Interactions involving N fertilization generally had little effect on the concentration of nutrients in plant tissue creating N fertilization when plants were inoculated with *G. versiforme*, but remained stable over N fertilization levels when they were inoculated with *G. intraradices* ( $P = 0.0036$ , the slope of the linear regression of mean Mg tissue concentration of N fertilization level was smaller than its SE when plants were inoculated with *G. intraradices*, but it was more than 3-5 times the size of its SE and positive when they were inoculated with *G. versiforme*).

Soil irradiation apparently had no effect on the P uptake of plants inoculated with *G. versiforme* but decreased that of plants inoculated with *G. intraradices* ( $P \leq 0.0001$  for the interaction between fungal species and the contrast between irradiated and natural soils. In the two irradiated soils, plants inoculated with *G. versiforme* had a higher P tissue concentration than plants inoculated with *G. intraradices*. The corresponding difference in natural soil did not seem substantial. On average over all soil types, plants inoculated with *G. versiforme* took up more P than plants inoculated with *G. intraradices* ( $P \leq 0.0001$  for the contrast between the two fungal species).

The addition of microflora to irradiated soil increased the N tissue concentration of plants inoculated with *G. intraradices* but decreased that of plants inoculated with *G. versiforme* [ $P = 0.0020$  for the interaction between fungal species and the presence of microflora in irradiated soils]. In irradiated soil amended with soil microflora, the N uptake of plants was approximately the same whether they had been inoculated with *G. versiforme* or with *G. intraradices*. In unamended irradiated soil, *G. versiforme* produced a higher N uptake than *G. intraradices*.

The addition of microflora to irradiated soil did not affect and Zn tissue concentration in the presence of *G. intraradices*, but decreased it when plants were inoculated with *G. versiforme* ( $P = 0.0033$  for  $I_2 \times S_2$ ). As for N uptake, the two fungal species were similarly efficient at Zn uptake in amended irradiated soil, but *G. versiforme* was more efficient than *G. intraradices* in unamended irradiated soil.

On average over soil types and N fertilization levels,

inoculation with *G. versiforme* yielded higher N, P and Zn tissue concentrations than inoculation with *G. intraradices*; the reverse was true for the Mn tissue concentration (mean for Mn, 0.0055% for *G. versiforme*, 0.0058% for *G. intraradices*; difference for Mn,  $0.0003 \pm 0.0001\%$ ,  $P \leq 0.0001$  for 12 of N, P, Mn and Zn. In natural soil, the P uptake of inoculated plants was higher than that of control plants ( $P = 0.0003$ ).

Plant tissue concentrations of N, Cu, Mn and Zn were higher in irradiated soils than in natural soil ( $P \leq 0.0001$  for  $S_1$  of N, Cu, Mn and Zn. Mean Cu and Mn tissue concentrations of onion plants grown in irradiated soil were 0.00109% and 0.0061% respectively, compared to 0.00097% for Cu and 0.0049% for Mn of onion plants grown in natural soil (differences,  $0.00011 \pm 0.00003\%$  for Cu and  $0.0012 \pm 0.0001\%$  for Mn). Tissue concentrations of Mn and Zn were also higher when plants were grown in unamended irradiated soil than when the irradiated soil was amended with microflora (means for Mn, 0.0062% in unamended irradiated soil, 0.0059% in amended irradiated soil,  $P \leq 0.0018$ ).

Plant tissue concentration of N and Ca increased proportionally with N fertilization level ( $P \leq 0.0018$ ).

## Discussion

Stunted growth of control plants grown in irradiated soils without AM fungal inoculation has been reported previously<sup>15</sup>. In the absence of AM fungi, there was lack of P limited growth. The addition of a non-mycorrhizal soil extract did not overcome the stunting of growth. It is our interpretation that the ecological equilibrium of the microflora could not be re-established in the short course of the experiment. It was mentioned that, despite the addition of a microbial filtrate from a natural soil to a sterilized soil, the microbial flora of broccoli was not re-established in a 28 or 40 day period<sup>16</sup>.

If soil nutrients are unbalanced, firmness may be affected, In this experiment, increasing N fertilization decreased bulb firmness. Some workers<sup>14</sup> observed a decrease in apple firmness under high N levels. Indeed, excessive N fertilization may depress the uptake of Ca which is a fundamental importance for membrane permeability and the maintenance of cell integrity<sup>13</sup>.

It was reported that Mg application reduced the tissue Ca concentration<sup>12</sup>. In this experiment, N, Ca and Mg increased proportionally with fertilization level when plants were inoculated with *G. versiforme*, while Mg concentration remained stable in the presence of *G. intraradices*. These results suggest that soil mineral contents of these elements were likely close to equilibrium because no interaction was observed between Ca and Mg.

All onion plants that grew in non-irradiated natural soil formed arbuscular mycorrhizae with native fungi. In this soil, onion plants preinoculated by *G. Intraradices* or *G. versiforme* produced firmer bulbs than those colonized by the native species. Even if our results show an advantage of preinoculating seedlings with the selected AM fungal species, it is possible that similar bulb firmness could have been achieved by preinoculation with native AM fungal species.

The greater N, Cu, Mn and Zn tissue concentrations of onions grown in irradiated soils, compared to those of plants grown in nonirradiated natural soil, may be explained by soil irradiation leading to increased nutrient availability<sup>3,11</sup>. Even in irradiation increases the availability of a macronutrient in the soil, growth of the host plant cannot improve unless a proper microflora including AM fungi is established.

The comparison of *G. versiforme* to *G. intraradices* in all soil treatments shows the greater efficiency of *G. versiforme* in supplying, in particular, N, P and Zn to the host plant. Yet, root colonization of onion plants by *G. versiforme* was lower than that by *G. intraradices*. Hence, a high root colonization level does not necessarily lead to better nutrient uptake or plant growth. This was also observed by earlier workers for strawberry plants grown in the field<sup>6,16</sup>. It was attributed to small differences among fungi to different rates of root colonization<sup>14</sup>.

In non-irradiated natural soil, the use of onion plants preinoculated by selected AM fungal species significantly improved P uptake compared to native species. Of the two fungal species tested, *G. versiforme* was the most efficient at nutrient uptake. This study provides no evidence that colonization by indigenous mycorrhizal fungi increased the dry biomass of onion plant relative to controls, as observed<sup>6</sup>.

## References

1. Baath E, Spokes J. The effect of added nitrogen and phosphorus on mycorrhizal growth response and infection in *Allium schoenoprasum*. *Can. J. Bot.* 1989; **67** : 3227-3232.
2. Cawse PA. Microbiology and Biochemistry of irradiated soils. In : Paul EA, McLaren AD (eds) Soil Biochemistry, Vol. 3 Dekker, New York. 1975; 213-267.
3. Charron G, Furlan V, Bernier-Cardou M, Doyon G. Response of onion plants to AM fungi. 1. Effects of inoculation method and phosphorus fertilization on biomass and bulb firmness. *Mycorrhiza* DOI 10.1007/s005720100121. 2001.
4. Crowder MJ, Hand DJ. Analysis of repeated measures Chapman and Hall, London. 1990; 257.
5. De Silva A, Patterson K, Mitchell J. Endomycorrhizae and growth of 'Sweetheart' strawberry seedlings. *Hortic Sci.* 1996; **21**: 951-954.
6. Furlan V, Bernier-Cardou M. Effects of N, P and K on formation of vesicular-arbuscular mycorrhizae, growth and mineral concentration of onion. *Plant Soil.* 1989; **12** : 167-174.
7. Harley JL, Smith SE. Mycorrhizal symbiosis. Academic Press, London. 1983.
8. Jakobsen I, Andersen AJ. Vesicular-arbuscular mycorrhiza and growth in barley : effects of irradiation and heating of soil. *Soil Biol Biochem.* 1982; **14**: 171-178.
9. Jarstfer AG, Farmer-Koppenol P, Sylvia DM. Tissue Magnesium and calcium affect arbuscular mycorrhiza development and fungal reproduction. *Mycorrhiza.* 1998; **7** : 237-242.
10. Mengel K, Kirkby EA. Principles of plant nutrition. 3<sup>rd</sup> edn. International Potash Institute, Bern, Switzerland. 1982.
11. Sanders FE, Tinker PE, Black RLB, Parmerley SM. The development of endomycorrhizal root systems. I. Spread of infection and growth promoting effects with four species of vesicular arbuscular endophyte. *New Phytol.* 1977; **78** : 257-268.
12. Sasa M, Zahka G, Jakobsen I. The effect of pretransplant inoculation with vesicular-arbuscular mycorrhizal fungi on the subsequent growth of leeks in the field. *Plant Soil.* 1987; **97** : 279-283.
13. Smith FA, Smith SE. Mycorrhizal infection and growth of *Trifolium subterraneum*; use of sterilized soil as a control treatment, *New Phytol.* 1981; **88** : 299-309.
14. Smock RM, Neubert AM. Apples and apple products. Inter-science, New York. 1950.
15. Steel RGD Torrie JH. Principles and Procedures of statistics : a biometrical approach, 2<sup>nd</sup> edn. McGraw-Hill, New York. 1980.
16. Williams SCK, Vestberg M, Uosukainen M, Dod JC, Jeffries P. Effects of fertilizers and arbuscular mycorrhizal fungi on the post-*vitro* growth of micropropagated strawberry, *Agronomie.* 1992; **12** : 851-857.