

## INFLUENCE OF MICROBIAL CONSORTIUM IN THE PRODUCTION OF CHINA ASTER AND GAILLARDIA SEEDLINGS

### Short communication

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#### ABSTRACT

China aster and gaillardia are flowering plants with high economic importance in floriculture. In the present investigation, response of China aster and gaillardia seedlings to inoculation with the arbuscular mycorrhizal fungus *Funneliformis mosseae* + the plant growth-promoting rhizobacterium *Bacillus sonorensis* was studied by growing in multipots (pro trays). The germination percentage and plant growth parameters: length of shoots, roots and whole seedlings, stem diameter, biovolume index, plant strength, vigor index, dry weight and nutrient uptake, were analyzed 60 days after sowing. The microbial parameters, mycorrhizal root colonization and spore count, and the population of *B. sonorensis* in the substrate were also determined. The results brought out that growth of inoculated seedlings was significantly improved as compared to uninoculated seedlings. Based on the plant growth and microbial parameters studied, it was concluded that inoculating the substrate in pro trays with the microbial consortium results in producing vigorously growing seedlings.

**Key words:** arbuscular mycorrhizal fungi, *Bacillus sonorensis*, *Funneliformis mosseae*, plant growth-promoting rhizobacteria

#### INTRODUCTION

Floriculture is an age-old farming activity in India, now emerging as an important commercial trade in agriculture. A lot of importance has been given to this sector because of its multiple uses, satisfying the aesthetic needs of the people and creating self-employment among small farmers and gardeners. In India, the floriculture industry comprises flower trade, production of nursery and potted plants, seeds and bulbs. These plants are also being used as raw materials in the manufacture of essence, perfumes, medicines and confectioneries for direct consumption. Because of the increase in demand for these plants, many new modern techniques are being adapted to improve the quality and quantity of plant resources (Kumar et al. 2012). Inoculation with beneficial microorganisms,

like arbuscular mycorrhizal fungi (AMF), including nitrogen fixers, phosphate solubilizers and plant growth-promoting rhizobacteria (PGPR), can promote plant growth and shorten time to flower (Sohn et al. 2003; Desai et al. 2020).

AMF constitute a group of obligate biotrophs that form symbiotic association with 80% of plants. The influence of AM symbiosis on plant growth depends mainly on the ability of fungi to take up and transfer diffusion-limited nutrients, especially P, Zn, Cu, etc., to plant roots in exchange for plant photosynthates (Jansa et al. 2011; Bagyaraj 2018; Desai et al. 2020). AMF benefits plants in several ways: improving the nutritional status, growth and development of plants, protecting plants against root pathogens and conferring resistance to drought and soil salinity (Jyothi et al. 2018).

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In recent years, there has been a considerable interest in PGPR. The PGPR is another group of beneficial soil microbes that stimulate plant growth directly or indirectly. The direct mechanism involves supplying plants with nutrients and phytohormones, and indirect mechanism reduced susceptibility to disease by producing antifungal metabolites, like HCN, phenazines, pyrrolnitrin, 2,4-diacetylphloroglucinol, etc. (Das et al. 2013; Shakuntala et al. 2019). The synergistic interaction of AMF and PGPR in soil resulting in plant growth acceleration was previously described (Anuroopa et al. 2017; Behera et al. 2019).

Microbial consortium is a mixture of beneficial microorganisms that benefits plant growth (Woo & Pepe 2018). They are known to enhance germination and growth of seedlings when inoculated in multipots (pro trays) (Desai et al. 2020) and nursery beds (Cely et al. 2016; Jyothi et al. 2018). The advantages of this technology are: shorter time in the nursery, which shortens the entire production cycle, homogeneity, easy operation, possibility of mechanization, labor saving, cheaper transport and more reliable yield prediction after transplanting to the main field. Inoculation of the planting medium or seeds with a beneficial microbial consortium is a biotechnological approach for producing healthy, vigorously growing seedlings (Desai et al. 2020).

China aster [*Callistephus chinensis* (L.) Nees] is one of the most popular and valuable seasonal flowers, used as a cut flower as well as in landscaping. Cultivation of China aster is a profitable enterprise. The flowers produce rich nectar, which attracts insects, thus helping in pollination of many flowering plants. The compounds of the plant are of pharmacological importance, as antiallergic, antiinflammatory, antiviral, antiproliferative, and anticarcinogenic (Bhargav et al. 2018). Gaillardia or blanket flower (*Gaillardia pulchella* Foug.) are known for their attractive, bright-colored flowers, used for various purposes – cut flowers, floral decorations, and garlands. The leaves have medicinal properties and are used as a diuretic when taken internally to get relief from pain during urination

and applied externally for treating gout disease (Kim et al. 2015). The objective of the present study was to evaluate the ability of the microbial consortium (*Bacillus sonorensis* + *Funneliformis mosseae*) in enhancing the growth of flowering plants China aster and gaillardia seedlings raised in pro trays under polyhouse condition.

## MATERIALS AND METHODS

The polyhouse experiment was conducted at the Centre for Natural Biological Resources and Community Development (CNBRCD), Bengaluru. The seeds of China aster and gaillardia used in the present study were obtained from the University of Agricultural Sciences, GKVK campus, Bengaluru, India.

### Inoculum preparation

The PGPR *B. sonorensis* used in the study was procured from the University of Hyderabad, Hyderabad, and maintained at the CNBRCD, Bengaluru, India. Sub-culturing was done on Luria-Bertani (LB) agar plates and incubated at 37 °C for 24 h. A single isolated colony was transferred to test tube containing 10 ml of LB broth and later transferred to 500 ml flasks containing LB medium and grown aerobically in flasks on a shaker (150 rpm) at 37 °C for 48 h. The bacterial suspension was then diluted with sterile water to a final concentration of  $10^8$  CFU·ml<sup>-1</sup>, and the resulting suspension was used as inoculum. *F. mosseae* is being maintained in the culture collection of CNBRCD using vermiculite: perlite: soilrite (3 : 1 : 1 v/v/v) as the substrate and Rhodes grass (*Chloris gayana*) grown as the host. Rhodes grass was harvested 75 days after sowing (DAS) and finely chopped roots along with the substrate were air dried and used as the AMF inoculum. The number of infective propagules in the inoculum was estimated through the most probable number (MPN) method with 10-fold dilution (Porter 1979). The PGPR *B. sonorensis* and AMF *F. mosseae* was selected based on the positive results reported in earlier studies on other vegetable and flower plants (Thilagar et al. 2014; Sukeerthi et al. 2020).

### Inoculation and growth condition

The China aster 'Kamini' and gaillardia 'Local Red' were raised in 50 cells pro trays, each cell holding 20 g of substrate. Cells were filled with 17.5 g of the mixture of vermiculite, soilrite and perlite in the ratio 3 : 1 : 1 (v/v/v), to which 1 g of sterile soil and 1.5 g of compost was added and mixed well with glass rod. The experiment consisted of two treatments for each crop (T1 – uninoculated control; T2 – inoculated with *B. sonorensis* + *F. mosseae*) using two pro trays of 100 cells each per species per treatment. A planting hole was made in the substrate and 1 g of *F. mosseae* inoculum (containing  $2.2 \times 10^3$  IP·g<sup>-1</sup>) and 2 ml of *B. sonorensis* inoculum (containing  $1.9 \times 10^8$  CFU·ml<sup>-1</sup>) was added. Uninoculated pro tray cells received autoclaved LB broth at the rate of 2 ml per cell, and washings of AMF inoculum suspension passed through a 45 µm filter, which contained associated microorganisms, but not AMF propagules, at the rate of 2 ml per cell. Four seeds were sown in each pro tray cell to ensure germination, and later, they were thinned out to one seedling per cell. Five ml of Ruakura's medium (Sreenivasa & Bagyaraj 1988) was added to each cell on day 15 after seeding and then 5 ml of the same medium without P was applied once every 10 days. The seedlings were maintained in a polyhouse and watered regularly.

### Parameters evaluated

The seedlings were harvested at 60 DAS. Plant parameters: length of shoot, root and cuttings (shoot + root), stem diameter, fresh and dry weight, were measured for all 100 seedlings from each treatment. Biovolume index (BI) was calculated by the formula  $BI = \text{plant height (cm)} \times \text{stem diameter (mm)}$ , as given by Hatchell et al. (1985). Germination rate (%) was calculated by dividing the number of seedlings germinated by total number of seeds sown. The plant strength was calculated using the formula given by Maskina et al. (1984), i.e., the plant strength equals the dry weight of the seedling (g) divided by the height of the seedling (cm). The seedling vigor index (SVI) was calculated using the formula  $SVI = \text{germination \%} \times \text{seedling length (cm)}$  (Abdul-Baki & Anderson 1973). Dry weight of the seedlings was recorded by drying the samples in a hot air oven at 60 °C.

The composite samples of each treatment were then powdered, and the nutrient status of seedlings was estimated with three replications per treatment. The nitrogen concentration was determined by micro Kjeldahl method (Jackson 1973). Phosphorus concentration was estimated by vanadomolybdate phosphoric yellow color method (Jackson 1973). Potassium concentration was determined by flame photometer method (Bowling Barnes et al. 1945). The micronutrient analysis of the samples was performed using atomic absorption spectrophotometer (AAS) (Ferreira et al. 2018), where the cathode lamp was set to standard wavelength. The percentage of mycorrhizal root colonization was determined by staining fine roots with trypan blue (Phillips & Hayman 1970), and estimated adopting the grid-line intersect method (Giovannetti & Mosse 1980). The AM fungal spore number in the root zone soil was determined by wet sieving and decantation method (Gerdemann & Nicolson 1963). The *B. sonorensis* population in the substrate was enumerated by following the spread plate technique (Sanders 2012). Data were subjected to Student's t-test at a significance level  $p = 0.01$  and sample effect size, i.e., D-value was calculated.

## RESULTS AND DISCUSSION

All the plant parameters studied showed significant difference between the inoculated and uninoculated plants. For most of the parameters tested, the effect was medium to high. It was observed that shoot, root, and the whole seedling length of inoculated plants showed significant increase in China aster by 133, 96, and 111%, respectively, compared to uninoculated seedlings (Table 1). A similar trend was observed in gaillardia, where shoot, root and the whole seedling lengths increased by 57, 192, and 131%, respectively, in inoculated compared to uninoculated seedlings. The increased length of shoots, roots and cuttings due to the inoculation of AMF + PGPR has been reported previously on other plants, such as tomato and pepper grown in pro trays (Behera et al. 2019; Desai et al. 2020).

Regarding stem diameter, inoculated plants showed significant increase by 58% in China aster and 68% in gaillardia seedlings, compared to uninoculated control, supporting our earlier study on flowering plants (Sukeerthi et al. 2020). The BI of the plants, which indicates the quality of seedlings, showed 270% increase in China aster and 167% increase in gaillardia compared to uninoculated seedlings (Table 1). Enhanced BI of seedlings due to AMF + PGPR inoculation has been reported earlier for French bean (Chauhan & Bagyaraj 2015) and chilly (Thilagar et al. 2016).

Seedlings inoculated with microbial consortium (*B. sonorensis* + *F. mosseae*) showed a significant increase in all the plant growth parameters. Germination rate, plant strength and SVI in inoculated treatment showed significant increase in both China aster and gaillardia seedlings compared to uninoculated control seedlings (Table 1). This indicates that the consortium helps in improving the germination of seeds and the seedling emergence (Paredes-Páliz et al. 2016). The total plant dry weight in inoculated seedlings was 140% higher in China aster and 172% higher in gaillardia compared to uninoculated seedlings (Table 1). This is in conformity with the results obtained for tomato, pepper, zinnia and balsam (Behera et al. 2019; Desai et al. 2020, Sukeerthi et al. 2020).

The content of N, P, and K macronutrients was respectively 13, 71, and 68% higher in the inoculated China aster seedlings as compared to uninoculated seedlings, and in gaillardia these increments were 6, 67, and 12%, respectively (Table 2). Micronutrients followed a similar trend. This could be mainly because of the activity of inoculated AMF and PGPR. Improved uptake of diffusion-limited nutrients like P, Zn, Cu, etc. is well documented (Bagyaraj 2014). Similar results of enhanced nutrient uptake because of dual inoculation with PGPR + AMF have been reported earlier in French bean and chilly (Chauhan & Bagyaraj 2015; Thilagar et al. 2014; Anuroopa et al. 2017).

PGPR may change the morphology of roots by the phytohormones that are synthesized, which results in an increased root surface area (Chauhan et al. 2015).

In China aster and gaillardia, 99% roots were colonized by AMF, while root colonization of uninoculated seedlings was only 2%. The mycorrhizal spore numbers were 41 and 45 in the inoculated substrate of China aster and gaillardia, respectively, while uninoculated substrate did not harbor any spores (data not given). The significantly higher percent mycorrhizal root colonization and spore number in the substrate of seedlings inoculated with microbial consortium, as compared to uninoculated control, indicates the better proliferating ability of *F. mosseae* with China aster and gaillardia as also in other flowering plants – zinnia and balsam (Sukeerthi et al. 2020). Enhanced root colonization and spore number in the root zone because of inoculation with AMF + PGPR have been reported earlier in different crops (Anuroopa et al. 2017; Pan et al. 2020). The CFU of *B. sonorensis* in the root zone substrate was found to be  $4 \times 10^4 \cdot \text{g}^{-1}$  substrate in China aster and  $3 \times 10^4 \cdot \text{g}^{-1}$  substrate in gaillardia, while these bacteria were not detected in the substrate of uninoculated treatment (data not given). The presence of inoculated PGPR in the root zone at the end of seedling stage was reported earlier in other crops (Desai et al. 2020, Sukeerthi et al. 2020). Synergistic interaction between *F. mosseae* and *B. sonorensis* promoting higher plant growth compared to single inoculation with either of them has been reported earlier in chilly (Thilagar et al. 2014). Considering the results obtained for all plant parameters in our study, it can be concluded that inoculation of China aster and gaillardia with the microbial consortium (*F. mosseae* + *B. sonorensis*) resulted in healthy vigorously growing seedlings in pro trays. The hypothesis that inoculated plants will grow faster and will have better quality also during the entire production cycle remains to be verified in further experiments.

Table 1. Influence of microbial consortium on plant growth parameters of China aster and gaillardia seedlings measured 60 days after seeding

| Growth parameters                     | China aster          |            |        |         | Gaillardia           |            |        |         |
|---------------------------------------|----------------------|------------|--------|---------|----------------------|------------|--------|---------|
|                                       | Uninoculated control | Inoculated | SE     | D-value | Uninoculated control | Inoculated | SE     | D-value |
| Shoot length (cm)                     | 3.05                 | 7.11       | 0.97   | 0.5**   | 4.99                 | 7.87       | 0.66   | 1.9**   |
| Root length (cm)                      | 4.48                 | 8.79       | 1.01   | 0.5**   | 6.15                 | 17.99      | 2.61   | 0.5**   |
| Seedling length (cm)                  | 7.53                 | 15.90      | 1.98   | 0.8***  | 11.14                | 25.84      | 3.23   | 1.3***  |
| Stem diameter (mm)                    | 1.58                 | 2.51       | 0.25   | 0.6**   | 1.29                 | 2.18       | 0.20   | 1.5***  |
| Biovolume index                       | 4.82                 | 17.85      | 3.24   | 1.2***  | 6.43                 | 17.15      | 2.37   | 2.0***  |
| Germination rate (%)                  | 52.00                | 80.50      | 7.26   | 0.6**   | 52.50                | 89.50      | 8.24   | 0.6**   |
| Plant strength (dry matter/unit area) | 0.0163               | 0.0169     | 0.001  | 0.3*    | 0.0088               | 0.0152     | 0.001  | 0.3*    |
| Vigor index                           | 389.2                | 1274.65    | 198.78 | 1.0***  | 583.45               | 2326.85    | 394.01 | 0.9***  |
| Dry weight (g)                        | 0.050                | 0.120      | 0.02   | 1.2***  | 0.044                | 0.120      | 0.02   | 1.2***  |

\*values are significant at  $p = 0.01$ ; increase: \*small, \*\*medium, \*\*\*large

Table 2. Effect of microbial consortium on the nutrient uptake of China aster and gaillardia measured in 60-day-old seedlings

| Nutrients                         | China aster          |            |        |         | Gaillardia           |            |        |         |
|-----------------------------------|----------------------|------------|--------|---------|----------------------|------------|--------|---------|
|                                   | Uninoculated control | Inoculated | SE     | D-value | Uninoculated control | Inoculated | SE     | D-value |
| N (%)                             | 1.87                 | 2.11       | 0.07   | 0.5**   | 1.94                 | 2.06       | 0.03   | 0.4*    |
| P <sub>2</sub> O <sub>5</sub> (%) | 0.07                 | 0.12       | 0.01   | 0.4*    | 0.06                 | 0.10       | 0.01   | 0.3*    |
| K <sub>2</sub> O (%)              | 1.60                 | 2.68       | 0.25   | 1.0***  | 1.95                 | 2.19       | 0.06   | 0.8***  |
| Ca (%)                            | 1.99                 | 2.25       | 0.06   | 0.4*    | 2.18                 | 2.67       | 0.11   | 0.6**   |
| Mg (%)                            | 1.54                 | 1.76       | 0.05   | 0.7**   | 0.93                 | 1.56       | 0.14   | 0.6**   |
| Zn (ppm)                          | 97                   | 208        | 24.76  | 0.7**   | 72.03                | 129.8      | 13.07  | 0.5**   |
| Fe (ppm)                          | 3181                 | 3937       | 169.12 | 1.1***  | 2460                 | 3775       | 293.41 | 0.8***  |
| Cu (ppm)                          | 108                  | 129        | 5.16   | 0.9***  | 59.03                | 105.1      | 10.48  | 0.3*    |
| Mn (ppm)                          | 81                   | 149        | 15.45  | 1.3***  | 74.75                | 92.05      | 3.94   | 1.2***  |
| B (ppm)                           | 57                   | 69         | 3.21   | 0.8***  | 56.47                | 83.62      | 6.05   | 1.3***  |
| Mo (ppm)                          | 80                   | 172        | 20.56  | 1.0***  | 62.16                | 99.24      | 8.48   | 1.2***  |

\*values are significant at  $p = 0.01$ ; increase: \*small, \*\*medium, \*\*\*large

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