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RESPONSE OF *SORGHUM BICOLOR* (L.) MONECH TO DUAL INOCULATION WITH *GLOMUS FASCICULATUM* AND *HERBASPIRILLUM SEROPEDICAE*

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Summary. Gramineous plants establish strong symbiosis with arbuscular mycorrhizal fungi that can improve the uptake of P from soil. They also associate with some diazotrophic bacteria and get biologically fixed nitrogen. *Sorghum bicolor* is a sugar-rich multipurpose fodder crop well suited to dry farming. In the present study, *S. bicolor* plants were grown in a green house and the impact of inoculation with *Herbaspirillum seropedicae* and *Glomus fasciculatum* on growth, N and P nutrition of the plant was studied. A single inoculation with either *H. seropedicae* or *G. fasciculatum* supported plant growth promotion effect resulting in enhanced root length (5-7%), shoot length (18-22%), root biomass (40-58%), shoot biomass (46%), total N content (21-49%), and total P content (46-167%) compared to the control. The dual inoculation with *H. seropediae* and *G. fasciculatum* resulted in enhanced root length (15%), shoot length (40%), root biomass (105%), shoot biomass (91%), total N content (108%), and total P content (138%) compared to the control. Within the single inoculated plants, N accumulation was higher by 23% after *H. seropedicae* inoculation compared with *G. fasciculatum* inoculation. In contrast, P accumulation in plants was higher by 82% after *G. fasciculatum* inoculation compared with *H. seropedicae* inoculation. There was little difference in hyphal (92-95%) and vesicular (82-83%) infection between *G. fasciculatum* inoculated plants and *G. fasciculatum* + *H. seropedicae* inoculated plants indicating no interference of bacteria on mycorrhizal colonization in roots of *S. bicolor.*

Key words: *Glomus fasciculatum*; growth; *Herbaspirillum seropedicae*; *Sorghum bicolor*; total nitrogen; total phosphorus.

Abbreviations: AM fungi – arbuscular mycorrhizal fingi; ANSA – 1-amino 2-naphthol 4-sulphonic acid; N – nitrogen; P – phosphorus; K – potassium; ANOVA – analysis of variance.

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INTRODUCTION

Arbuscular mycorrhizal fungi are ubiquitous in occurrence and colonize root systems of nearly all plants (Barea, 1991). The external hyphae of these fungi extend beyond the limits of the rhizosphere, and hence improve the plant's capacity for uptake of P from soil (Marschner and Dell, 1994), the prime factor responsible for plant growth improvement. Other benefits of AM fungi include suppression of soil-borne plant pathogens, tolerance to water stress, production of plant growth hormones and mobilization of minor nutrients. In general, diazotrophs like *Azospirillum*, *Azotobacter*, *Beijerinkia*, *Klebsiella*, *Bacillus* and *Derxia* are found to be beneficial to grasses and cereals by fixing atmospheric nitrogen. A Gram-negative diazotrophic spirillum has been found in association with some cereal crops and sugarcane in Brazil (Baldani et al., 1986) and has been named as *Herbaspirillum seropedicae*. Although the dual inoculation with AM fungi and nitrogen fixing bacteria is known to enhance the inoculation effect in several crop plants (Bagyaraj and Menge, 1978), literature pertaining to endophytic *Herbaspirillum* and AM fungi interaction on plant growth is scanty. *Sorghum bicolor* is an important dry land crop, which ranked fifth among the major cereals following wheat, maize, rice and barley (House, 1995). It is widely cultivated for obtaining sugar, alcohol, syrup, jaggery, fodder and fuel. Although India is the second largest producer of sorghum in the world, the yield of 840 kg per hectare is the lowest amongst the major sorghum-producing counties in the world (Sheorain et al., 2000). This poor yield is due to inadequate agricultural practices and dependency on rainwater and hence Indian farmers get lesser profit from sweet sorghum cultivation. Hence, the present study is aimed to elucidate the interaction between *H. seropedicae* and *G. fasciculatum* on plant growth, N and P concentrations in *S. bicolor*.

MATERIALS AND METHODS

Soil from Marikundu village, Theni District, Tamil Nadu was collected for growing plants. *G. fasciculatum* and *H. seropedicae* were obtained from the Department of Botany, Thiagarajar College, Madurai. The soil was subjected to pH and NPK analyses. Soil N and K were analyzed following the method of Black (1965). Soil P was estimated by the method of Olsen et al., (1954). Soil pH was determined in an 'Elico' pH meter after mixing 1 g of soil with 10 ml of distilled water. The soil pH was 6.9. Each kg of soil contained 56 mg N, 3.8 mg P and 285 mg K. Spores and sporocarps of *G. fasciculatum* were multiplied in *Eleusine coracana* (variety Co-11) plants in sterile soil under potted conditions. One gram of soil-based inoculum containing 300 to 800 spores and sporocarps was spread over the lower layer of 0.5 kg soil and then 1 kg of soil was layered over the inoculum. Seeds of *S. bicolor* variety Co27 obtained from Tamil Nadu seed testing centre, Madurai were surface sterilized with 0.1 % $HgCl_2$ for 5 min and sown at the rate of 10 per pot (12.5 cm height and 15 cm diameter) containing red loamy soil. After germination, the seedlings of *S. bicolor* were thinned out to 5 per pot. *H. seropedicae* culture was grown in JNFb broth (Baldani et al., 1986) with 20 mg yeast extract l^{-1} for 3

days in a 'Schigenics' rotary shaker with a speed of 120 strokes min^{-1}. Seven-day-old seedlings were inoculated with 2 ml of *H. seropedicae* culture (approximately $1x10^9$ cells/ml) by introducing the cell suspension in the soil around the base of each seedling. The following treatment schedule was followed:

T1: Uninoculated control.
T2: *H. seropedicae* inoculation.
T3: *G. fasciculatum* inoculation.
T4: *H. seropedicae* + *G. fasciculatum* inoculation.

The plants were grown in a greenhouse under conditions of broad day light and a temperature range of 28 to 30°C. Tap water was used to water the plants. Each treatment was replicated 5 times for harvest at 45 days after inoculation with *H. seropedicae*. On harvest, root and shoot lengths were measured in a meter scale. For evaluation of biomass yield 3 plants from each treatment were used. The root, stem and leaves were dried separately at 90°C for 72 h and their dry weight was determined.

The root samples were cleared and stained with tryphan blue following the method of Phillips and Hayman (1970). The root segments in 10% KOH were incubated at 90°C for 2 h and bleached by immersing in 30% H_2O_2 for 10-15 min. After rinsing in water they were acidified with 5N HCl and stained by simmering in 0.05% tryphan blue in lactophenol for 30 min. The root segments were squashed on slides containing few drops of a solution containing 3g of polyvinyl alcohol, 25 ml of lactic acid, 20 ml of phenol and 50 ml of glycerol. The infection was measured by the gridline intersect method of Giovannetti and Mosse (1980) using the formula:

% infection = 100 x total number of infected roots intersecting gridlines / total number of roots intersecting gridlines.

The dried plant material (root, stem and leaves) was ground separately and mixed together, and total N and acid-soluble total P in these samples were estimated. Total nitrogen was estimated by the modified micro-Kjeldahl method (Umbreit et al., 1972). Ten milligrams of finely powdered plant material in a micro-Kjeldahl flask were digested with 50 mg of catalyst (preparation of catalyst: 1 g of $CuSO_4$, 8 g of K_2SO_4, and 1 g of selenium dioxide were pulverized separately and mixed together), and 0.5 ml of concentrated H_2SO_4 in a 'Tempo' digestion rack. After the digest turned to apple green color, an aliquot of 0.1 ml was mixed with 2 ml of water, 2 ml of color reagent (preparation of color reagent: 4 g of KI and 4 g of HgI_2 were dissolved in 25 ml of distilled water. About 1.75 g of gum arabic was dissolved in 750 ml of boiling distilled water and cooled. The gum arabic solution was mixed with the potassium iodide - mercuric iodide solution and made up to 1000 ml with distilled water) and 3 ml of 2N NaOH, in series. The resultant colored solution was measured at 490 nm with a 'Bausch & Lomb' Spectronic – 20 colorimeter. Authentic NH_4Cl was used as a standard.

Acid-soluble total phosphorus was determined by the method of Bertlett (1959). An aliquot of dried sample was extracted with 0.2 N perchloric acid and kept in an ice bath for 15 min prior to clearing by centrifugation. The pellet was re-extracted with perchloric acid for three

times and the extracts were pooled together and analyzed for total phosphorus. For estimation of total phosphorus, 1 ml of the extract was mixed with 1 ml of 60% trichloroacetic acid and digested at 160-180°C. After digestion, each sample received 4.5 ml of 2.5% ammonium molybdate in 5N H_2SO_4 and 0.2 ml of 0.25% ANSA reagent. The contents were heated in a boiling water bath for 10 min and the volume was adjusted to 100 ml with distilled water. The resultant solution was read at 650 nm using a colorimeter. Potassium dihydrogen phosphate was used as a standard. The data were subjected to statistical analysis by using Costat package for one-way ANOVA and student Newman Keuls test.

RESULTS AND DISCUSSION

Plant growth can be measured in a variety of ways. In this study, root and shoot length, root and shoot biomass were used to illustrate the impact of *H. seropedicae* and *G. fasciculatum* on the growth of *S. bicolor*. The inoculation with either *G. fasciculatum* or *H. seropedicae* or both induced a significant increase in root and shoot length and root and shoot biomass over the uninoculated control (Table 1). This increase was more expressed after the dual inoculation with *H. seropedicae* + *G. fasciculatum,* compared to the single inoculation with either *H. seropedicae* or *G. fasciculatum*. The increase in root length was by 5-7% after single inoculation, in contrast to 15% after dual inoculation. In the case of shoot length, the increase was by 18-22% and 40% after single and dual inoculation, respectively. The increase in root biomass was by 40-58% and 105% after single and dual inoculation, respectively. Similarly, the increase in shoot biomass was by 46% and 91% after single and dual inoculation, respectively. Further, *H. seropedicae* induced more root biomass with or without *G. fasciculatum* inoculation compared to shoot biomass. The improved growth of *S. bicolor* could

Table 1. Effect of inoculation with *Herbaspirillum seropedicae* and *Glomus fasciculatum* on root and shoot length and root and shoot dry weight of *Sorghum bicolor* at 45 DAI.

Treatments	Root length [cm plant^{-1}]	Shoot length [cm plant^{-1}]	Root dry weight [g plant^{-1}]	Shoot dry weight [g plant^{-1}]
Control	$28.33^{a} \pm 3.70$	$36.80^{b} \pm 6.44$	$0.185^{b} \pm 0.040$	$0.313^{b} \pm 0.136$
Herbaspirillum seropedicae	$29.83^{a} \pm 0.28$ (5.29)	$43.50^{ab} \pm 3.50$ (18.21)	$0.293^{ab} \pm 0.040$ (58.37)	$0.458^{ab} \pm 0.080$ (46.32)
Glomus fasciculatum	$30.33^{a} \pm 0.57$ (7.06)	$44.83^{ab} \pm 1.25$ (21.82)	$0.259^{b} \pm 0.080$ (40)	$0.459^{ab} \pm 0.014$ (46.64)
H. seropedicae + *G. fasciculatum*	$32.66^{a} \pm 2.08$ (15.28)	$51.50^{a} \pm 2.78$ (39.94)	$0.381^{a} \pm 0.015$ (105.94)	$0.600^{a} \pm 0.105$ (91.69)
L.S.D. (P<0.05)	4.040^{NS}	7.473^{*}	0.093^{**}	0.178^{*}

± Standard deviation. Values suffixed with different letters on the same column indicate significant differences. *; **; *** = Extent of significance; NS = Not significant. Values in parenthesis represent % increase over control.

be due to hormonal and or nutritional effects of the inoculated microbes. Pacovsky (1990) reported a positive impact of *Azospirillum* inoculation on sorghum growth. In addition, it has been shown that some species of *Azospirillum* stimulate plant growth by means of phytohormones, in addition to nitrogen fixation (Roesch et al., 2007). Inoculation with *G. fasciculatum* or *H. seropedicae* or both induced a significant increase in total N and P content over the uninoculated control, but the dual inoculation expressed its superiority over any of the single inoculations (Table 2). The diazotroph, *H. seropedicae* induced an increase by 49.75% and 46.31% for N and P, respectively. In the case of *G. fasciculatum* inoculation, the increase was by 21.46% and 167.28% for N and P, respectively. Further, the dual inoculation induced an increase by 108.27% and 138.70% for N and P, respectively. There was little difference in hyphal infection (92-95%) and vesicular infection (82-83%) between two categories of mycorrhizal plants – the ones co-inoculated with *H. seropedicae* and the ones without *H. seropedicae* co-inoculation. In an earlier report, it has been shown that dual inoculation of cowpea with *Rhizobium* and AM fungus increased the shoot N and P content over the single inoculation (Thiagarajan et al., 1992). While studying *Digitaria adscendens* under water logged conditions, Rao and Shukla (2002) found a higher per cent infection in roots, dry weight of seedlings and N and P content upon dual inoculation with *Azospirillum brasilense* and *G. mossae* than with single inoculation. However, the results of the present study showed that *H. seropedicae* did not influence the per cent hyphal and vesicular infection since there was little difference in per cent infection in roots of single inoculated and dual inoculated mycorrhizal plants. The enhanced growth of *S. bicolor* upon dual inoculation is attributed to better

Table 2. Effect of inoculation with *Herbaspirillum seropedicae* and *Glomus fasciculatum* on per cent infection in roots, and whole plant total nitrogen and total phosphorus in *Sorghum bicolor* at 45 DAI.

Treatments	% Hyphal infection	% Vesicular infection	Total nitrogen [mg N g^{-1} dry weight]	Total phosphorus [mg P g^{-1} dry weight]
Control	–	–	$21.99^{c} \pm 1.341$	$0.434^{b} \pm 0.025$
Herbaspirillum seropedicae	–	–	$32.92^{b} \pm 1.210$ (49.70)	$0.635^{b} \pm 0.011$ (46.31)
Glomus fasciculatum	92.16 ± 3.53	82.13 ± 7.04	$26.71^{bc} \pm 0.832$ (21.46)	$1.160^{a} \pm 0.048$ (167.28)
H. seropedicae + *G. fasciculatum*	95.30 ± 4.80	83.00 ± 10.18	$45.80^{a} \pm 8.014$ (108.27)	$1.036^{a} \pm 0.355$ (138.70)
L.S.D. ($P<0.05$)			7.774^{***}	0.338^{**}

± Standard deviation. Values suffixed with different letters on the same column indicate significant differences. *; **; *** = Extent of significance; NS = Not significant. Values in parenthesis represent % increase over control.

nutritional status especially N and P since AM fungi sequestrate P from soil by extending hyphae beyond the limits of the rhizosphere (Marschner and Dell, 1994) and diazotophs fix atmospheric nitrogen. This investigation clearly showed the positive additive effect of dual inoculation involving *G. fasciculatum* and *H. seropedicae* on the growth and nutrition of *S. bicolor*. Further, the triple interaction can be exploited for the productivity of sweet sorghum.

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