Synergistic Effects of Diazotrophic Phosphate Solubilizing *Azotobacter chroococcum* and AM Fungus *Glomus mosseae* on Yield Improvement in Finger Millet (*Eleusine Coracana* (L.) Gaertn.)

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Abstract*—Field experiments were conducted to study the response of finger millet Eleusine Coracana* (L.) Gaertn. *to combined inoculation with the P-solubilizing diazotrophic Azotobacter chroococcum (DT) isolated from the rhizosphere of finger millet and the P-mobilizing Arbuscular Mycorrhizal fungus Glomus mosseae under graded levels of N & P fertilizers (100, 75 and 50 percent).The inoculated seedlings were transplanted to the laid out plots (RCBD) with red sandy loam soil having medium levels of NPK and OC. The combined inoculation resulted in significant increase in yield over all the other treatments with 31.00, 27.99 and 18.10 percent grain yield and 36.69, 33.55 and 15.01 percent straw yield respectively in presence of 100, 75 and 50 percent N and P fertilizers in comparison with uninoculated control indicating efficient N supplementation and P- solubilization by A. chroococcum and P-mobilization by the AM fungus. The results indicated that 25-50 percent of N and P fertilizers can be curtailed in presence of combined inoculants, saving money without decrease in the yield performance of finger millet. The combination of these inoculants can be included as one of the important agronomic inputs in finger millet cultivation both under subsistence and affluent farming systems*.

Keywords—Azatobacter chroococcum, Biofertilizers, Eleusine coracana, finger millet, Glomus mosseae.

I. INTRODUCTION

Finger millet (*Eleusine Coracana* (L.) Gaertn.) popularly known as ragi is one of the important minor millets grown in India and ranks second in importance among the millets cultivated in India assuming growing importance due to its dietary role among rural folk and the diabetics. The crop is grown in 2.5 million hectares in the country and Karnataka ranks first with an area of 0.99 m. ha under ragi cultivation [1]. To a large extent, the finger millet is cultivated by poor and marginal farmers under subsistence farming with low nutrient input. Nitrogen and phosphorus determine the yield in combination with potash. However, the soils grown for ragi are of low fertility soils resulting in wide regional variations in yield averaging 1500-1700 kg/ha as against the varietal potential of 4000 to 5000kgs. Nitrogen fixing Bacteria (NFB), phosphate solubilizing bacteria (PSB) and Arbuscular Mycorrhizal Fungal (AMF) inoculants play a major role in supplementing and mobilizing these nutrients to plants under low input conditions while reducing their application under affluent farming systems. To harness the synergistic effects, these three groups of inoculants have to be used in combination which is not a common practice among farmers. However, this problem can be solved to a great extent by using bacterial isolates which perform both the functions of nitrogen fixation and phosphate solubilization. In nature there exist bacteria which perform dual functions of nitrogen fixation and phosphate solubilization in a single application. Such phosphate solubilizing diazotrophs among *Azotobacter, Bacillus* and *Azospirillum* species have been reported from the rhizosphere of plants Halder *et al.,* [2], Abd alla [3][4], Sheshadri *et al.,* [5]; Vivek Kumar *et al*.,[6]; Monica *et al*.,[7]; Rahim *et al.,* [8] and Hafsa *et al*.,[9]. Chandana and Venkataramana [10] isolated and evaluated phosphate solubilizing diazotrophic *Azotobacter chroococcum* and *Bacillus* sp. from the rhizosphere of finger millet with significant grain and straw yield improving influences. A few reports are available on the effects of tripartite inoculation of NFB, PSB and AMFs with encouraging results [11]. However there is lack of information on the synergistic influences of phosphate solubilizing diazotrophic *Azotobacter chroococcum* in combination with AM fungus *Glomus mosseae* on finger millet. The present study reports for the time the growth and yield response of finger millet to the combined inoculation with phosphate solubilizing diazotrophic *Azotobacter chroococcum* (DT) in combination with AM fungus *Glomus mosseae.*

II. MATERIAL AND METHOD

The field experiments were conducted at the campus of M/s. Chaitra Biofertilizers and Chemicals (P) Ltd., Mysore, Karnataka, India during 2016-2018 using the finger millet variety MR1 under irrigated condition. The experiment was laid out randomized complete block design with three replicates per treatment. Each plot was 2 x 1 m size. The recommended fertilizers are NPK 100:50:50 kg/ha. There were 12 treatments and all the treatments received 10 tons of farmyard manure and full dose of potash fertilizer (50 kg/ha) while N & P fertilizer dosage varied (100, 75, 50 percent/ha) depending on the treatments. *A. chroococcum* (DT) having the dual trait of nitrogen fixing and phosphate solubilization, isolated from the rhizosphere of finger millet and evaluated under pot culture conditions (unpublished) was used in comparison with uninoculated control. The lignite based inoculant of *A. chroococcum* (DT) was used at 25gm/kg seeds with carboxy methyl cellulose as sticker. The treatments imposed are given in the table 1.

The treated seeds were sown in nursery trays with cavities and irrigated on daily before transplantation to the field. Prior to sowing, the potting material (sterilized cocopith) was inoculated with one gram of *G. mosseae* inoculum containing 20 spores/gm of carrier material depending on the treatment imposed. Care was taken to avoid cross contamination. The 20 days old seedlings were transplanted along with the soil holding the seedlings in the nursery tray cavities, to the holes made in the soil at 22.86 x 15.24cms spacing in each plot. The plots were irrigated once in 8-10 days depending on the rains received. Data on plant height and number of productive tillers were collected on 90^{th} day selecting 8 plants/replicate avoiding the boarder rows. Harvest was made on 120^{th} day from each replicate on whole plot basis avoiding the boarder rows. Grain weight/earhead, grain and straw yield were collected after sun drying till a constant weight was obtained. Yield per hectare was calculated. The data was statistically analyzed following the method developed by Sundaraj *et al.*,[12].

III. RESULTS AND DISCUSSION

The results obtained on the response of finger millet to combined inoculation with the phosphate solubilizing diazotrophic *Azotobacter chroococcum* (DT) and phosphate mobilizing AM fungus *Glomus mosseae* are presented in the table 1. Significant differences were recorded on the following parameters studied.

TABLE 1
RESPONSE OF FINGER MILLET TO DUAL INOCULATION WITH P SOLUBILIZING DIAZOTROPHIC *AZOTOBACTER CHROOCOCCUM* (DT) AND AM FUNGUS *GLOMUS MOSSEAE* UNDER FIELD CONDITIONS (AVERAGE DATA OF FOUR HARVESTS)

Treatments	Plant height (cm)	No. of Productive tillers	Grain weight/ Earhead (gm)	Grain yield/ha (kg)	Straw yield/ha (kg)
T1 Uninoculated control - 100% N & P	112	4.25	4.53	3890	8947
T2 75% N & P	99	4.17	3.84	3210	7704
T3 50% N & P	70	4.13	2.88	2380	5712
T4 100% N & P + *Ac* (DT)	121	4.30	5.15	4429	10629
T5 75% N & P + *Ac* (DT)	108	4.28	5.00	4280	9416
T6 50% N & P + *Ac* (DT)	106	4.19	4.93	4128	8668
T7 100% N & P + *Gm*	118	4.30	4.66	4010	9223
T8 75% N & P + *Gm*	100	4.26	4.27	3651	8560
T9 50% N & P + *Gm*	80	4.25	3.35	2888	6931
T10 100% N & P + *Gm* + *Ac*	130	4.31	5.85	5096	12230
T11 75% N & P +*Gm*+ *Ac*	124	4.27	5.75	4979	11949
T12 50% N & P + *Gm*+ *Ac*	120	4.28	5.30	4594	10290
CD @ 5%	**4.20**	**0.05**	**0.60**	**453.02**	**573.00**

Ac- Azotobacter chroococcum; Gm- Glomus mosseae

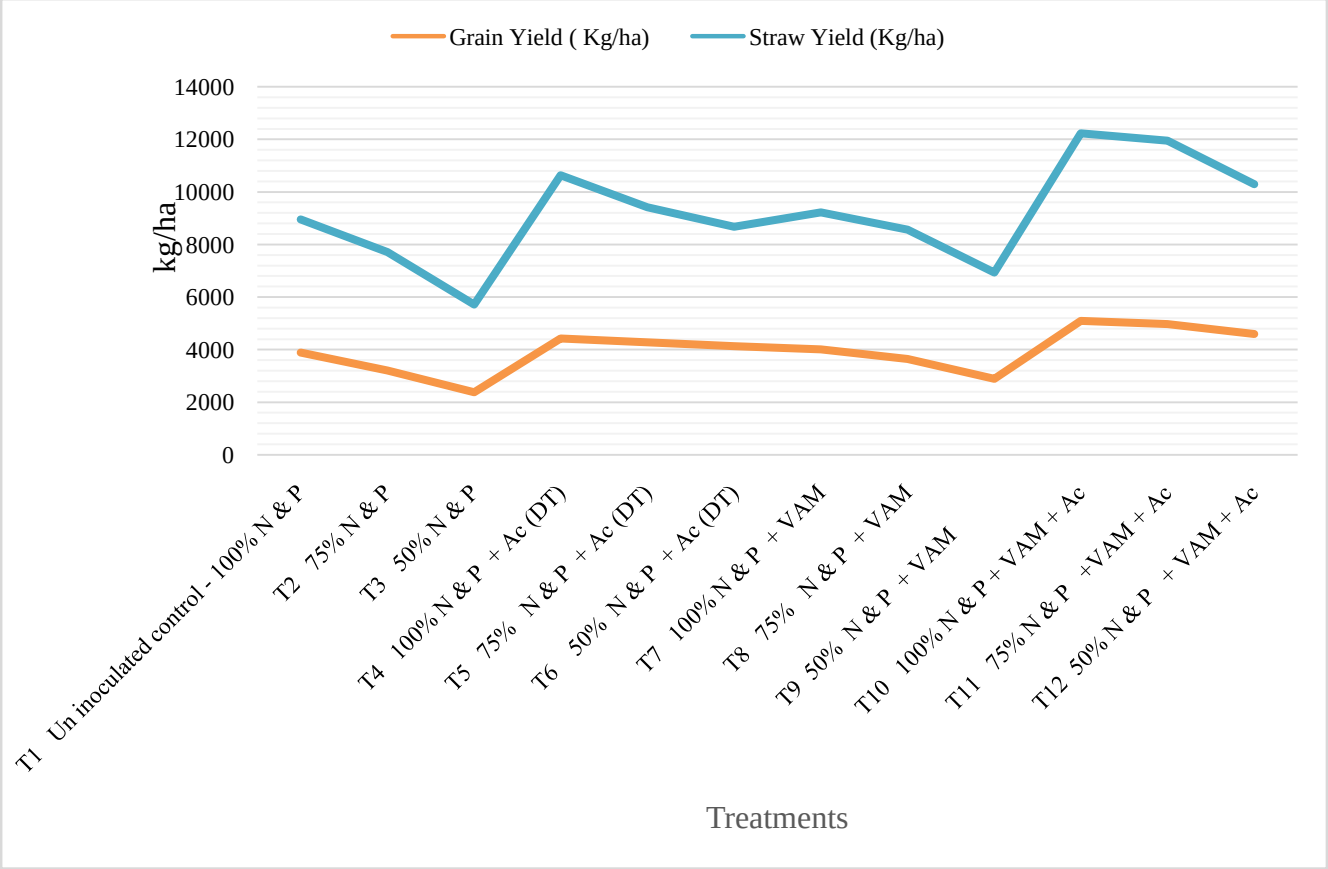

FIGURE 1: Grain and Straw yield in finger millet in response to combined inoculation with *A.chroococcum* (DT) and *G. mosseae*

3.1 Growth Parameters

Plant height varied significantly in response to combined inoculation in treatments T-10 (130 cm), T-11 (124 cm) and T-12 (120 cm) provided with 100, 75 and 50 percent of the recommended dose of N & P fertilizers (100 : 50 kg/ha) as compared to the uninoculated control T-1 (112 cm) receiving 100% recommended N and P fertilizers. The increase recorded was 16.07, 10.71 and 7.14 percent respectively over control. The plants receiving individual inoculants of *A. chroococcum* (DT) and *G. mosseae* (T-4 and T-7) in presence of 100% N and P also recorded significant increase in height (121 and 118 cm) over control but not over T-10 but were on par with T11 receiving combined inoculation in presence of 75% N and P. Plant height was not significant over control in T-5 and T-8 receiving 75% N and P and T-6 and T-9 with 50% N and P in all treatments inoculated singly with *A. chroococcum* (DT) or *G. mosseae.*

The number of productive tillers per plant ranged from 4.13 in uninoculated treatment (T-3) receiving 50% N and P to 4.31 in T-10 receiving combined inoculation and 100% N and P. The plants receiving combined inoculation in presence of 100% N and P (T-10) produced significantly higher number of tillers (4.31) compared to control and other treatments. The number of tillers in plants provided with single inoculants and 100% N & P were on par with control. Other treatment was not significant with control.

3.2 Yield parameters

The grain weight per earhead, grain and straw yield per hectare recorded more significant differences among treatments. Grain weight per earhead in plants inoculated combined in presence of graded levels of N and P fertilizers (T10, T11 and T12) increased significantly (5.85, 5.75 and 5.30gm) over control T1 (4.53 gm) by 29.14, 26.93 and 17.20 percent respectively. Among the treatments receiving individual inoculations with 100% N & P, only T4 inoculated with *A. chroococcum* (DT) recorded significant increase (5.15gm) over control with an increase of 13.68% while other treatments were less significant compared to control.

Similar to the trend observed in grain yield per earhead, the grain yield per hectare also showed significant differences among different treatments. Highest increase was recorded in T10 inoculated combined in presence of 100% N and P fertilizers

yielding 5096 kg/ha followed by T11 (4979 kg) and T12 (4594 kg) provided with 75 and 50 percent N and P inoculated together with *A. chroococcum (DT)* and *G. mosseae* registering an increase of 31.00, 27.99 and 18.10 percent respectively over control. Among the singly inoculated treatments in presence of 100% N and P, T4 with *A. chroococcum* (DT) yielded 4429 kg/ha which was significant over control with an increase of 13.85% while the same inoculant with 75 and 50 percent N and P (T5 and T6) recorded yield which was on par with control but with an increase of 10.02 and 6.2 percent respectively. Other individually inoculated treatments were not significant over control.

Straw yield also registered an improvement in treatments corresponding to those treatments showing increased grain yield. Significant increase was recorded in three treatments T10, T11 and T12 receiving combined inoculation in presence of all the three levels of fertilizers. The yield was 12230, 11949 and 10290 kg/ha, an improvement of 36.69, 33.55 and 15.01 percent over control. The yield levels were significant over all the other inoculated and uninoculated treatments. Straw yield in response to single inoculation with *A. chroococcum* (DT) in presence of 100% N and P (T4) was significant over control with 10,629 kg/ha, an increase of 18.80% while with 75 and 50% N and P (T5 and T6) the yield was on par with control recording 9416 and 8668 kg/ha an increase of 5.24 and 3.12 percent respectively. Straw yield with *G. mosseae* as single inoculant in presence of 100% N and P was on par with control (T1) with 9223 kg/ha, an increase of 7.57% while treatments T8 and T9 with 75 and 50% N and P were not significant over control (fig 1).

The present study reports the synergistic interactions of phosphate solubilizing diazotrophic *A. chroococcum* and the P mobilizing AM fungus *G. mosseae* on grain and straw yield improvement in finger millet. The synergistic interactions between plant growth promoting bacteria and AM fungi on crop plants are on account of a wide range of mechanisms including nitrogen supplementation and available P transportation especially in low fertility soils. A few reports are available on possession of dual traits of P solubilizing abilities in *Azotobacter*, *Azospirillum* species among nitrogen fixers and nitrogen fixing abilities in *Bacillus* species among phosphate solubilizing bacteria and their positive influence in improving crop yield in wheat, maize and finger millet (Kumar *et al*., [13]; Monica *et al*., [7]; Rahim *et al*.,[8]; Chandana and Venkataramana, [10]). Synergistic interactions between AM fungi and *Azotobacter* having single trait of nitrogen fixation have been reported by Bhagyaraj [11]; Kumar *et al*., [13] ; Rishi *et al*., [14]. However, there are no such studies on the synergistic interactions involving P-solubilizing diazotrophic *A. chroococcum* in association with AM fungi in crop plants in general and finger millet in particular. This is the first report on such interactions between P solubilizing, *A. chroococcum* and AM fungus *G. mosseae* on finger millet. Several possible mechanisms mediating the interactions have been reported such as nitrogen fixation and hormone production by *Azotobacter* and phosphate acquisition, uptake and transportation by AM fungi and both together stimulating root hair and lateral root formation, and increased root biomass production, expanding the nutrient and water absorptive capacity of root system in the soil and also increased root colonization by AM fungus (Bhagyaraj [11]; Rishi *et al*., [14]). The results obtained in the present study suggest such interactions between *A. chroococcum* (DT) and *G. mosseae* reflecting on the increased grain and straw yield in finger millet. Phosphorus is critical for biological nitrogen fixation (Singleton *et al*., [15] and Arun[16]).The presence of both the traits in *A. chroococcum* (DT) act complementarily in increasing nitrogen fixation as the phosphorus required is made available by the same bacterium through P-solubilization. The AM fungi not only absorb the soil available P but also the P released through solubilization process by the bacterium. The increments in grain and straw yield recorded in the present study can be attributed to the above mechanisms. The study also reports an important finding that 25 to 50% of N and P fertilizers can be reduced in presence of these organisms thus saving money on fertilizers without reduction in the yield parameters. It can be concluded that the P solubilizing diazotrophic *A. chroococcum* (DT) can be the next generation microbial inoculant for sustainable finger millet production with enhanced activity in combination with AM fungus *G. mosseae*. The combination not only helps in supplementing the N & P nutrients under subsistence farming but also to reduce their application under affluent farming.

REFERENCES

[1] Anonymous, Agriculture Statistics, Dept. of Agriculture, Government of Karnataka, India, 2017.

[2] A.K. Halder, A. K. Mishra, P. Bhattacharyya, and P.K. Chakrabartty, "Solubilization of rock phosphate by *Rhizobium* and *Bradyrhizobium*," *J. Gen. Appl. Microbiol.*,vol.36, 1990, pp. 81-92.

[3] M.H. Abd-alla, "Phosphates and nitrification of organic phosphorus by *Rhizobium* biovar *viceae*," *Letters in Appl. Microbiology*, vol. 18, 1994a, pp.294-296.

[4] M.H. Abd-alla, "Solubilization of rockphosphate by *Rhizobium* and *Bradyrhizobium", Folia Microbiologica*, vol.39, 1994b, 130-134.

[5] S. Sheshadri, R. Muthukumaraswamy, C. Lakshminarasimhan, and S.Ignacimuthu, "Solubilization of inorganic phosphates by *Azospirillum halopraeferans,"Curr Sci.*, vol. 79, 2000, pp. 565-567.

[6] Vivek Kumar, Rishi Kumar Behl, and Neeru Narula, "Establishment of phosphate solubilizing strains of *Azatobacter chroococcum* in the rhizosphere and their effect on wheat cultivars under greenhouse conditions,"*Microbiol. Res.,* Vol. 156, 2001, pp. 87-93.

[7] Monica del Pilar Lopez Ortega, Poola Jimena Criolla Campoh, Ruth Milena Gomez-Vargas, Mauricio Came Rusinque, German Estroda Bonilla, Maria Fernanda Garrido-Rubiano and Ruth Bonilla-Buitrago, "Charactrization of diazotrophic phosphate solubilizing bacteria as growth promoters of maize plants,"*Rev. Colomb. Biotechnol.*,Vol. XV(2), 2013, pp. 115-123.

[8] Rahim Nosrati, Parviz Owlia, Horieh Saderi, Iraj Rasooli and Mohammad Ali Malboobi, "Phosphate solubilization characteristics of efficient nitrogen fixing soil *Azatobacter* strains", *Iran. J. Microbiol*., vol. 6(4), 2014, pp. 285-295.

[9] M.E. Hafsa Mohamed, F.M.AShaieb and H.M.A EL-Komy, "Solubilization of inorganic phosphates by isolated *Azospirillum lipoferum* (H3) as free or alginate immobilized inoculation, *Control*, vol.1, 2017, pp.14.

[10] M. Chandana and G.V. Venkataramana, "Influence of phosphate solubilizing *Azotobacter chroococcum* on growth and yield of finger millet (*Eleusine Coracana* (L.) Gaertn.) under graded levels of N & P fertilizers," *Trends in Biosciences*, vol. 11 (36), 2018, pp.3930-3933.

[11] D.J Bhagyaraj, "Mycorrhizal Fungi". Proc. Of Indian Natn. Scad., vol. 80(2), 2014, pp. 415-428.

[12] N. Sundarraj, S. Nagaraju, M.N. Venkataramu, and M.K. Jagannath, Design and analysis of field experiments, Misc. Series No. 22.University of Agricultural Sciences, Bangalore, 1972,424 pp.

[13] V. Kumar, N. Kumar Aggarwal, B.P. Singh, "Performance and persistence of phosphate solubilizing *Azatobacter chroococcum* in wheat rhizosphere", *Folia Microbiol.*, Vol. 45 (4), 2000, pp. 343-347.

[14] Rishi K. Behl, Sabine Ruppel, Erika Kothe and Neeru Narula, "Wheat x Azotobacter x VA Mycorrhiza interactions towards plant nutrition and growth – a review", *Journal of Applied Botany and Food Quality*, vol. 81, 2007, pp. 95-109.

[15] P.W. Singleton, H.M. Abdel Magid and J.W. Tavares, "Effect of phosphorus on the effectiveness of strains of *Rhizobium japonicum", Soil Science society of America journal*, vol. 49(3), 1985, pp. 613-616.

[16] Arun K Sharma, Biofertilizers for Sustainble Agriculture 2.Jodhpur, Agrobios, India, 2005, 407. pp.163-166.