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Growth Response of *Amaranthus gangeticus* to *Azotobacter chroococcum* Isolated from Different Agroclimatic Zones of Karnataka

C. Sandeep*, S.N. Rashmi, V. Sharmila, R. Surekha, R. Tejaswini, C.K. Suresh

Department of Biotechnology, University of Agricultural Sciences, GKVK Campus, Bangalore -5600651, India

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*Corresponding Author

Tel : + 91-9886778079
Fax : +91-803330277

Email:
sandeep.c.naidu@gmail.com

Summary

In the present study *Azotobacter chroococcum* was isolated from various agro climatic zones of Karnataka. The effect of *A. chroococcum* isolates on seed germination of *Amaranthus.gangeticus* was studied and also the effect of *A.chroococcum* isolates on growth, biomass and nutrient content of *Amaranthus gangeticus* was studied under green house conditions. In seed germination studies the length of plumule and radicle was higher with inoculation of *A. chroococcum* isolates than uninoculated control plants. Treatments of *A.chroococcum* isolates from ten different zones of Karnataka were given to seedlings of *Amaranthus gangeticus* to study plant growth parameters such as plant height, number of leaves, number of branches, root length, shoot and root fresh and dry weight and nutrient uptake. Plants inoculated with *Azotobacter* isolates performed well when compared to uninoculated control plants. In Biochemical analysis chlorophyll content, nitrogen, phosphorous and potassium content was higher when compared to uninoculated control plants. The results of these experiments concluded that plants inoculated with *Azotobacter* isolates showed better growth response, biomass yield and nutrient content when compared with uninoculated control plants. Hence plants inoculated with *A.chroococcum* isolates were found to enhance the plant growth, biomass and nutrient content.

Key Words: *Amaranthus gangeticus*, *Azotobacter chroococcum*, Growth response, biochemical analysis and seed germination

Introduction

Azotobacter spp. are Gram negative, aerobic, asymbiotic free living nitrogen fixing bacterium belonging to family Azotobacteriaceae, section VI of Bergey's Manual of Determinative Bacteriology that play an important role in improving plant growth and yield by producing plant hormones and antimicrobial substances. *Azotobacter chroococcum* is a well known free living nitrogen fixing bacterium capable of synthesizing various plant hormones and is frequently used as a nitrogenous biofertilizers for a number of crops. Several field trials have demonstrated that under certain environmental conditions, inoculation with *Azotobacter* has beneficial effects on plant yields, due to the increase of fixed nitrogen content in soil [1-3] and due to the microbial secretion of stimulating hormones, like gibberellins, auxins and cytokinins [4-6].Several authors have shown the beneficial effects of *A.chroococcum* on vegetative growth and yields of maize [7-8], as well as the positive effect of inoculation with this bacterium on wheat [9-10].The geographical area of Karnataka is classified into ten agro-climatic zones *viz.*, North eastern transition zone, North eastern dry zone, Northern dry zone, Central dry zone, Eastern dry zone, Southern dry zone, Southern transition zone, Northern transition zone, Hilly zone and Coastal zone. Each zone has its own characteristic feature in relation to climatic condition, soil type, vegetation *etc.,* which has influence on the establishment of diversified flora and fauna.

Amaranthus gangeticus belongs to family Amaranthaceae and is a drought tolerant plant. Amaranth is a traditional seed crop grown by the Aztecs and Southwest peoples as a grain. The grain is high in Lysine and the young leaves are high in Iron and Calcium. Seeds can be cooked as a hot cereal or ground and used as flour.

In the present study isolation and identification of *A. chroococcum* from different agroclimatic zones of Karnataka was carried out .Seed germination studies of *A. gangeticus* inoculated with *A.chroococcum* isolates was carried out and growth response of *A.gangeticus* to *A. chroococcum* isolated from different agroclimatic zones of Karnataka was studied under green house conditions. Also seed germination studies of *A.gangeticus* inoculated with *A.chroococcum* isolates was carried out.

Materials and Methods

Isolation of Azotobacter: The soil samples collected were dried inside the laboratory at 28°C. The four soil samples collected from each soil type were mixed well to get a pooled soil sample for a zone. Totally ten soil samples were obtained for the study. Each soil sample was sieved through 1000µmesh to remove the bigger soil particles and debris. The sieved soil samples were used for the isolation of the *Azotobacter* species. *Azotobacter* species were isolated using Waksman No. 77 N-free medium by employing serial dilution plate technique. Then plates were incubated for 72 hours or

more at 28°C±2. The plates were checked for *A. chroococcum* growth and pigmentation on prolonged incubation. The isolated colonies of *A. chroococcum* were re-streaked for purification and the pure isolates thus obtained were maintained on the agar slants prepared with Waksman No. 77 medium.

Identification of the Isolates: For identification, presumptive tests were carried out following standard methods as outlined in Bergey's Manual of Systematic Bacteriology [11]. The observations were taken as follows:

Morphology: Cell shape was observed by simple staining and cell motility was observed Hanging Drop Technique.

Microscopic Observation: Gram's staining was carried out for all the isolates grown on W-77 liquid medium. Observation was recorded.

Capsule: A.chroococcum isolates were grown on W-77 N-free agar medium for 3 days at room temperature and negative staining with nigrosine was done.

Cyst formation and pigmentation: A.chroococcum isolates were grown on W-77 N-free agar medium for 7 days. These isolates were simple stained with crystal violet for cyst formation and change of colour from white to dark brown was recorded.

Inoculum preparation: The isolated colonies of *Azotobacter chroococcum* maintained on the agar slants prepared with Waksman No. 77 medium was inoculated in 250 ml conical flask containing 100 ml W-77 broth and incubated at 28 ± 2 ^{o}C under shaking at 100 rpm for six days.

The grown cultures were homogenized and 5ml each culture (12.4x10^{6}cfu/ml) inoculated to each pot.

Plant Growth Response under Green House Conditions and seed germination studies: Sand: soil mixture in the ratio of 1:1 v/v was filled into pots containing 4 kg soil (red sany loam). Planting holes were made at the centre of the pots to enable the inoculation of *Azotobacter chroococcum* isolates and 5ml inoculum representing each zone *A. chroococcum* isolate was separately added to the pot as per the treatment allocation. There were three replications each treatment. The treatments of experiment includes: C - Control (uninoculated control), T1 – *A. chroococcum* isolate from Zone 1, T2 - *A.chroococcum* isolate from Zone 2, T3 - *A.chroococcum* isolate from Zone 3, T4 - *A.chroococcum* isolate from Zone 4, T5 - *A.chroococcum* isolate from Zone 5, T6 - *A.chroococcum* isolate from Zone 6, T7 - *A.chroococcum* isolate from Zone 7, T8 - *A.chroococcum* isolate from Zone 8, T9- *A.chroococcum* isolate from Zone 9, T10- *A. chroococcum* isolate from Zone 10 (T- Treatments of isolates of zones 1 to 10).

An experiment pertaining to seed germination of *Amaranthus gangeticus* was also conducted to study the effect of *A.chroococcum* isolates on *A.gangeticus*. The length of plumule and radicle was recorded after one week. In this study we report the influence of *A. chroococcum* isolates on growth and biomass of *Amaranthus gangeticus*. Then, one week old *Amaranthus gangeticus* having uniform height were planted in the pots. One plant per pot was maintained and there were three replications per each treatment. These pots were watered as and when required until harvest. The plants were harvested 45 days after planting. The observations for growth parameters like plant height, number of leaves and girth of stem were recorded at 15, 30, 45 days intervals. The plants were harvested at 45 days and the plant biomass was recorded after drying the harvested plants at 60°C in a hot air oven for 7 days to reach constant weight. The plant growth parameters like shoot fresh weight and dry weight, root fresh weight and dry weight were recorded at the time of harvest. Biochemical analysis like total chlorophyll content, nitrogen, phosphorous, potassium content were recorded after the plants were harvested.

Total chlorophyll content of the leaf was estimated following DMSO method [12]. The nitrogen estimation for root and shoot was carried out by Micro–Kjeldahl method [13]. Phosphorus concentration was estimated colorimetrically following the vanadomolybdate yellow colour method [14]. Potassium concentration in the plant tissues was estimated by using flame photometer [15].

The data obtained from the experiments were subjected to one-way analysis of variance for completely randomized design (CRD) using MSTAT software. The treatment means were separated by Duncan's Multiple Range test (DMRT) a 5% level of significance [16].

Results and Discussion

Isolation and Identification: Ten *Azotobacter* isolates from different agroclimatic zones of Karnataka were isolated on Waksman 77 N-free agar medium. Waksman No.77 [17] was used for isolation of the *Azotobacter chroococcum*. Observations for growth characters on Waksman 77 N-free agar medium were recorded and presented in Table-1. *A.chroococcum* produces characteristic brown to black pigment. Pigmentation and colony characters of *Azotobacter* spp were studied [18-20]. The morphological characters of the colonies were found to be oval to round in shape while some were blunt ended long cells. Cells were motile, gram negative and formed capsule and microcyst. It was reported that bacteria isolated from barely roots were gram negative, motile and identified as *A.chroococcum*[21]. All the isolates representing each zone were tested for growth on different carbon source viz., mannitol, glucose and sucrose. All the 10 isolates grew on media containing mannitol, glucose and sucrose.

Table 1: Growth characters of *Azotobacter chroococcum* isolates

Azotobacter chroococcum isolates	Cultural Characters	Pigmentation	Oxygen Requirement
Zone-1 Isolate	Good growth, flat entire slimy colony	Light Brown	Aerobic
Zone-2 Isolate	Moderate growth, flat entire slimy colony	Dark Brown	Aerobic
Zone-3 Isolate	Good growth, flat entire slimy colony	Pale Brown	Aerobic
Zone-4 Isolate	Good growth, raised entire slimy colony	Dark Brown	Aerobic
Zone-5 Isolate	Good growth, raised entire slimy colony	Dark Brown	Aerobic
Zone-6 Isolate	Moderate growth, flat entire slimy colony	Dark Brown	Aerobic
Zone-7 Isolate	Moderate growth, flat entire slimy colony	Dark Brown	Aerobic
Zone-8 Isolate	Moderate growth, flat entire slimy colony	Dark Brown	Aerobic
Zone-9 Isolate	Moderate growth, flat entire slimy colony	Light Brown	Aerobic
Zone-10 Isolate	Moderate growth, raised entire slimy colony	Dark Brown	Aerobic

Table 2- Seed Germination of *Amaranthus gangeticus* as influenced by *Azotobacter chroococcum* isolates

Zones	Plumule(cm)	Radicle(cm)
C	0.6	0.4
T 1	4.4	2.2
T 2	5.1	2.8
T 3	5.8	3.4
T 4	4.7	2.5
T 5	5.5	2.1
T 6	6.2	4.5
T 7	6.0	3.5
T 8	5.0	2.7
T 9	4.9	2.6
T 10	4.5	2.4
SEM±	0.044	0.048
CD at 5%	0.1319	0.1425

Isolate with maximum response
T1 to T10: Treatments for isolates from zone 1 to 10

Table 3: Growth parameters of *Amaranthus gangeticus* influenced by Azotobacter chroococcum isolates

Zones	Plant height(cm) 45 DAT	Number of Leaves/plant 45 DAT	Number of Branches (cm)	Root length(cm) 45 DAT	Fresh weight (g/plant) 45 DAT		Dry weight (g/plant) 45 DAT	
					Shoot	Root	Shoot	Root
C	33.8	18.6	1.6	8.4	7.32	0.94	1.94	0.34
T 1	52.68	19.6	2.3	9.0	9.12	1.37	2.39	0.49
T 2	57.29	29.3	5.6	15.3	14.94	2.04	3.34	0.94
T 3	46.03	26.0	3.3	19.0	17.05	2.18	3.82	0.99
T 4	53.96	28.3	5.3	11.3	10.68	1.71	2.81	0.79
T 5	58.11	37.6	6.3	16.3	16.41	2.06	3.58	0.92
T 6	58.89	30.6	6.3	20.6	18.41	2.64	4.11	1.09

T 7	49.62	28.0	5.0	20.0	17.59	2.36	4.05	0.97
T 8	46.48	29.3	3.0	14.3	14.53	1.93	3.25	0.88
T 9	41.35	22.3	3.0	13.0	12.15	1.73	3.19	0.81
T 10	39.01	21.0	2.0	10.5	10.22	1.50	2.65	0.71
SEM ±	0.2503	0.56	0.39	0.5013	0.1633	0.0577	0.0707	0.0483
CD at 5%	0.7385	1.658	1.68	1.479	0.4817	0.1703	0.2086	0.1425

DAT: Days after treatment
T1 to T10: Treatments for isolates from zone 1 to 10

Table 4- Biochemical parameters of *Amaranthus gangeticus* influenced by *Azotobacter chroococcum* isolates

Zones	Total Nitrogen Content (mg/plant dry wt)	Total Phosphorous Content (mg/plant dry wt)	Potassium Content (mg/plant dry wt)
C	1.63	0.007	0.89
T 1	3.03	0.020	1.61
T 2	2.74	0.120	1.38
T 3	6.41	0.124	1.32
T 4	2.54	0.165	1.46
T 5	4.17	0.092	1.69
T 6	4.62	0.124	2.68
T 7	6.07	0.139	2.86
T 8	2.92	0.313	1.79
T 9	2.26	0.054	1.35
T 10	1.73	0.012	1.55
SEM±	0.1807	0.0730	0.0605
CD at 5%	0.5332	0.2154	0.1786

Table 5- Total Chlorophyll Content of *Amaranthus gangeticus* influenced by *Azotobacter chroococcum* isolates

zones	Chlorophyll-a (mg/g fw)	Chlorophyll-b (mg/g fw)	Total Chlorophyll (mg/g fw)
C	0.74	0.38	1.12
T 1	0.79	0.45	1.24
T 2	1.02	0.55	1.57
T 3	1.06	0.64	1.70
T 4	0.88	0.56	1.45
T 5	1.03	0.63	1.66
T 6	1.00	0.74	1.75
T 7	1.10	0.69	1.79
T 8	0.96	0.54	1.51
T 9	0.86	0.50	1.36
T 10	0.82	0.53	1.36
SEM±	0.0316	0.0182	0.0316
CD at 5%	0.0932	0.0538	0.0932

Biochemical and Physiological Tests: The test isolates were found positive when further examined for their biochemical properties for Indole, Methyl red and Vogus proskauer (MRVP) test, citrate test, catalase test and oxidase tests .Based on the colony characters, cell shape, presence of cyst, capsule, gram reaction, and utilization of different carbohydrates tested and biochemical tests, the isolates were confirmed as *A.chroococcum* isolates.

Plant Growth Response and seed germination studies: Plant Growth Response: Sand: soil mixture in the ratio of 1:1 v/v was filled into pots of uniform size. Planting holes were made at the centre of the pots to enable the inoculation of Azotobacter isolates and 5ml inoculum representing each zone *Azotobacter chroococcum* isolate was separately added to the pot as per the treatment allocation. An experiment pertaining to seed germination of *Amaranthus gangeticus* was conducted to study the effect of *A.chroococcum* isolates on *A.gangeticus*. The length of plumule and radicle was maximum in seeds inoculated with zone 6 isolate which is presented in Table 2 and least length of plumule and radicle was recorded in uninoculated control plants.

The plant height of *Amaranthus gangeticus* was significantly increased in the inoculated treatments at 15, 30

and 45 days after treatment (DAT), compared to uninoculated control plants. At 45 days after treatment the Zone-6 isolate recorded maximum plant height, which is followed by zone 5 isolate and least height was recorded in uninoculated control plants which is presented in Table 3. The increased growth might be attributed to the nitrogen fixation and production of growth hormones by *A. chroococcum.* Thus agreeing with earlier observations [22]. The average number of leaves/plant was more in the plants inoculated with Zone 5 isolate at 45 days after treatment, which was followed by the isolate of Zone 6 and uninoculated control plants recorded least number of leaves at the time of harvest. Number of branches was found maximum in Zone 5 and Zone 6 isolates than uninoculated control plants.

The data pertaining to the total fresh and dry weight of shoot and root are presented in Table 3. The isolate from Zone 6 showed maximum shoot fresh weight and dry weight. Similarly, the highest root fresh weight and dry weight was also observed in the treatments inoculated with Zone 6 isolate and least was observed in uninoculated control plants. Increased dry weight is due to enhanced growth, number of leaves and branches, which was influenced probably by greater availability of nitrogen in the soil to the plants inoculated with *A. chroococcum.* These results were similar with the earlier findings, which reported improved yields of Banana varieties by using biofertilizers [23].

Plants inoculated with different isolates of *A. chroococcum* revealed significant increase in nitrogen content of shoots compared to the uninoculated control plants as shown in Table 4. The highest total nitrogen was observed in the plants treated with Zone 3 isolate and least in uninoculated control. These results are in agreement with the earlier findings [24] that reported increased N content in *Azotobacter* inoculated plants. Earlier reports have shown that an increased growth, biomass, nitrogen and phosphorus in Ocimum sanctum and Ocimum kilimandascharicum inoculated with *Glomus fasciculatum*, *A. chroococcum* and *Aspergillus awamori* singly and in combinations [25]. Similarly total phosphorous content was maximum in plants treated with Zone 3 isolate and least in uninoculated control plants as shown in Table 4. But maximum amount of total potassium content was recorded in zone 7 isolate plants and least in uninoculated control plants as shown in Table 4. Chlorophyll content was maximum in Zone 7 treated plants and least in uninoculated control plants as shown in Table 5.

Conclusion

Results of the present study revealed enhanced growth, biomass, chlorophyll content and nitrogen, potassium and phosphorous content of *Amaranthus gangeticus* due to inoculation with *Azotobacter chroococcum* strains isolated from different agro-climatic zones of Karnataka and the zone 6 isolate as the most efficient compared to other strains.

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