Plant Archives Vol. 25, No. 2, 2025 pp. 2839-2846 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org

DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2025.v25.no.2.409

RESPONSE OF PHYSIOLOGICAL AND BIOCHEMICAL TRAITS IN CROSSANDRA VAR. ARKA CHENNA BY MICROBIAL INOCULANTS AND THEIR METHOD OF INOCULATION

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(Date of Receiving-12-07-2025; Date of Acceptance-17-09-2025)

ABSTRACT

A field experiment was conducted during the Rabi season of 2023 at the College of Horticulture-Anantharajupeta, Dr. Y.S.R. Horticultural University to evaluate the influence of different plant growth promoting root endophytes (PGPRE's) and their methods of inoculation on the physiological and biochemical traits of Crossandra variety Arka Chenna. The experiment was carried out in a two-factor factorial randomized block design comprising 60 treatment combinations with three replications. The microbial inoculants included *Piriformospora indica*, *Glomus fasciculatum* and Arka Microbial Consortium. Results indicated that the interaction treatment of M_1P_8 (Soil application with PGPRE's 10 days prior to Crossandra seedling planting x *Piriformospora indica*+ *Glomus fasciculatum*+ Arka Microbial Consortium + 25% RDF) significantly enhanced plant biomass (143.23 g plant^{-1}) and biochemical constituents such as phenol content (5.69 mg 100g^{-1}), proline content (5.67 mg 100g^{-1}) and carotenoid content (3.83 mg g^{-1}) compared to other methods and treatments. The combined inoculation of *Piriformospora indica*, *Glomus fasciculatum* and Arka Microbial Consortium with 25% RDF consistently outperformed other PGPRE treatments in promoting growth and physiological performance. These findings reveal that microbial inoculation, particularly when applied as a soil treatment before planting, can effectively improve growth and antioxidative biochemical responses in Crossandra, offering sustainable alternatives for enhanced floriculture production.

Key words: Crossandra, Microbial inoculants, Plant biomass, Phenol, Carotenoids.

Introduction

Crossandra, commonly known as 'Tropical Flame' or 'Fire Cracker' plant, is one of the important native flower crops in India. It is an ornamental flowering plant, belonging to the family Acanthaceae (Beese and Saravanan, 2020). Among the species, *Crossandra infundibuliformis* is the most commonly cultivated species and supposed to be native of Southern India and Sri Lanka. The cultivation of crossandra is being taken up by large number of farmers in South India, particularly in Tamil Nadu due to assured and regular income (Tejaswi *et al*., 2020). The plants are quite hardy and can be grown for flowerbeds and /or for loose flowers (Supriya *et al*., 2024). According to the survey conducted in Andhra Pradesh, the area and production of Crossandra was 1,865 ha and 7,461 MT (Dept. of Horticulture, Govt. of Andhra Pradesh, 2020-21).

Piriformospora indica is a root-endophytic fungus isolated from the rhizosphere soil of *Prosopis juliflora* and *Ziziphus nummularia* in the Thar Desert of India. Unlike AMF, P. indica can be easily cultured on laboratory media, where it forms typical pear-shaped chlamydospores. It mimics typical AMF and greatly improves the growth and overall biomass production of a broad spectrum of hosts (Ahmadvand and Hajinia, 2018). The plants colonized by *P. indica* have higher concentrations of proline that is known to be responsible for tolerance against osmotic stress. Plants colonized with

P. indica has low level of malondialdehyde (MDA), which is used as a biomarker to measure the level of oxidative stress, suggesting that *P. indica* prevents the colonized plant from oxidative stress (Trivedi *et al.*, 2016).

AMF grows in roots and then extends outwards into soil as extra radical mycelium to intensify the uptake of nutrients. AM fungi play a dominant role for increasing phosphorus solubilization and uptake of Phosphorus (P), Potassium (K), Sodium (Na), Calcium (Ca), Sulphur (S), Magnesium (Mg), Manganese (Mn) and Chloride (Cl) by plants. *G. fasciculatum* is also known to protect plants against root pathogens and offer resistance during drought and salinity (Khatun, 2020). This AMF can interact with wide variety of microorganisms in the rhizosphere (Aslanpour *et al.*, 2019). AMF increases the root surface area, allowing for the exploration of larger soil volumes and addressing zones of water and nutrient depletion surrounding active root surfaces (Othman *et al.*, 2022). Arka Microbial Consortium is a carrier-based product which contains N fixing, P & Zn solubilizing and plant growth promoting microbes viz. *Bacillus aryabhattai*, *Pseudomonas taiwanensis* and *Azotobacter tropicalis* as a single formulation (Aswathi *et al.*, 2020).

As per various estimates by FAO, total fertilizer requirement is predicted to grow at 1.6% per annum globally. Demand for essential nutrients like N, P_2O_5 and K_2O were grown by 1.5%, 2.2% and 2.4% respectively, from 2015 to 2020. This will result in an increase in the overall production and consumption of fertilizers in the next 5 years. In recent times, the consumption of P fertilizer increased by 3.2% from 2002 to 2010 (Lun *et al.*, 2018). The application of PGPRE's helps in soil remediation by solubilizing and mobilizing the insoluble nutrients into soluble form. Another beneficial aspect is that after continuous use of PGPRE's for 3–4 years, there is no need for their application, as parental inoculation is sufficient for growth and multiplication. This will help in minimizing the cost of FYM and other fertilizer inputs. By keeping this in view, the present investigation was carried out to study the influence of PGPRE's on the physiological and biochemical attributes of Crossandra var. Arka Chenna.

Materials and Methods

During the *Rabi* season of 2023, a field experiment was carried out at the open field conditions of College of Horticulture, Anantharajupeta, Dr. Y.S.R. Horticultural University, Andhra Pradesh, situated at 13.99° N latitude and 79.32° E longitude. Healthy and uniform rooted cuttings of Crossandra variety Arka Chenna were used as the planting material. These cuttings, along with the plant growth promoting microbial inoculant Arka Microbial Consortium (AMC) were obtained from ICAR–Indian Institute of Horticultural Research (IIHR), Bengaluru. Additional PGPRE's included were *Piriformospora indica* and *Glomus fasciculatum*. The former PGPRE was procured from Amity University, Noida while later PGPRE was supplied by the Agricultural Research Station, Amaravathi, Andhra Pradesh.

The experiment was laid in a two-factor factorial randomized block design (RBD) with 60 treatment combinations replicated thrice. All the data recorded for physiological and biochemical parameters were analyzed using two-way ANOVA under a two-factorial RBD, at 5% level of probability ($p<0.05$) to determine significant differences among the treatments. The details of the experimental factors are outlined below:

Factor-1: Method of application

M_1 - Soil application with PGPRE's 10 days prior to Crossandra seedling planting

M_2- Inoculation of PGPRE's to cuttings prior to planting

M_3- Seedling root inoculation with PGPRE's through root treatment just prior to planting

M_4- Seedling root inoculation with PGPRE's through soil drenching on 10th day after transplanting

M_5- Seedling root inoculation with PGPRE's through soil drenching on 20th day after transplanting

M_6- Seedling root inoculation with PGPRE's through soil drenching on 30th day after transplanting

Factor-2: Plant Growth Promoting Root Endophytes (Treatments)

T_1- *Piriformospora indica*

T_2- *Glomus fasciculatum*

T_3- Arka Microbial Consortium

T_4- *Piriformospora indica* + *Glomus fasciculatum* + Arka Microbial Consortium

T_5- *Piriformospora indica* + 50% RDF

T_6- *Glomus fasciculatum* + 50% RDF

T_7- Arka Microbial Consortium + 50% RDF

T_8- *Piriformospora indica* + *Glomus fasciculatum* + Arka Microbial Consortium + 25% RDF

T_9- RDF alone (Dr. YSRHU recommendation for Crossandra)

T_{10}- Absolute control

Physiological studies

Physiological attributes like plant biomass and stomatal conductance were recorded. Plant biomass was measured by uplifting the plant along with the roots and clean them under the tap water gently. Then, the plants were placed in a hot air oven and dried at 70ºC - 80ºC for a period of 72 hours. After that, the plants were weighed and values were recorded. While, stomatal conductance was measured by using LCi-SD portable photosynthetic system *viz*., Infra-Red Gas Analyzer (IRGA).

Biochemical studies

Phenol

One gram of plant sample was homogenized with 20ml of 80% methanol and the extract is pooled and brought to 50ml. A 0.5ml of aliquot is mixed with 0.2ml of Folin-Ciocalteaus's reagent followed by 3.3 ml distilled water and 1ml of 20% Na_2CO_3. After settling for 30 minutes, the blue colour developed was read in spectrophotometer at 700nm. Phenols are quantified using gallic acid standard curve and expressed as mg $100g^{-1}$ (Singleton *et al*., 1965).

Proline

0.5g of fresh leaf tissue was homogenized with 10ml of 3% sulfo-salicylic acid and filtered. Then, 2ml of filtrate was mixed with 2ml of acid-ninhydrin and 2ml of glacial acetic acid and placed in water bath for 1hr at 100°C. After, the mixture was extracted with 4ml toluene and absorbance was read at 520nm and expressed as µg g^{-1} FW (Bates *et al*., 1973).

Flavonoid

Two grams of plant sample was homogenized with 20ml of 80% methanol and the extract is pooled to 50ml. 1ml extract is sequentially reacted with 0.3ml of 5% $NaNO_2$, followed by 0.3ml of 10% $AlCl_3$ and 3.4ml of 0.1N NaOH. After allowing it for 10 mins, absorbance was read at 510nm and expressed as mg $100g^{-1}$ (Chang *et al*., 2003).

Carotenoid

500mg of fine and chopped leaf sample was immersed in 10ml of dimethyl sulphoxide (DMSO) and incubated for 4 hours at 70°C. Later, 1ml extract was diluted to 5ml with DMSO and absorbance was read at 645, 663 and 480 nm and expressed as mg g^{-1} (Lichtenthaler and Wellburn, 1983).

Results

The application of microbial inoculants significantly influenced the physiological attributes of Crossandra variety Arka Chenna among the methods of inoculation, PGPRE's and the interactions ($p \leq 0.05$). M_1 (Soil application with PGPRE's 10 days prior to Crossandra seedling planting) emerged as the significant inoculant method and recorded superior plant biomass (115.66 g $plant^{-1}$) among all other methods of inoculation. While, M_2 (Inoculation of PGPRE's to cuttings prior to planting) had shown least plant biomass (86.41 g $plant^{-1}$). Regarding the PGPRE's, P_8 (*Piriformospora indica*+ *Glomus fasciculatum*+ Arka Microbial Consortium + 25% RDF) exhibited significantly superior plant biomass (121.87 g $plant^{-1}$) among all other PGPRE's. Meanwhile, least plant biomass (40.46 g $plant^{-1}$) was noted in P_{10} (Absolute control). Greater plant biomass (143.23 g $plant^{-1}$) was enhanced in the interaction treatment M_1P_8 (Soil application with PGPRE's 10 days prior to Crossandra seedling planting x *Piriformospora indica*+ *Glomus fasciculatum*+ Arka Microbial Consortium + 25% RDF), marking the consistent trend of improved performances under microbial based interventions. Although, minimum plant biomass (33.08 g $plant^{-1}$) was recorded in M_2P_{10} (Inoculation of PGPRE's to cuttings prior to planting x Absolute control).

In view of stomatal conductance, M_2 (Inoculation of PGPRE's to cuttings prior to planting) had shown best stomatal conductance (0.14 µmol m^{-2} s^{-1}) while lowest stomatal conductance (0.06 µmol m^{-2} s^{-1}) was showed in M_5 and M_6. Among the PGPRE's, greater stomatal conductance (0.18 µmol m^{-2} s^{-1}) was found in P_8 (*Piriformospora indica*+ *Glomus fasciculatum*+ Arka Microbial Consortium + 25% RDF) and least stomatal conductance (0.02 µmol m^{-2} s^{-1}) was noted in P_{10}. Among the interactions, maximum stomatal conductance (0.31 µmol m^{-2} s^{-1}) was exhibited in M_2P_4 (Inoculation of PGPRE's to cuttings prior to planting x *Piriformospora indica*+ *Glomus fasciculatum*+ Arka Microbial Consortium) whereas minimum stomatal conductance (0.01 µmol m^{-2} s^{-1}) was recorded in M_2P_{10}.

Biochemical traits

The results confirmed that microbial interactions significantly promote the anti-oxidant and biochemical activities in Crossandra variety Arka Chenna among the methods of inoculation, PGPRE's and the interactions ($p \leq 0.05$). It was proven that greater phenol content (5.69 mg $100g^{-1}$), proline content (5.67 mg $100g^{-1}$) and carotenoid content (3.83 mg g^{-1}) were recorded in M_1 (Soil application with PGPRE's 10 days prior to Crossandra seedling planting) among all other methods of inoculations. While, M_2 (Inoculation of PGPRE's to cuttings prior to planting) had shown least levels of phenols (1.95 mg $100g^{-1}$), proline (3.19 mg $100g^{-1}$) and carotenoid (0.91 mg g^{-1}). Highest flavonoid content (2.04 mg $100g^{-1}$) was recorded in M_2 (Inoculation of PGPRE's to cuttings prior to planting) and lowest flavonoid content

Table 1 : Effect of methods of inoculation, Plant Growth Promoting Root Endophytes (PGPRE's) and their interaction on plant biomass (g) content in Crossandra var. Arka Chenna.

Method of inoculation (M)	Plant Growth Promoting Root Endophytes (P)										
	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	Mean M
M1	128.94	127.72	137.39	139.10	130.08	121.13	124.45	143.23	60.94	43.63	115.66
M2	89.68	93.77	98.46	104.20	96.04	94.93	90.71	108.20	55.03	33.08	86.41
M3	117.62	113.73	125.93	114.48	117.96	119.41	113.20	128.91	65.72	39.20	105.62
M4	99.97	106.01	111.39	114.89	98.54	109.62	92.76	120.74	66.51	42.84	96.33
M5	91.67	96.45	98.31	109.90	93.42	86.57	90.14	117.60	65.97	40.11	89.01
M6	99.26	97.03	90.54	109.36	103.36	101.34	94.41	112.56	62.60	43.92	91.44
Mean P	104.52	105.79	110.34	115.32	106.56	105.50	100.95	121.87	62.79	40.46	
Source	**Method of inoculation (M)**				**PGPRE's (P)**				**M x P**		
S.Em±	0.736				0.951				2.329		
CD (*P*=0.05)	2.062				2.662				6.522		

Table 2 : Effect of methods of inoculation, Plant Growth Promoting Root Endophytes (PGPRE's) and their interaction on stomatal conductance content in Crossandra var. Arka Chenna.

Method of inoculation (M)	Plant Growth Promoting Root Endophytes (P)										
	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	Mean M
M1	0.08	0.16	0.14	0.07	0.09	0.04	0.24	0.22	0.03	0.03	0.11
M2	0.19	0.11	0.16	0.31	0.02	0.14	0.20	0.29	0.02	0.01	0.14
M3	0.09	0.08	0.06	0.10	0.06	0.04	0.04	0.20	0.03	0.02	0.07
M4	0.03	0.04	0.14	0.04	0.04	0.11	0.04	0.21	0.04	0.03	0.07
M5	0.07	0.07	0.07	0.11	0.03	0.06	0.09	0.09	0.02	0.02	0.06
M6	0.09	0.03	0.06	0.03	0.20	0.06	0.03	0.07	0.04	0.02	0.06
Mean P	0.09	0.08	0.10	0.11	0.07	0.07	0.11	0.18	0.03	0.02	
Source	**Method of inoculation (M)**				**PGPRE's (P)**				**M x P**		
S.Em±	0.003				0.004				0.011		
CD (*P*=0.05)	0.010				0.012				0.031		

(1.30 mg 100g^{-1}) was showed in M_5. While coming to the microbial inoculants, different microbes shown different impacts on biochemical traits. P_8 (*Piriformospora indica*+ *Glomus fasciculatum*+ Arka Microbial Consortium + 25% RDF) exhibited superior performance and recorded maximum proline content (6.62 mg 100g^{-1}) and carotenoid content (3.11 mg g^{-1}) while phenol content (6.60 mg 100g^{-1}) was more in P_5 (*Piriformospora indica* + 50% RDF) and flavonoid content (2.04 mg 100g^{-1}) was greater in P_4 (*Piriformospora indica*+ *Glomus fasciculatum*+ Arka Microbial Consortium). Yet, minimum levels of phenols (1.38 mg 100g^{-1}), proline (1.77 mg 100g^{-1}), flavonoid (0.73 mg 100g^{-1}) and carotenoid (1.03 mg g^{-1} were recorded in P_{10} (Absolute control). Similarly, the interaction treatments also shown different effects. M_1P_8 (Soil application with PGPRE's 10 days prior to Crossandra seedling planting X *Piriformospora indica*+ *Glomus fasciculatum*+ Arka Microbial Consortium + 25% RDF) recorded maximum proline content (9.68 mg 100g^{-1}) and carotenoid content (7.09 mg g^{-1}), while flavonoid content (2.86 mg 100g^{-1}) was more in M_2P_4 (Inoculation of PGPRE's to cuttings prior to planting X *Piriformospora indica*+ *Glomus fasciculatum*+ Arka Microbial Consortium) and phenol content (15.17 mg 100g^{-1}) was more in M_1P_5 (Soil application with PGPRE's 10 days prior to Crossandra seedling planting X *Piriformospora indica* + 50% RDF). Whereas, lower levels of phenols (0.46 mg 100g^{-1}), proline (0.96 mg 100g^{-1}), flavonoid (0.22 mg 100g^{-1}) and carotenoid (0.46 mg g^{-1}) were recorded in M_2P_{10} (Inoculation of PGPRE's to cuttings prior to planting x Absolute control).

Proline, phenols, flavonoids and carotenoids were the biochemical traits that were recorded during this experiment. These were first line defense mechanisms released by the host plants to counterattack the production of reactive oxygen species such as H_2O_2, O_2^- and HO^- (Nadarajah, 2020). These mechanisms help in suppressing the oxidative stress caused by reactive oxygen species (Bhatt *et al*., 2021). In our experiment, co-inoculation of

Table 3 : Effect of methods of inoculation, Plant Growth Promoting Root Endophytes (PGPRE's) and their interaction on phenol content in Crossandra var. Arka Chenna.

Method of inoculation (M)	Plant Growth Promoting Root Endophytes (P)										
	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	Mean M
M_1	3.67	3.80	3.36	11.53	15.17	1.67	3.20	10.92	1.41	2.22	5.69
M_2	2.41	1.36	1.48	1.69	0.97	5.37	1.80	2.39	1.63	0.46	1.95
M_3	3.38	3.21	7.47	7.78	9.03	2.76	6.42	4.28	1.98	2.36	4.87
M_4	1.93	2.81	2.08	5.23	3.33	2.28	1.26	4.16	1.68	1.11	2.59
M_5	1.52	0.82	2.83	4.14	4.28	7.68	2.60	3.53	1.84	1.14	3.04
M_6	3.34	2.55	2.89	5.06	6.82	1.61	0.80	3.89	2.71	0.97	3.06
Mean P	2.71	2.42	3.35	5.90	6.60	3.56	2.68	4.86	1.87	1.38	

Source	Method of inoculation (M)	PGPRE's (P)	M x P
S.Em±	0.037	0.048	0.118
CD (*P*=0.05)	0.105	0.135	0.331

Table 4 : Effect of methods of inoculation, Plant Growth Promoting Root Endophytes (PGPRE's) and their interaction on proline content in Crossandra var. Arka Chenna.

Method of inoculation (M)	Plant Growth Promoting Root Endophytes (P)										
	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	Mean M
M_1	6.12	6.49	4.66	7.69	5.17	7.27	6.29	9.68	1.88	1.41	5.67
M_2	2.38	5.13	2.42	5.19	2.99	2.29	2.71	5.98	1.84	0.96	3.19
M_3	5.25	7.74	4.22	7.25	4.68	3.47	4.50	7.44	1.43	2.39	4.84
M_4	3.92	3.44	3.25	5.82	3.19	3.29	6.18	6.82	2.97	2.56	4.14
M_5	2.46	2.55	2.46	3.89	4.25	2.98	6.28	3.20	3.31	1.18	3.26
M_6	3.68	4.82	2.82	7.15	3.60	3.29	3.40	6.58	2.28	2.09	3.97
Mean P	3.97	5.03	3.30	6.16	3.98	3.76	4.89	6.62	2.29	1.77	

Source	Method of inoculation (M)	PGPRE's (P)	M x P
S.Em±	0.013	0.016	0.040
CD (*P*=0.05)	0.036	0.046	0.113

Piriformospora indica, *Glomus fasciculatum* and Arka Microbial Consortium successfully enhance the levels of proline, flavonoid and carotenoid activities when applied directly to the soil 10 days before sowing, while phenol was shown higher when *Piriformospora indica* alone along with 50% RDF was applied directly to the soil 10 days prior to planting. It helps in allowing sufficient time for the PGPRE's to multiply and colonize the soil environment, establishing ecological niches and eventually forming a biologically active zone which improves root development once the planting material is introduced. Similar findings were also found by Chen *et al*. (2021) by inoculating the *Piriformospora indica* alone to gerbera. Plants that were colonized with mycorrhizal fungi accumulate more antioxidant compounds such as carotenoids, phenols and flavonoids (Baslam and Goicoechea, 2012). Bhuyan *et al*. (2015) studied that co-inoculation of *Piriformospora indica* with *Azotobacter chroococcum* enhanced the C:N ratio and provided major nutrients to the plants, which in turn improved the photosynthesis molecules which results in carotenoids production. Noorjahan *et al*. (2018) stated that *Piriformospora indica* promotes the carbohydrate precursors which were required for the phenolic compounds synthesis and also helps in producing secondary metabolites such as flavonoids. This was found in coordinate with Arora *et al*. (2017), where he revealed the maximum carotenoids and flavonoids in *Artemisia annua* plants inoculating with *Piriformospora indica* and *Azotobacter chroococcum* alone or in combination. Devi *et al*. (2022) reported that increase in phenolic and flavonoid contents by inoculating the chilli plant with the combination of three compatible microbes. This finding was shown similar with the findings of Devi *et al*. (2024), where he found higher phenol and flavonoid content in wheat when the seeds were inoculated with the combination of strains EU-A3Rb1, EU-A2RNL1, EU-C3SK2 and EU-WRSe4 1-2 hours prior to sowing. Rayavarapu and Padmavathi (2016) reported maximum proline content in tomato by the soil inoculation of *Bacillus*

Table 5 : Effect of methods of inoculation, Plant Growth Promoting Root Endophytes (PGPRE's) and their interaction on carotenoid content in Crossandra var. Arka Chenna.

Method of inoculation (M)	Plant Growth Promoting Root Endophytes (P)										
	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	Mean M
M_1	3.17	5.54	2.00	6.97	3.38	3.37	3.22	7.09	1.81	1.71	3.83
M_2	1.22	0.81	0.69	0.80	0.64	0.92	2.27	0.65	0.70	0.46	0.91
M_3	2.50	3.35	4.59	4.94	1.67	1.77	5.50	4.31	2.11	1.50	3.22
M_4	1.55	0.81	1.56	1.27	1.11	0.91	0.54	3.37	0.78	0.79	1.27
M_5	2.01	1.74	1.08	3.17	1.60	1.55	3.21	0.96	0.69	0.68	1.67
M_6	0.79	0.86	0.94	0.84	1.15	0.53	1.59	2.26	1.11	1.08	1.11
Mean P	1.87	2.19	1.81	3.00	1.59	1.51	2.72	3.11	1.20	1.03	
Source	**Method of inoculation (M)**				**PGPRE's (P)**				**M x P**		
S.Em±	0.025				0.032				0.079		
CD (*P*=0.05)	0.070				0.091				0.222		

Table 6 : Effect of methods of inoculation, Plant Growth Promoting Root Endophytes (PGPRE's) and their interaction on flavonoid content in Crossandra var. Arka Chenna.

Method of inoculation (M)	Plant Growth Promoting Root Endophytes (P)										
	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	Mean M
M1	1.36	1.68	2.20	1.40	2.35	1.15	2.56	2.62	1.20	0.41	1.69
M2	2.54	2.51	2.49	2.86	2.63	2.73	1.40	1.12	1.91	0.22	2.04
M3	1.64	1.85	0.75	1.43	2.03	0.80	2.03	2.59	0.95	0.70	1.48
M4	1.10	1.91	1.53	2.19	1.11	1.65	2.02	2.09	0.47	1.05	1.51
M5	1.60	0.64	1.94	1.90	0.95	1.54	1.56	1.39	0.67	0.84	1.30
M6	0.54	2.19	1.77	2.44	1.59	1.75	1.41	2.28	0.80	1.17	1.59
Mean P	1.46	1.80	1.78	2.04	1.78	1.60	1.83	2.01	1.00	0.73	
Source	**Method of inoculation (M)**				**PGPRE's (P)**				**M x P**		
S.Em±	0.034				0.044				0.108		
CD (*P*=0.05)	0.095				0.123				0.301		

sp and *Glomus monosporum* before planting.

Among the physiological attributes, plant biomass and stomatal conductance was shown greater when the treatments were inoculated the soil with the combination of *Piriformospora indica*, *Glomus fasciculatum* and Arka Microbial Consortium along with the supplementary nutrients 10 days prior to planting. Improved plant biomass in the PGPRE's treatment is a result of multifaceted interactions *i.e.*, involving hormonal production, improved nutrient acquisition and enhanced rhizosphere dynamics. Smith and Read (2008) stated that soil application of *Glomus* sp. helps in absorption of immobile nutrients, particularly phosphorus through hyphal networks. On the other hand, *Piriformospora indica* enhances auxin and cytokinin synthesis, promoting plant cell division and elongation, which directly influences more plant height, number of branches and leaf proliferation, thereby plant biomass (Varma *et al.*, 2012). The bacterial strains particularly *Bacillus* sp, *Azotobacter* sp and *Pseudomonas* sp which were used as a carrier-based product of AMC, contributes to the plant biomass by producing siderophores, phytohormones (particularly IAA), antibiotics, enzymes, nitrogen fixation and phosphorus solubilisation (Glick, 2012). Arora *et al.* (2017) reported that co-inoculation of *Piriformospora indica* and *Azotobacter chroococcum* helps in promoting plant biomass of *Artemisia annua* by production of plant hormones *viz.*, auxins and cytokinin. Application of PGPRE's helps in influencing photosynthetic efficiency there by promoting plant biomass (Long *et al.*, 2015; Shoukat *et al.*, 2019). Li *et al.* (2020) found that successful promotion of plant biomass in sweet potato with *Piriformospora indica* colonization. It was in favour with Sayed *et al.* (2011), who found that enhanced biomass in sweet basil with the co-inoculation of *Bacillus subtilis* and AMF. In *Phaseolus vulgaris*, increased biomass was observed with the inoculation of *Glomus mosseae* and *Pseudomonas florescence* (Younesi and Moradi, 2014). It was found in accordance with Rayavarapu and Padmavathi (2016), who observed increased biomass content in bell pepper with the application of *Bacillus* sp. and *Glomus monosporum*. Kumar *et al*. (2021) reported that tri-inoculation of *Arthrobacter chlorophenolicus*, *Enterobacter* sp. and *Pseudomonas aeruginosa* promoted the crop biomass in wheat. The similar finding was shown by Kaur *et al*. (2022), who found that combination of *Erwinia* sp. EU-B2SNL1, *C. arthrosphaerae* EU-LWNA-37 and *P. gessardii* EU-MRK-19 strains in barley crop resulted in more crop biomass.

Conclusion

From this study, it was concluded that the application of plant growth promoting root endophytes substantially improves the physiological and biochemical attributes of Crossandra var. Arka Chenna. Among the different inoculation methods, soil application of PGPREs 10 days prior to planting emerged as the most effective in enhancing plant biomass and antioxidant-related biochemical traits. The combination of *Piriformospora indica*, *Glomus fasciculatum* and Arka Microbial Consortium supplemented with 25% RDF, contributed to superior plant growth and metabolite accumulation compared to individual or other combined treatments. These results underscore the potential of integrated microbial inoculants as a sustainable and eco-friendly approach to improve plant vigor and productivity in ornamental horticulture. Adoption of such PGPRE-based biofertilization practices can reduce chemical fertilizer usage, enhance soil health and support economical cultivation of Crossandra and potentially other floriculture crops.

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