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Evaluation of Potential PGPRs with Biocontrol Activity against Dry Root of Chickpea caused by *Rhizoctonia bataticola* and Mitigating Physiological Stress

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ABSTRACT: In this present study, out of thirty one root colonizing chickpea (*C. arietinum*) rhizosphere bacterial isolates, three isolates reported highly antagonistic to *R. bataticola* and found to be potential on the basis of 16S rRNA under accession number KP966499 for the strain PR31 identified as *Bacillus subtilis.* PR30 strain identified as *Bacillus subtilus* under accession number KP966505. PR10 strain identified as *Enterobacter cloacae* under accession number KP226575 and was deposited in Gen-Bank sequence database. The synergistic effects of PGPR as single and consortium treatments T9 (B+C) with *E. cloacae* and *B. subtilis* in this study ameliorated drought effects by reducing the degradation of chlorophyll 'a' ranged 0.67 mg/g, chlorophyll 'b' ranged 0.43 mg/g and total chlorophyll content 1.12 mg/g by improving water balance and osmoregulation by acting as osmoprotectant. Likewise by increased carotenoid in T6 with 80.31 µg/g content according to the mean data, assisted in declining singlet oxygen and can even helps in maintaining healthy photosystem by improved protein content as well. Therefore PGPR treatments could be solution for overcoming drought and against soil borne fungal pathogen effectively.

Keywords: PGPR, *R. bataticola*, drought, chickpea, biochemical.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the leguminous pulse crop which is predominantly cultivated in arid, semi-arid, poor source of regions under rainfed conditions globally and well known as poor mans' meat, a substitute of animal proteins (FAOSTAT 2013). India alone producing more than 75% share of chickpea, while comparing to any other countries. Eventhough, it is unable to achieve the desirable protein requirement due to the rapid growing population (Gaur *et al.*, 2019). Chickpea production faces a great number of challenges on the account of several non biotic stress factors like salinity, drought, high and low temperature. The change occurs in climate is highly erratic, due to increasing frequency of water deficit leading to a great loss in yields (Kasim *et al.*, 2013).

Drought effects turgor potential, plant water potential and modifications takes place in morphological, physiological traits like electrical conductivity in leaves, relative water content (RWC), leaf water potential (w), transpiration rates, stomatal conductance (gs), chlorophyll fluorescence, malondialdehyde (MDA) and chlorophyll content which comes under photo oxidation symptom and decrease in photosynthetic carbon assimilation. Water deficit stress negatively affects cell organ levels, several sub cellular compartment and at whole plant stage. The disproportion of above traits leads to increase in harshness of drought (Rahdari *et al.*, 2012). In chickpea, among numerous factors related to biotic and non-biotic, susceptibility to diseases is the most important cause for the low productivity. Among several diseases, dry root rot caused by *Rhizoctonia bataticola* is one of the key constraints registered so far which declines 10-60% production every year (Sundravandana *et al.*, 2012).

Bacteria that colonize roots and encourage plant growth are denoted as plant growth promoting rhizobacteria (PGPR) (Drogue *et al.*, 2013). Earlier studies have highlighted that PGPR performs as plant helpers in fighting stress by improving tolerance effectively and alleviating the impact of various stress factors through several mechanisms and beneficial effect on the growth enhancement in chickpea. The triggering of protective responses within the plants occurs through synthesis of signaling molecules by these PGPR, that affects susceptibility to stress (Patel *et al.*, 2015).

Generally, fungal pathogens which are considered as soil borne are managed by chemicals, but this process leads to other health and environmental issues. Approximately 2.5 million tons of pesticides are utilized per annum worldwide also accumulates into the

environment (Rao *et al.*, 2015). Some of these PGPR belong to genera such as *Burkholderia, Azotobacter, Azospirillum, Micrococcus, Flavobacterium, Serratia* and *Erwinia* etc. and the strains of *Enterobacter, Bacillus and Pseudomonas* assists by producing antibiotic compounds and inducing plant immune defenses from pathogen attachment and invasion under stress conditions (Singh, 2015). The objective performed for the assessment of biochemical effects of PGPR in chickpea KWR-108 genotype at *in vivo* conditions against *R. bataticola* under drought for the fulfilment of present investigation.

METHODOLOGY

Estimation of total chlorophyll content: Chlorophyll was determined according to (Wellborn, 1983). 1 gram of leaf sample weighed and crushed with 80% acetone and made up the volume to 25 ml with 80% acetone. The centrifugation was at 800 rpm for 5 minutes. The supernatant was read under 663, 645 nm under spectrophotometer. The total chlorophyll content was calculated by using the following formula and expressed in mg/g fresh weight^{-1}

$$\text{Chlorophyll 'a'} \ (mg\ g-1\ F.W.) = 12.7\ x\ (A663) - 2.69\ x\ (A645)\ x\ \frac{V}{1000\ x\ w\ x\ a}$$

$$\text{Chlorophyll 'b'} \ (mg\ g-1\ F.W.) = 22.9\ x\ (A645) - 4.68\ x\ (A663)\ x\ \frac{V}{1000\ x\ w\ x\ a}$$

$$\text{Total chlorophyll} \ (mg\ g-1\ F.W.) = 20.2\ x\ (A645) + 8.02\ x\ (A663)\ x\ \frac{V}{1000\ x\ w\ x\ a}$$

where,
A645 = Absorbance of the extract at 645 nm
A663 = Absorbance of the extract at 663 nm
a = Path length of cuvette (1 cm)
V = final volume of the chlorophyll extract (10 ml)
W = Fresh weight of the sample (0.10 g)

Estimation of Carotenoid content: Carotenoid was determined according to (Wellborn, 1983). 1 gram leaves sample weighed and crushed with 80% acetone made up the volume to 25 ml with 80% acetone and the centrifugation was at 3000 rpm at 10 min. The absorbance was recorded at 470 nm by spectrophotometer. It was calculated by the formula

$$\text{Total carotenoids} = \frac{[1000\ A470 - (3.27\ Chl\ a + 104\ Chl\ b)]}{229}$$

Estimation of Protein: Protein was estimated as suggested by (Bradford, 1976). 0.5 gm leaf was weighed and grinded in mortar pestle with 1ml of phosphate buffer. This centrifuged at 12000 rpm for 10 min at 4°C. Supernatant was collected and transferred to another tube and stored at -20° C for 1-2 hrs. 10 µl of protein sample in 990 µl distilled water and 2 ml Bradford reagent was added in it as sample and 1 ml of distilled water, 2 ml of Bradford reagent served as a blank sample. Finally measured at 595 nm. This was calculated by Bovine serum albumin (BSA) by different concentrations.

Pot trial: Pot trial was prepared in composition of around 25% vermiculite to the soil and pathogen was added excluding control treatments (T1 and T2), conducted for reported genotype in greenhouse conditions against *R. bataticola* under drought during 2019-2020 at research field, Department of Molecular Biology and Genetic Engineering, Rashtrasant Tukadoji Maharaj Nagpur University (RTMNU), Nagpur, Maharashtra.

Field trial: To assess the efficacy of PGPR isolates *Bacillus subtilis* and *Enterobacter cloacae* against *R. bataticola* under *in vivo* conditions during 2020-2021, field trials were conducted at three replications in RBD layout comprised of ten PGPR treatments with single and consortium along with controlled treatments (T1), (control with drought as T2) and (control with pathogen as T3) at research field, Department of Molecular Biology and Genetic Engineering, Rashtrasant Tukadoji Maharaj Nagpur University (RTMNU), Nagpur, Maharashtra. The treatment details and recorded parameters were the same used for greenhouse trial.

Source of Variety details: Chickpea variety- KWR 108, Resistant to wilt, seeds are small, dark and brown in color.
Released Year-1996
Released by- Chandra Shekar Azad University of Agriculture Technology (CSAUAT)
Zone- North East Plain Zone, at East Uttar Pradesh, Bihar, West Bengal
Production- 20-23q/hec
Days to maturity- 130-135
Obtained from- Plant Protection Department, ICAR-IIPR, Kanpur, U.P.

Treatment details:
T1- control
T2- control with drought
T3- control with *R.bataticola*
T4- Isolate A *(Bacillus subtilis)*
T5- Isolate B *(Enterobacter cloacae)*
T6- Isolate C *(Bacillus subtilis)*
T7- Isolate A+B
T8- Isolate A+C
T9- Isolate B+C
T10- Isolate A+B+C

Seed treatment: Seeds were inoculated with three reported potential isolates in broth medium in single and consortium treatments according to compatibility test results and incubated in shaking incubator overnight for PGPR growth formerly before sowing.

Statistical analysis. The data obtained in respect of various biochemical and antioxidant observations were statistically analyzed in two factor RBD analysis (Randomized Block Design) through OPSTAT software. Data was subjected to analysis of variance (ANOVA) at 5% level of significance (P<0.05) was found to be significant (Gomez and Gomez, 1984).

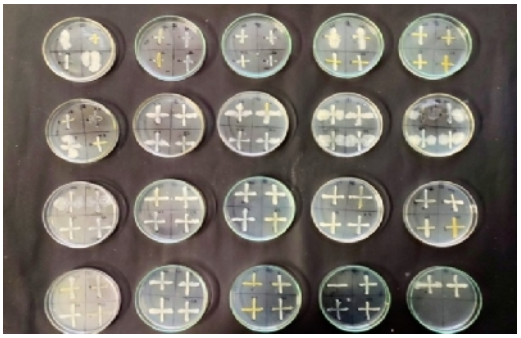

Plate 1: Compatibility test.

Plate 2: Pathogencity test.

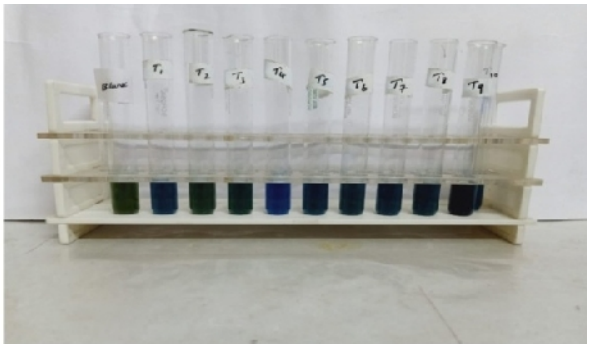

Plate 3: Estimation of protein content in leaves of chickpea genotype KWE-108.

RESULTS

The observations showed significant enhancement in biochemical parameters such as chlorophyll 'a', chlorophyll 'b' and total chlorophyll content, carotenoids, and protein against *R. bataticola* during drought conditions.

Chlorophyll 'a' content (mg/g): In drought conditions, leaf chlorophyll 'a' content was more susceptible at reproductive stage, but the PGPR consortium with *E. cloacae* and *B. subtilis* ranged highest in T9 (B+C) even under drought during 2019-2020 followed by 2020-2021 with disease tolerance against *R. bataticola*, whereas the mean data of both consecutive years recorded highest in T9 (B+C) treatment.

During first trial in pot experiment, genotype KWR-108 recorded highest in T9 treatment in chlorophyll 'a' ranged between 0.65 mg/g to 0.23 mg/g as lowest in T3 treated as control with pathogen *R. bataticola* alone during drought conditions in year 2019-2020. Followed by 2020-2021, in field trial the similar treatments ranged from 0.69 mg/g to 0.29 mg/g shown in Table 1. However in mean data of recorded genotype ranged highest with 0.67 mg/g in T9 and 0.26 mg/g in T3 was recorded least of all.

Chlorophyll 'b' content (mg/g): In drought conditions, leaf chlorophyll 'b' content declined at reproductive stage, but the PGPR consortium with *E. cloacae* and *B. subtilis* ranged highest in T9 (B+C) even under drought during 2019-2020 followed by 2020-2021 with disease tolerance against *R. bataticola*, whereas the mean data of both consecutive years recorded highest in T9 (B+C) treatment.

During first trial in pot experiment, genotype KWR-108 recorded highest in T9 treatment in chlorophyll 'b' ranged between 0.41 mg/g to 0.08 mg/g as lowest in T3 treated as control with pathogen *R. bataticola* alone during drought conditions in year 2019-2020. Followed by 2020-2021, in field trial the similar treatments ranged from 0.46 mg/g to 0.13 mg/g shown in Table 1. However in mean data of recorded genotype ranged highest with 0.43 mg/g in T9 and 0.10 mg/g in T3 was recorded least of all.

Total Chlorophyll content (mg/g): Total chlorophyll content increased due to the PGPR consortium with *E. cloacae* and *B. subtilis* T9 (B+C) ranged highest even under drought over controlled treatments during 2019-2020 followed by 2020-2021 and in mean data along with disease tolerant against *R. bataticola* in KWR-108 genotype in both consecutive years.

During first trial in pot experiment, genotype KWR-108 recorded highest in T9 treatment in total chlorophyll content ranged between 1.06 mg/g to 0.64 mg/g as lowest in T3 treated as control with pathogen *R. bataticola* alone during drought conditions in year 2019-2020. Followed by 2020-2021, in field trial the similar treatments ranged from 1.19 mg/g to 0.73 mg/g shown in Table 1. However in mean data of recorded

genotype ranged highest with 1.12 mg/g in T9 and 0.68 mg/g in T3 was recorded least of all.

Carotenoid content (µg/g): Carotenoids are pigments assists in neutralizing oxidative stress by declining lipid peroxidation, scavenging singlet oxygen and lipid peroxy radicles. These content generally declines during drought, but the PGPR single treatment T6 (C) with *B. subtilis* ranged highest over control even under drought during 2019-2020 followed by 2020-2021 and in mean data with disease tolerant against *R. bataticola* in KWR-108 genotype in both consecutive years.

During first trial in pot experiment, genotype KWR-108 recorded highest in T6 treatment in carotenoid content ranged between 0.46 µg/g to 0.11 µg/g as lowest in T3 treated as control with pathogen *R.bataticola* alone during drought conditions in year 2019-2020. Followed by 2020-2021, in field trial the similar treatments ranged from 0.51 µg/g to 0.19 µg/g shown in Table 1. However in mean data of recorded genotype ranged highest with 0.48 µg/g in T6 and 0.15 µg/g in T3 was recorded least.

Protein content (%): Protein content assists in inhibition of lipid peroxidation, which leads to cell damage during drought. But by PGPR single treatment T6 (C) with *B. subtilis* ranged highest even under drought during 2019-2020 followed by 2020-2021 and in mean data with disease tolerant against *R. bataticola* in KWR-108 genotype over control in both consecutive years.

During first trial in pot experiment, genotype KWR-108 recorded highest in T6 treatment in protein content ranged between 18.24 % to 5.54 % as lowest in T3 treated as control with pathogen *R. bataticola* alone during drought conditions in year 2019-2020. Followed by 2020-2021, in field trial the similar treatments ranged from 25.32 % to 11.75 % shown in Table 1. However in mean data of recorded genotype ranged highest with 21.78 % in T6 and 8.65 % in T3 was recorded least.

Table 1: List of biochemical parameters in chickpea variety KWR-108 against *R. bataticola* under drought.

Treatments (KWR-108)	Chlorophyll 'a' (mg/g)			Chlorophyll 'b' (mg/g)			Total Chlorophyll (mg/g)			Carotenoid (µg/g)			Protein (%)		
	Pot trial	Field trial	Mean data	Pot trial	Field trial	Mean data	Pot trial	Field trial	Mean data	Pot trial	Field trial	Mean data	Pot trial	Field trial	Mean data
T1 (control)	0.35	0.41	0.38	0.12	0.19	0.15	0.76	0.85	0.8	0.25	0.32	0.28	10.98	16.12	13.55
T2 (control with drought)	0.33	0.38	0.35	0.09	0.14	0.11	0.74	0.83	0.78	0.15	0.23	0.19	6.54	12.25	9.4
T3 (control with *R.bataticola*)	0.23	0.29	0.26	0.08	0.13	0.1	0.64	0.73	0.68	0.11	0.19	0.15	5.54	11.75	8.65
T4 (A) *B. subtilis*	0.45	0.52	0.48	0.19	0.2	0.19	0.86	0.92	0.89	0.35	0.46	0.4	14.05	22.02	18.03
T5 (B) *E. cloacae*	0.52	0.53	0.52	0.35	0.37	0.36	0.96	1.05	1	0.32	0.45	0.38	11.96	20.41	16.21
T6 (C) *B. subtilis*	0.58	0.64	0.61	0.39	0.44	0.41	1.01	1.15	1.08	0.46	0.51	0.48	18.24	25.32	21.78
T7 (A+B)	0.42	0.46	0.44	0.14	0.22	0.18	0.83	0.95	0.89	0.29	0.4	0.34	7.33	14.14	10.73
T8 (A+C)	0.36	0.41	0.38	0.15	0.2	0.17	0.77	0.86	0.81	0.28	0.39	0.33	12.01	20.31	15.65
T9 (B+C)	0.65	0.69	0.67	0.41	0.46	0.43	1.06	1.19	1.12	0.42	0.51	0.46	13.03	24.01	21.43
T10 (A+B+C)	0.48	0.57	0.52	0.32	0.4	0.36	0.99	1.08	1.03	0.38	0.5	0.44	12.97	22.36	17.7
MEAN	0.437	0.49	0.461	0.224	0.275	0.246	0.862	0.961	0.908	0.301	0.396	0.345	11.265	18.869	15.313
CD	0.453	1.013	0.733	1.564	3.497	2.531	0.500	1.119	0.810	0.030	0.067	0.049	0.614	1.373	0.994
SE(d)	0.223	0.498	0.360	0.77	1.721	1.246	0.246	0.551	0.399	0.015	0.033	0.024	0.302	0.676	0.489
SE(m)	0.158	0.352	0.255	0.544	1.217	0.881	0.174	0.389	0.282	0.010	0.023	0.017	0.214	0.478	0.346

DISCUSSION

It had been reported that chlorophyll content in chickpea leaves decrease due to drought stress, but PGPR inoculated plants with consortium of *Bacillus* spp. and *Enterobacter* spp. significantly enhanced the leaf chlorophyll 'a', 'b' and total chlorophyll content that assisted in osmoregulation and ameliorated oxidative stresses in this study and helped in inducing new proteins (Khan *et al.,* 2018). The chlorophyll content indicates better physiological health of inoculated plants, as high chlorophyll content has been linked with drought tolerance (Kumar *et al.,* 2015).

Carotenoids were considered regardless of their ability to scavenge lipid peroxyradicals and singlet oxygen, in order to inhibit MDA contents under drought conditions. However, due to PGPR treatment with *Bacillus* spp. even under adverse conditions carotenes form a key part of the plant antioxidant defense system, but they are very susceptible to oxidative destruction. The β-carotene in the chloroplasts is completely bound to the core complexes of PS-I and PS-II. Protection at this site is important in photosynthetic tissue may be through direct quenching of triplet chlorophyll, which prevents the generation of singlet oxygen against ROS for chloroplast functioning is constantly gained by this reported PGPR treatments in this study. Here, β-carotene, in addition to functioning as an accessory pigment, acts as an effective antioxidant and plays a distinctive role in protecting photochemical processes and sustaining them even under *R. bataticola* and water deficit stress (Wahid, 2007).

PGPR provides drought tolerance in our study by improved levels of protein content in photosynthetic tissues that condensed ROS damage to photosynthetic structure. This change enhanced in the cell viability, photosynthetic rate and increased growth, productivity under water deficit conditions (Allakhverdiev and Murata, 2004). Protein damage is a common consequence of stress, so maintaining proteins in a functional form is necessary for plant survival under stress conditions**.** Significant increase in the leaf protein content was evident after being treated with PGPRs single and consortium in this current study with *B*acillus spp. in comparison to control in legumes shown in plate 3 (Afzal, 2008). The increased level of proteins due to PGPRs might have assisted plants to mitigate ROS effects normally synthesize heat shock proteins, antioxidant enzymes and several plant hormones to cope with environmental stresses (Wani *et al.,* 2016).

CONCLUSION

From the results, it is concluded that T9 (B+C) PGPR inoculation with consortium of *E. cloacae* and *B. subtilis* pronounced to be actively potential in comparison to other PGPR treatments. The attributes of biochemical parameters like chlorophyll 'a', 'b', total chlorophyll content, carotenoids and protein content in chickpea genotype extremely improved by T9 consortium treatment over control under drought stress. Therefore PGPR would be suitable reason to overcome reported pathogen even under water deficit conditions.

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Conflict of Interest. None.

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