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Effect of plant growth-promoting rhizobacterium (PGPR) on chlorophyll content of chickpea plant (*Cicer arietinum* L.)

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Abstract

Green plants produce a varied arrangement of primary and secondary metabolites. Chlorophylls are the principal metabolites that give leaves and fruits their colour. Chlorophylls spectrum characteristics are critical for capturing light energy and transducing absorbed light energy for photosynthesis. Like other plants, Chlorophyll content affects leaf colour and photosynthetic activity. The amount of Chlorophyll in a plant's leaves impacts its photosynthetic capability per unit area of its leaf and stress and nutritional inadequacies. The Arnon method was used to analyze the concentrations of Chlorophyll a and b. This work investigates the PGPR element regulating Chlorophyll content and supply defence in chickpea plants. After that, the chickpea plant was examined for a Chlorophyll estimation test for 30, 60, and 90 days. We noticed treated crops having CHL content, which was found to have different values after thirty, sixty, and ninety days.

Keywords: PGPR, *Cicer arietinum* L., chickpea, *Fusarium oxysporum*, *Bacillus*, *Pseudomonas*

Introduction

The commercial worth of plant organs is determined by colour, while the freshness of most vegetables is determined by texture. Pigments are responsible for the perception of colour. Pigments are abundant in fruits and vegetables, which appeal to customers. Plant pigments are classified into different groups based on their chemical makeup. Chlorophyll, carotenoids, anthocyanins, and flavonoids can be identified (Manolopoulou *et al.*, 2016)^[18].

The amount of Chlorophyll in leaves is a crucial feature studied to measure Chloroplast concentration, photosynthetic activity, and plant metabolism. Chlorophyll is an antioxidant found in green leaf plants' Chloroplasts, primarily in the roots, branches, florae, and leaves (Mirza *et al.*, 2013; Srichaikul *et al.*, 2011)^[19, 25]. Instead, CHL product primarily depends on sunlight piercing and is the plant's primary energy source (Srichaikul *et al.*, 2011)^[25]. Extract the pigments; it's commonly done in the lab with a pestle and mortar and a carbon-based solvent like acetone (Arnon 1949; Porra *et al.*, 1989)^[1]. Plant photosystems require the pigments Chlorophyll A and Chlorophyll B. (Richardson *et al.*, 2002)^[21]. Furthermore, CHL a is the most abundant colourant for photosynthesis in plants, supporting producing energy (Srichaikul *et al.*, 2011)^[25]. On the other hand, Chalcone in plants is 2-3 times larger than subordinate CHL b conc (Srichaikul *et al.*, 2011)^[25]. Chlorophyll absorbs light of various wavelengths, especially in the visible spectrum's red (650–700 nm) and Amethyst colour (400–500 nm) areas. The green colour comes from the fact that green light (550 nm) is reflected rather than absorbed. Chlorophyll A is an Aquamarine colour pigment, whereas CHL B is a Chartreuse colour. The volume of solar energy consumed by a leaf is primarily determined by the conc of photosynthetic pigments on its foliar surface. As a result of low CHL levels, photosynthetic capacity and primary production may be limited (Da Matta *et al.*, 2008; Kumari *et al.*, 2018)^[4, 16].

Compared to Chlorophyll, non-Chlorophyll accessory pigments such as carotenoids are found in a and b. It absorbs light and sends it to a different photosystem. By absorbing and dispersing excess light energy, carotenoids also serve as antioxidants. The spectra of CHL and non-CHL pigments differ for 1-2 reasons.

Chickpea is a frequently consumed member of the pulse plant family. It is the most commonly submitted Pod in South Asia and its third most popular edible bean. It is a vital basis of protein for many persons in evolving nations, notably in Asia's south region, where most people are vegetarians, either voluntarily or because of economic constraints (Gaur *et al.*, 2010)^[8].

Plant rehabilitation is a requirement that can help meet the food needs of an ever-increasing population (Lwin *et al.*, 2012) [17]. It is healthy knowledge that bacteria born in the soil directly impact plant development and thus ecosystem maintenance. The notion of PGPR is well-known in PGP (Yadav *et al.*, 2017) [32]. It could help for employing to grow plants in any environment. The goal of this work was to identify and extract PGP rhizo bacteria from soil that had been exposed to bacterial stress, as well as to establish their impact on black chickpea development. During the inquiry, several activities were revealed, including phenolic, CH₂O, flavonoids, Chlorophyll, and bacterial stress. Chickpeas are an outstanding source of water, protein, minerals (iron, magnesium, phosphorus, zinc, and calcium), and -carotene; their healthy protein content is far superior to that of the majority of other bean plants (Jukanti *et al.*, 2012; Siddique *et al.*, 2012) [14, 23]. Chickpea farming is a decent option for rice in insufficient zones, as it improves the fertility of soil and control of weeds (Singh and Mukherjee, 2009) [24]. Leguminous plants use symbiotic microbes in their origin blemishes to fix nitrogen. Using PGP organisms-based bio fertilisers improves plant return by addressing weather N and civilizing the P schedule in leguminous plants (Selvakumar *et al.*, 2012) [22].

Furthermore, PGP organisms-based bio-fertilizers in leguminous plants instead of natural P plant food decrease the phosphate plant food used. It increases uptake, resulting in more sustainable plant growth (Uddin *et al.*, 2014; Welley *et al.*, 2005) [26, 31]. These bio-fertilizers have gotten a lot of attention because of their low cost and environmental friendliness, reliability, and decreased use of finite resources in numerous farming worldwide (Caliskan *et al.*, 2013; Gopala Krishnan *et al.*, 2015) [2, 9].

Although various vaccinate, microbes PGPR is known to boost plant vigour and returns, particularly in chickpea, the rhizosphere of leguminous plants provides a distinct eco-friendly niche in which knot development is a vital surface of metabolic tasks, the rhizosphere of leguminous plants provides a particular-friendly place in which knot development in the critical character of metabolic studies tasks (Valverde *et al.*, 2006; Verma *et al.*, 2012; Verma *et al.*, 2013) [28, 30, 29]. Nonetheless, relatively little work has been done on alternative bio-fertilizers other than symbiotic associations with Rhizobium.

Fusarium wilt, produced by the *Fusarium oxysporum f. sp. ciceris* microbe, is a particularly critical limiting factor in the triumph of this plant's development worldwide (Jiménez & Jiménez, 2011) [12]. The mortality produced by this fungus range from 10 to 40% of the annual crop, and they can eventually wreak havoc on yield if there are problems with the growth of the subsequent following (Guerrero *et al.* 2015) [10].

These bacteria are difficult to control due to their resistance to fungicides and fumigants. The fungus can remain latent in the ground with Chlamydo spores, which works as the first inoculums for subsequent plant cycles. The primary technique is soil fumigants and fungicides. Another hand, some of these contents d, can harm plants due to their phytotoxic effects, and because of their lack of specialization, they can also wipe out profitable species. This situation, together with the safety and public health issues related to pesticide manufacturing and the USA and the potential for widespread groundwater pollution, mandates the search for and implementation of

environmentally sensitive management strategies.

Disease pathogens attack the root and block the xylem tissue, an essential plant portion, due to the poorer defensive system and virulent efficacy. It primarily distributes water from the roots to the stems and leaves and carries another dissolved component. The lack of water in the leaves caused by the wilt illness damaged the floral leaves and lowered the Chlorophyll content in the chickpea crop.

As a result, the PGPR can reduce these infectious agents while improving the efficacy of plant defence mechanisms. The biocontrol agents create PR proteins that suppress pathogen activity while also exhibiting PGPR activity, promoting plant development.

The current study was undertaken with the good impact of PGPR on chickpea wilt infections in mind. Rhizospheric wilt strains harmed the chickpea crop. This PGPR protects chickpea plants. In this treatment, we discovered that T3 *Pseudomonas Aeruginosa*, *Pseudomonas chlororaphis*, *Fusarium oxysporum*, Resistant variety JAKI- 9218, and Susceptible Variety (NDG18-4) had the highest Chlorophyll content, followed by T4 *Fusarium oxysporum*, Resistant variety JAKI- 9218, and Susceptible Variety (NDG18-4). The goal of this work is to investigate how the PGPR factor affects Chlorophyll content and provides pathogen protection in chickpea plants.

Material and Methods

Treatments

- T1:** *Pseudomonas Aeruginosa*+ Resistant variety JAKI- 9218+ Susceptible Variety (NDG18-4)
T2: *Pseudomonas Chlororaphis*+ Resistant variety JAKI- 9218+ Susceptible Variety (NDG18-4)
T3: *Pseudomonas Aeruginosa*+ *Pseudomonas Chlororaphis*+ *Fusarium oxysporum* + Resistant variety JAKI- 9218+ Susceptible Variety (NDG18-4)
T4: *Fusarium oxysporum* + Resistant variety JAKI- 9218+ Susceptible Variety (NDG18-4)
T5: - control + Resistant variety JAKI- 9218+ Susceptible Variety (NDG18-4)
T6: + control + Resistant variety JAKI- 9218+ Susceptible Variety (NDG18-4)

Estimation of Chlorophyll content by acetone method (Arnon method 1949) [1].

Chicken leaf = 100mg

80% Acetone buffered – 80 ml acetone + 20 ml Distilled water=100 ml

Procedure

- Fresh leaf 200 mg + 5 ml acetone
- Gridding with marter pestle
- Centrifuge 4000rpm for 20 minute
- The supernatant was collected & residue was extracted with 5ml of 80% acetone.
- Centrifuge 5 minute
- Take the supernatant
- 20 ml volume was made with 80% acetone.

O.D was calculated at 645 nm & 663nm on a spectra photometer

Note: 80% acetone was blank

Extraction of chlorophyll (Arnon method 1949)^[1]

The total content and the Chlorophyll a and b concentrations were estimated using Arnon's method and conveyed as mg per g fresh mass.

In a pre-cooled mortar, one gm of fresh leaves sample was mixed with 1 ml of 80 percent (v/v) acetone containing a nip of CaCO₃ and homogenized properly. The homogenate was centrifuged at 5000 rpm for fifteen mins. The supernatant was collected and the volume was maintained to two millilitres using 80 percent acetone. Compared to a control of 80 per cent acetone, the OD was measured at 645 and 663 nanometers.

Calculation

The amount of CHL a, b and total CHL was determined as follows:

Chlorophyll a = $12.7 \times \text{O.D. (663)} - 2.69 \times \text{O.D. (645)} \times \text{V}/1000 \times \text{W}$

Chlorophyll b = $22.9 \times \text{O.D. (645)} - 4.68 \times \text{O.D. (663)} \times \text{V}/1000 \times \text{W}$

Total Chlorophyll = $20.2 \times \text{O.D. (645)} + 8.02 \times \text{O.D. (663)} \times \text{V}/1000 \times \text{W}$

Where

V = Final volume

W = Weight of sample

OD = Optical density

Statistical analysis: One-way ANOVA is used to measure statistical analysis.

Result and Discussion

PGPR's stimulatory role in leaf Chlorophyll concentration was previously reported (Vafadar *et al.*, 2014; Fahad *et al.*, 2015)^[27, 6]. According to (Kamble *et al.*, 2015)^[15], most plants have more Chlorophyll content in adult leaves than in young leaves.

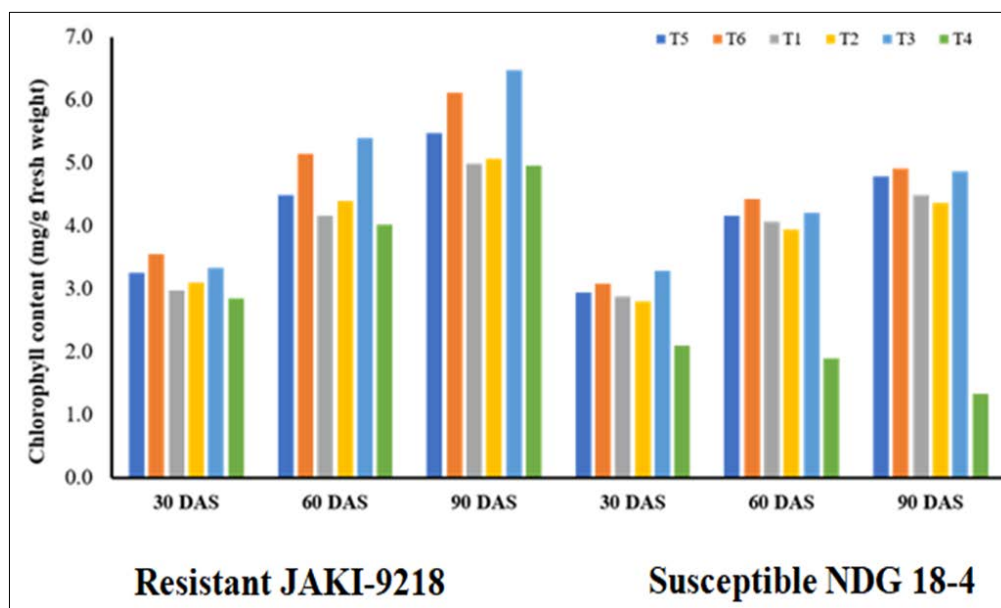
Both the plant varieties i.e. JAKI-9218 and NDG18-4 were treated with two screened PGPRs *P. aeruginosa* (T1), *P. chlororaphis* (T2) individually, and in combination along with the wilt pathogen (T3, *P. aeruginosa* + *P. chlororaphis* + *F. oxysporum*). Further, the plants were treated with known PGP bacterium *viz. Bacillus subtilis* (T6, positive control) and the pathogen *F. oxysporum* (T4) also. The plant varieties without any treatment were considered as control for the study.

When compared to the negative control, all PGPR treatments in the resistant variety JAKI- 9218 and the Susceptible variety (NDG18-4) revealed differing Chlorophyll content values at all stages of observation. At 30, 60, and 90 DAS, all bio-agent treatments had different levels of Chlorophyll content than the negative control (T5), but the groups treated with *Pseudomonas Aeruginosa* only had the lowest values (T1). A comparable level of chlorophyll was estimated in both the plant types in the presence of *P. chlororaphis* at all the days of observations. Interestingly, at 30 DAS, 60 DAS, and 90 DAS, significantly greater Chlorophyll content was found in the resistant (2.969, 3.099, and 3.333 mg/g fresh weight) and

susceptible variety (NDG18-4, 3.292, 4.210, 4.859 mg/g fresh weight) when treated with T3 combination which was comparable to the positive control. As per the expectation, the Chlorophyll content was highest in the positive control in both the varieties (JAKI-9218: 3.546, 5.142, 6.119 and NDG18-4: 3.088, 4.430, 4.918 mg/g fresh weight) while lowest in the pathogen treated plant (JAKI-9218: 2.855, 4.026, 4.951 and NDG18-4: 2.101, 1.891, 1.343 mg/g fresh weight). The whole data suggest that the screened PGPRs were not able to improve the chlorophyll content individually, but when treated in combination they had better effect on chlorophyll content in both the plant varieties. According to another study, paddy crops grown in topsoil cured with *Azospirillum* and *Bacillus* had the maximum levels of CHL a (0.821 mg/g fresh weight), CHL b (0.671 mg/g fr. wt.), total CHL (1.598 mg/g fresh weight), and tetraterpenoid (0.721 mg/g fresh weight). (José Francisco and his associates 2008)^[13] Researchers used a portable Chlorophyll meter and appropriate correction methods to assess CHL Concentration in tropical tree types. The portable Chlorophyll type is a simple and non-destructive method of estimating Chlorophyll concentrations. CHL a (0.91 0.19 mg/g FW) and CHLb (0.61 0.09 mg/g FW) levels in micro proliferated *Psoralea corylifolia* plants were greater than CHL a (0.83 0.31 mg/g FW) and CHL b (0.53 0.14 mg/g FW) levels in saplings, according to (Faisal and Anis *et al.*, 2006)^[7]. Compared to *Ulvarigita* L., *Codium tomentosum*, and *Cladostephus verticillatus* Ag, (Sukran Dere 1998)^[5] found the degree of CHL in the freshwater form *Cladophora glomerata* was relatively high. *Ulvarigita* also has a higher concentration of Chlorophyll. Previous research found that the Chlorophyll-to-pigment-level relationship was nearly identical in all algal taxa. The quantity of CHL a and b was lower than in the redeveloped leaf in the normal leaf. (Indira Priyadarsini *et al.*, 2015)^[11] calculated the CHL content of *Tridax procumbens* growing in normal and contaminated habitats, finding 2.99 mg/g in normal and 2.56 mg/g in polluted circumstances, respectively. (Croft *et al.*, 2017)^[3] discovered that when developing GPP models in forest ecosystems, it is superior to utilize CHL as a proxy to exchange photosynthetic efficacy rather than the conventional substitute-leaf N content for specific plant species. This method was novel and fascinating for physiological ecology and macro ecology experts. The maximum carboxylation rate (V-max) is represented by leaf CHL content, which could be used as a proxy in future models (Croft *et al.*, 2017)^[3]. It isn't easy to find data on CHL in natural populations, particularly on a large scale. According to (Kamble *et al.*, 2015)^[15], leaf Chlorophyll content is critical for maintaining photosynthetic processes and plant metabolism. Apart from these factors, the conc of CHL in the leaf is affected by seasonal variation and leaf maturity. The Chlorophyll a: b ratio is significantly lower in plants developing in high CO₂ environments. Chlorophyll content changes can be caused by various factors, including water, soil, temperature stress, and others, which indirectly impact leaf area, shape, thickness, and Chloroplast dispersion.

Table 1: Leaf treated chickpea resistant variety JAKI-9218 and susceptible variety NDG18-4 total Chlorophyll concentration ($\mu\text{g/ml}$) at 30, 60, and 90 days

S. No	Treatments	Total Chlorophyll content (mg g^{-1} fresh weight)					
		Resistant (JAKI-9218)			Susceptible NDG18-4		
		30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
T5	-ve control	3.251	4.486	5.473	2.949	4.158	4.794
T6	+ve control	3.546	5.142	6.119	3.088	4.430	4.918
T1	<i>Pseudomonas aeruginosa</i>	2.969	4.157	4.988	2.888	4.072	4.491
T2	<i>Pseudomonas chlororaphis</i>	3.099	4.401	5.061	2.797	3.944	4.365
T3	<i>Pseudomonas aeruginosa</i> + <i>Pseudomonas chlororaphis</i> + <i>Fusarium Oxysporum</i>	3.333	5.399	6.479	3.292	4.210	4.859
T4	<i>Fusarium oxysporum</i> f. sp. <i>ciceris</i>	2.855	4.026	4.951	2.101	1.891	1.343
	S.E.M	0.39	0.49	0.49	0.10	0.36	0.48
	CD at 5%	1.048			0.807		



*Significant changes were originated at $p < 0.05$

Fig 1: Total CHL content (mg/g fresh weight) of resistant JAKI-9218 and susceptible NDG18-4 varieties at 30, 60, and 90 days of treatment. The mean SEM was used to represent all of the data

Conclusion

Chlorophyll concentration can assess the health of a plant's canopy and the pace of photosynthesis. Chickpea leaf Chlorophyll was isolated and quantified. With different wavelengths, the concentration of Chlorophyll can change. CHL levels varied significantly between 30DAS, 60DAS, and 90DAS. The Arnon (1949) [1] method estimates Chlorophyll in this Chlorophyll Estimation. The amount of Chlorophyll collected in chickpea plants exhibits a significant difference. In addition, the Chlorophyll content can be used to detect plant stress and nutritional deficits. The amounts of CHL a, CHL b, and total CHL are all different. The value of Chlorophyll diminishes with leaf senescence, as can be shown.

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