

Interaction between nutrition, PGPR, and ambient and increased tropospheric ozone levels on chickpea (*Cicer arietinum*) development and yield

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ABSTRACT

In 2020 and 2021, an experiment was carried out at the ICAR-Indian Agricultural Research Institute in New Delhi to investigate how nutrients and PGPR (plant growth promoting rhizobacteria) interacted to affect the growth and yield of chickpea (*Cicer arietinum* L.) cv. Pusa 3043 under ambient and elevated ozone levels in FAOE (free air ozone enrichment). When compared to ambient ozone (AO₃) settings, high ozone (EO₃) was shown to reduce dry matter production by 15–16%. PGPR seed treatment was shown to make up for this loss, as evidenced by an 18–19% increase in seed production compared to untreated seeds. When compared to the prescribed dosage of fertilizer (RDF) application, the PGPR treatments with 75% nitrogen demonstrated a significant gain in seed output. PGPR like RPAN8 (*Anabena laxa*) and An-Rh (*Anabena torulosa* with *Mesorhizobium ciceri*) (chickpea rhizobium) showed yield increases of 12% and 14%, respectively, indicating that An-Rh has an advantage over RPAN8 under EO₃. Combining both PGPRs produced a 19% yield improvement over RDF, outperforming either PGPR alone. This demonstrates how treating seeds with PGPR (RPAN8 and An-Rh) might lessen the stress that tropospheric ozone causes in chickpea plants.

Keywords: Growth, Nitrogen, Ozone, PGPR, Yield, Chickpea

Introduction

In India, tropospheric ozone (O₃), which damages flora and human health, is produced by photochemical interactions of volatile organic molecules with nitrogen oxides (Sharma et al. 2022). The troposphere's background O₃ concentration has grown by 36% since pre-industrial times, and it is now increasing at a pace of 0.5–2.5% annually (Wang et al. 2022). Large agricultural regions in China and India are severely polluted by O₃ (Gao et al. 2020). In the northern region of India, climate change is predicted to raise the O₃ level by 4% regardless of O₃ precursor emissions (Pommier et al. 2018). O₃ enters leaves through stomata and quickly changes into reactive oxygen species (ROS), which harm plant tissues and change their physiological and biochemical characteristics, including reduced photosynthesis, stomatal conductance, leaf damage, and decreased plant yield, which results in production losses (Ashrafuzzaman et al. 2017). India's most prolific and rich agricultural regions are the Indo-Gangetic Plains (IGPs), which also confront

1 SRM College of Agricultural Sciences, SRM Institute of Science and Technology, Baburayanpettai, Tamil Nadu; 2 Sher-e-Kashmir University of Agriculture Sciences and Technology, Chatha, Jammu, Jammu and Kashmir; and 3 ICAR-Indian Agricultural Research Institute, New Delhi. * Corresponding author email: dineshpusa69@gmail.com Grain yield loss ranges from 7 to 13% under ambient O₃ concentration (~30ppb) in the northern area of India, with the greatest yield losses for the majority of crop species under current and projected O₃ concentrations (Mukherjee et al. 2021). Legumes and pulse crops, which are more vulnerable to ozone stress, lost 10–66% of their production in Asia when ambient ozone levels ranged from 35–75 ppb throughout crop growth (Emberson et al. 2018). India is the world's top producer of chickpeas (*Cicer arietinum* L.), accounting for 70% of the world's output; nevertheless, its susceptibility to biotic and abiotic stresses has also grown, leading to yield swings (Muehlbauer and Sarker 2017). Studies measuring the effects of ozone stress on chickpeas are quite rare. Research has shown the advantages of PGPR as a biofertilizer and in mitigating abiotic stressors such as metal toxicity, salinity, and drought in plants (Emberson 2020). However, there is no known research that uses PGPR to lessen ozone stress in legume crops like chickpeas. Elevated ozone (EO₃) levels cause abiotic stress in plants. The current study attempts to clarify how nutrients and PGPR might mitigate ozone stress on chickpea development and productivity.

METHODS AND MATERIALS

In the Free Air Ozone, a marijuana experiment was carried out.

Enrichment facility (FAOE) during the winter (rabi) season of 2020 and 2021 at the ICAR-Indian Agricultural Research Institute's experimental farm in New Delhi (28°35'N and 77°12'E, 228.16 m amsl). The silty clay loam soil used in the pot experiment had a pH of 7.6, an electrical conductivity of 0.47 dS/m, 0.51% organic carbon, and accessible N, P, and K concentrations of 212.8 kg/ha, 24.2 kg/ha, and 255.2 kg/ha, respectively. Elevated ozone exposure lasted from 9.30 AM to 4.30 PM for seven hours every day (except from wet days). Throughout the crop development period, the enhanced O₃ concentration in FAOE was around 60 ± 10 ppb (using an O₃ generator), whereas the average ambient O₃ concentration was 30 ± 10 ppb. A factorial fully randomized design (FCRD) with three replications was used to assess the treatments, which included two ozone exposure levels (Ambient-AO₃; Elevated-EO₃), a soil application of three distinct nitrogen dosages, and two strains of PGPR (seed treatment). For this investigation, the chickpea cultivar Pusa 3043 was used. Eight treatment combinations were used in the experiment: T1, Absolute control; T2, 100% RDF (Recommended Dose of Fertilizers); T3, 75% RDN + RPAN8 (*Anabena laxa*); T4, 50% RDN + RPAN8; T5, 75% RDN + An-Rh (*Anabena torulosa* with *Mesorhizobium ciceri*) (chickpea rhizobium); T6, 50% RDN + An-Rh; T7, 75% RDN + RPAN8 + An-Rh; T8, 50% RDN + RPAN8 + An-Rh. The recommended NPK dosage for chickpea crops was 20:40:20 kg/ha. The Division of Microbiology at the ICAR-Indian Agricultural Research Institute in New Delhi provided the two PGPR utilized in this investigation. The first cyanobacterial culture, RPAN8 (*Anabena laxa*), is a terrestrial nitrogen-fixing cyanobacterium that can fix up to 20–30 kg/ha and is utilized in agriculture to generate biopesticides and fertilizers. The second PGPR is a cyanobacterial biofilm called An-Rh *Anabena torulosa* with *Mesorhizobium ciceri* (chickpea rhizobium), which is used to treat seeds and has been shown to be resistant to abiotic stress. Seeds were coated with carrier-based weight for microbiological treatment, and the test weight of 100 seeds was also noted. The following formula was used to determine the crop's harvest index.

(Seed yield/Total biological yield) times 100 is the harvest index.

Using OPSTAT software, an analysis of variance (ANOVA) was performed for factorial totally randomized design (FCRD) to determine if the treatment differences were statistically significant. The R software program was used to do multivariate analysis.

OUTCOMES AND TALK

Growth criteria: In all seasons, there were notable variations in plant height across the treatments (Fig. 1). Under ambient ozone (AO₃) treatment, the maximum plant height in T7 (75% N + RPAN8 + An-Rh) was 48.13 cm in the first year and 48.84 cm in the second. In comparison to ambient ozone levels, a decrease in plant height was seen under EO₃ treatments (29.69 cm and 30.09 cm in T1). The overall number of leaves and, thus, the total photosynthetic area are determined by the number of secondary branches. The quantity of secondary branches under AO₃ and EO₃ differed significantly (Fig. 1). At the blooming stage, the AO₃ PGPR treatment (T7) produced a greater number of secondary branches/plant (10.65 and 11.85). In both trial years, secondary branch output decreased by 7.2 to 8% under EO₃ compared to AO₃. On the other hand, PGPR had a 7.9–9% compensatory impact over EO₃. This was due to the favorable conditions that PGPRs created in the rhizosphere for plant development and productivity. In comparison to AO₃ conditions, the EO₃ treatments considerably reduced the dry matter production (biomass g/pot) by 16% in 2020–21 and 15% in 2021–22 (Table 1). In comparison to untreated seeds, PGPR-treated seeds showed an increase in dry matter accumulation of 19% in 2020–2021 and 18% in 2021–2022.

creation of the microbial consortia at 50 g/kg seed using an adhesive agent of 3% sucrose solution. The covered seeds were immediately planted in pots after being shade-dried. Five randomly chosen and tagged plants from each condition were used for observations. Plant height and the quantity of secondary branches were measured at the harvest stage. The total biomass was calculated as g/plant by adding the dry and fresh weights of all plant parts. yield metrics such as the quantity of plants or pods, the quantity of seeds or pods, the weight of the pods, and seed

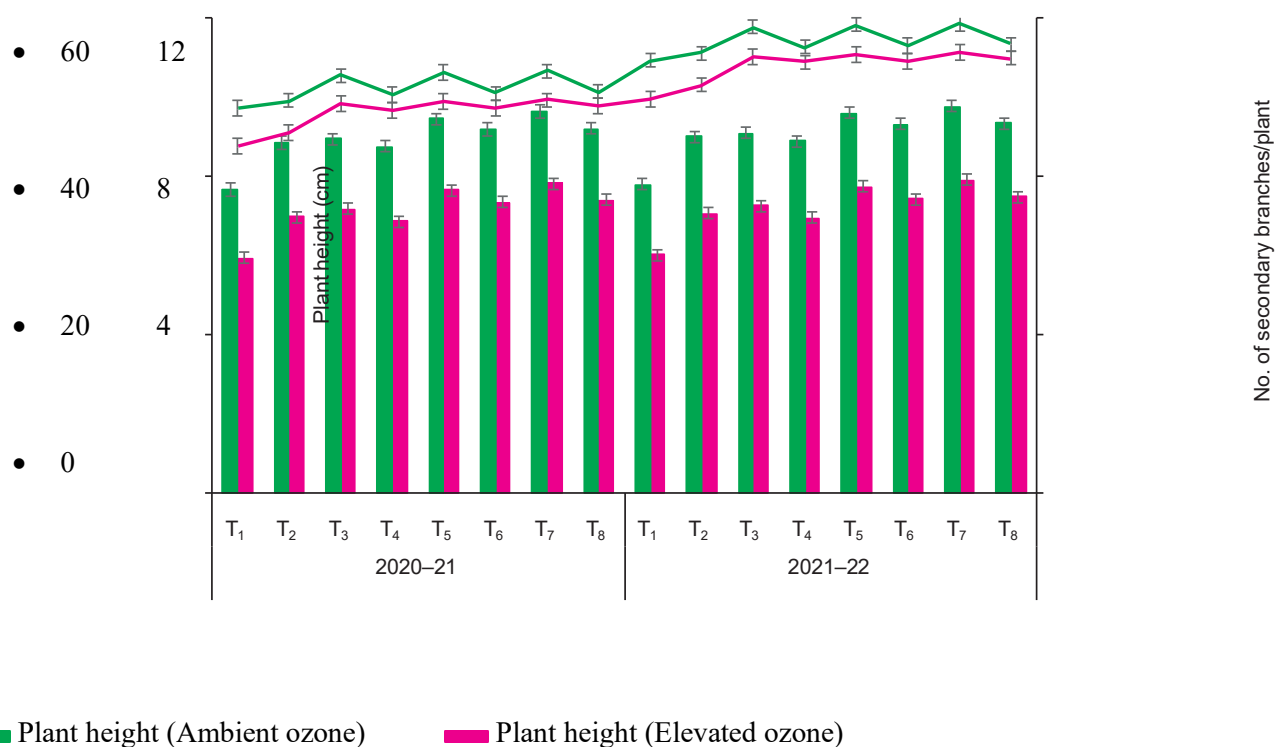


Fig. 1 Interactive effect of tropospheric ozone, nutrient and PGPR on plant height and number of secondary branches of chickpea under ambient and elevated levels. Treatment details are given under Materials and Methods.

Table 1 Interactive effect of ambient and elevated O₃ and PGPR on total biomass (g/pot) of chickpea

Treatment	2020-21		2021-22	
	AO3	EO3	AO3	EO3
T1	41.47 ± 1.03a	34.1 ± 0.5g	43.06 ± 1.06a	35.69 ± 0.76g
T2	44.58 ± 0.86d	37.61 ± 0.24f	46.17 ± 1.06d	39.2 ± 0.33f
T3	49.75 ± 0.16b	38.67 ± 0.51f	51.34 ± 0.11b	40.26 ± 0.42f
T4	44.08 ± 0.28d	38.06 ± 0.81f	45.67 ± 1.09d	39.65 ± 0.31f
T5	51.24 ± 0.38ab	38.98 ± 0.79f	52.83 ± 0.74ab	40.57 ± 0.61f
T6	43.96 ± 0.44d	38.39 ± 0.58f	45.55 ± 0.69d	39.98 ± 0.77f
T7	53.12 ± 0.69a	39.14 ± 0.71f	54.71 ± 0.91a	40.73 ± 0.04f
T8	47.61 ± 0.99c	38.51 ± 0.7f	49.2 ± 0.92c	40.1 ± 0.69f
Mean	46.97	37.93	48.56	39.52
Factors	C.D.	SE(m)	C.D.	SE(m)
AO3	0.67	0.23	0.74	0.25
EO3	1.34	0.46	1.49	0.51

(AO3 + EO3) 1.89 0.65 2.11 0.73
× (Fertilizer
and PGPR)

± Standard error; ambient ozone (AO3); elevated ozone (EO3). At $P \leq 0.05$, values that are followed by the same letters within columns do not vary substantially.

Details of the treatment are provided under Materials and Methods.

There is proof in the scientific literature that EO3 has an impact on critical metabolic processes in O3-sensitive species and cultivars, which reduces growth and total biomass output (Hoshika et al. 2013). According to a recent research on common beans with PGPR, increasing phytohormones improves root development, shoot growth, yield, and biochemical activity while reducing toxicity in plants under challenging circumstances (Kumar et al. 2020).

Yield attributes: The EO3 levels had a detrimental impact on the yield contributing features, such as the number of pods/plant, pod weight/plant, number of seeds/pod, seed weight, 100 seed weight, and harvest index. According to the pod weight/plant and number of pods/plant data (Table 2), the T7 (75% RDN + RPAN8 + An-Rh) had the maximum range under AO3. The EO3 significantly decreased in the range of 11% (2020–21) and 10.1% (2021–22) among the treatment effects of the number of pods/plant.

The percentage difference between PGPR-treated and untreated seeds increased significantly, ranging from 8.3–12% in AO3 conditions to 25–22.2% in EO3 conditions. Pod weight/plant findings showed a range of 16.24–26.80 g at AO3 and 13.16–22.30 g at EO3. At the harvest stage, however, the variation in the pod weight/plant was

Table 2 Interactive effect of ambient and elevated O3 and PGPR on number of pods/plant in chickpea

Treatment	2020–21		2021–22	
	AO3	EO3	AO3	EO3
T1	31 ± 0.54ij	26 ± 0.49k	34.28 ± 0.17h	29.28 ± 0.22j
T2	36 ± 0.49ef	32 ± 0.78hi	39.28 ± 0.25f	35.28 ± 0.28h
T3	38.5 ± 0.32cd	37.25 ± 0.43de	41.78 ± 0.74de	40.53 ± 0.36ef
T4	33.5 ± 0.75gh	29.5 ± 0.61j	36.78 ± 0.11g	32.78 ± 0.07i
T5	40 ± 0.44c	39 ± 0.53c	43.28 ± 0.23c	42.28 ± 0.44cd
T6	34.6 ± 0.67fg	31.3 ± 0.77i	37.88 ± 0.97g	34.58 ± 0.34h
T7	50 ± 0.21a	49 ± 0.26a	53.28 ± 0.19a	52.28 ± 0.54a
T8	46 ± 0.84b	45.5 ± 0.05b	49.28 ± 0.51b	48.78 ± 0.48b
Mean	38.69	36.19	41.98	39.47
Factors	C.D.	SE(m)	C.D.	SE(m)
AO3	0.56	0.19	0.44	0.15
EO3	1.13	0.39	0.89	0.30
(AO3 + EO3) × (Fertilizer and PGPR)	1.60	0.55	1.26	0.43

AO3 stands for ambient ozone, EO3 for elevated ozone, and ± for standard error. Values in columns that are followed by the same letters do not vary substantially at $P \leq 0.05$.

Details of the treatment are provided under Materials and Methods.

determined to be insignificant. Stress-related reductions in photosynthetic activity may impact assimilate translocation and carbon fixation, leading to fewer pods, poor seed set, and decreased sink activity in chickpeas (Nadeem et al. 2019). Microbes may have used the increased availability of photosynthates for detoxification

and repair mechanisms against EO3 stress (Backer et al. 2018), leading to a much greater output. Yield qualities are closely correlated with the decline in growth characteristics.

Seed treated with PGPR in AO3 treatments showed significantly increased numbers of seeds/pod (Fig. 2) and seed yield (Table 3), but EO3 treatment showed lower numbers of seeds and seed weight compared to ambient ozone levels. The maximum number of seeds/pod range of 1.8–2.1 and seed weight of 23.96–25.19 g in AO3 levels were reported by treatment T7 (75% N+RPAN8+An-Rh). However, in untreated seed treatment, the number of seeds/pod dropped by 11% (2020–21) and 10% (2021–22) from ambient to EO3 condition; in contrast, T7 (75% N+RPAN8+An-Rh) showed an increase of 18–21% in comparison to EO3 condition. While the T7-EO3 treatment resulted in a 19.5–21.1% increase in seed weight, the exposure to EO3 produced a reduction in seed weight relative to ambient conditions. It was discovered that the relationships between PGPR treatments, fertilizer, and O3 levels were

al. 2021). This shorter reproductive time may have led to lower seed weight and yield in our experiment. Under stress, the pace at which pollen tubes expand may decrease, which might have an impact on the production of pods and seeds (Kaloki et al. 2019). The impact of stress-tolerant PGPRs on growth and yield was shown by Kumar et al. (2020).

Figure 2 shows how tropospheric ozone, nutrients, and PGPR interact to affect the number of seeds per pod and the chickpea harvest index at both ambient and increased levels. Details of the treatment are provided under Materials and Methods.

that 32 stress-tolerant PGPR strains' combined impact (8 Rhizobium, 8

not significant. Furthermore, the findings show that the PGPR significantly reduced crop damage caused by O3. Table 3 Interactive effect of ambient and elevated O3 and PGPR on seed yield per plant in chickpea

Treatment	2020–21		2021–22	
	AO3	EO3	AO3	EO3
T1	9.74 ± 0.17g	3.43 ± 0.07i	10.97 ± 0.24g	4.66 ± 0.07i
T2	11.98 ± 0.05f	4.83 ± 0.02k	13.21 ± 0.15f	6.06 ± 0.14k
T3	15.79 ± 0.35d	6.93 ± 0.08i	17.02 ± 0.21d	8.16 ± 0.14i
T4	11.78 ± 0.17f	4.85 ± 0.12k	13.01 ± 0.28f	6.08 ± 0.02k
T5	17.46 ± 0.2c	8.38 ± 0.06h	18.69 ± 0.27c	9.61 ± 0.05h
T6	13.6 ± 0.26e	5.63 ± 0.03j	14.83 ± 0.14e	6.86 ± 0.09j
T7	23.96 ± 0.04a	12.15 ± 0.29f	25.19 ± 0.04a	13.38 ± 0.09f
T8	19.85 ± 0.15b	10.13 ± 0.07g	21.08 ± 0.41b	11.36 ± 0.06g

Mean	15.52	7.04	16.75	8.27
Factors	C.D.	SE(m)	C.D.	SE(m)
AO3	0.16	0.05	0.18	0.06
EO3	0.33	0.11	0.37	0.12
(AO3 + EO3) × (Fertilizer and PGPR)	0.47	0.16	0.52	0.18

AO3 stands for ambient ozone, EO3 for elevated ozone, and $\pm$ for standard error. Values in columns that are followed by the same letters do not vary substantially at $P \leq 0.05$.

Details of the treatment are provided under Materials and Methods.

When compared to the control condition, *Pseudomonas*, eight potassium-releasing bacteria, and eight zinc-solubilizing bacteria provide the maximum output. Under AO3, the 100-seed weight varied between 21.01 and 22.0 g, but in EO3, it varied between 20.91 and 21.76 g. There was a notable difference between the treatments in both seasons according to the harvest index (HI) (Fig. 2). EO3 had a greater reduction in HI than AO3, indicating that the former had more unfilled seeds per plant than the latter (Singh et al. 2012). Less dry matter was partitioned into seeds under ambient air pollution stress, according to the HI decrease. The synthesis of carbohydrates and the transfer of assimilates from the source to seeds are known to be hampered by tropospheric O₃. Chickpeas (Bhatia et al. 2021), mungbean (Chaudhary and Agrawal 2015), turnips (Sethupathi et al. 2018), rice (Kumar et al. 2021), and cauliflower (Kovilpillai et al. 2023) all showed comparable reduced yields..

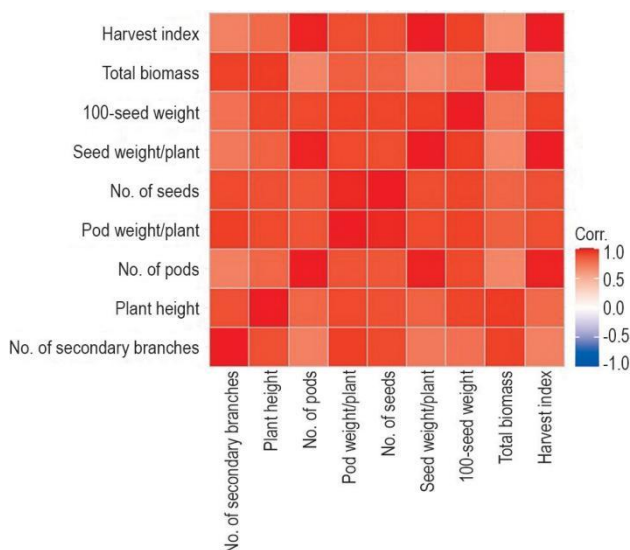


Fig. 3 Multivariate analysis correlation between growth and yield parameters of chickpea.

In chickpeas, PGPR treatment increased the number of pods/plant, number of seeds/plant, and seed production by 50, 63, and 37%, respectively (Laranjeira et al. 2022). Increased rice production might result from the PGPR inoculation's ability to reduce MDA buildup brought on by EO3 and boost chlorophyll content (Autarmat et al. 2023). The chickpea yield showed a favorable correlation with the growth and yield characteristics (Fig. 3).

The results of this research demonstrated that the administration of 75% N + RPAN8 and 75% N + An-Rh enhanced seed yield by 12% and 14%, respectively, when compared to the recommended fertilizer dosage. On the other hand, the yield was 19% higher when both PGPR (75% N + RPAN8 + An-Rh) were combined. This result suggests that An-Rh was more effective than RPAN8 in reducing the effects of ozone stress, but the combined effect of these two PGPR was more than the sum of their individual contributions. Using PGPR in addition to nutrients may be an economical and sustainable way to mitigate the negative impacts of increasing tropospheric O₃. Future research should focus on examining the effects of tropospheric ozone on various crops and determining the optimal PGPR to counteract the effects of tropospheric ozone in order to ensure sustainable

agriculture, as there was little evidence regarding the amelioration effects of nutrients and PGPR over O₃.

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