Poll Res. 43 (1–2) : 171-176 (2024)

ISSN 0257–8050
DOI No.: http://doi.org/10.53550/PR.2024.v43i01-02.029

EFFECT OF PLANT GROWTH PROMOTING RHIZOBACTERIA AND NUTRIENT ON GASEOUS EXCHANGE AND YIELD OF CHICKPEA UNDER TROPOSPHERIC OZONE

SETHUPATHI NEDUMARAN[1], D.K. SHARMA[1*], ARTI BHATIA[1], DEEPASRI MOHAN[2], BOOMIRAJ KOVILPILLAI[3], G.K. DINESH[4] AND KOKILA MURUGESAN[1]

[1]*Division of Environment Science, ICAR-Indian Agricultural Research Institute, New Delhi, India*
[2]*Division of Environmental Sciences, Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir, India*
[3]*Department of Environmental Sciences, Agro Climate Research Centre, Tamil Nadu Agricultural University, Coimbatore, India*
[4]*SRM College of Agricultural Sciences, SRM Institute of Science and Technology, Baburayanpettai 603 201, T.N., India*

(Received 24 October, 2023; Accepted 25 December, 2023)

ABSTRACT

An experiment was conducted during 2020–21 and 2021–22 under FACE (Free air concentration enrichment) to assess the effect of plant growth promoting rhizobacteria (PGPR) and nutrient on gaseous exchange and yield of chickpea (*Cicer arietinum* L.) under tropospheric ozone (O_3). Chickpea PUSA 3043 (Desi) crop grown with PGPR treated and untreated seed and three fertilizers doses (100% urea N, 75% urea N and 50% urea N) under ambient and elevated O_3 exposure. Gaseous exchange (Photosynthetic rate, Stomatal conductance, Transpiration rate) and yield (seed weight per plant) were negatively impacted under elevated ozone treatment. In seed yield under interaction treatment, the PGPR ameliorates the negative effects of elevated ozone by about 21% and 20% in both years. Thus, in our study the PGPR treated seed was able to ameliorate the negative impact of EO_3 exposure in chickpea crop.

KEY WORDS: Ozone, Chickpea, Gaseous exchange, yield, PGPR, Nutrient

INTRODUCTION

Air pollution as a result of increased industrial and anthropogenic activities has caused major impact on agricultural activities. Tropospheric ozone (O_3) is a most critical secondary air pollutant, generated byphotochemical reactions of volatile organic compounds with nitrogenoxides. Its increasing concentration in India particularly in Indo-Gangetic plains is an issue of major concern as it is posing a threat to agriculture (Singh and Agrawal, 2016). A high concentration of O_3 in India's IGP region is mostly attributed to the transport sector and long-range transport from distant sources (Gao *et al*., 2020). Ground level ozone being phytotoxic causes substantial losses to agricultural productivity. O_3 enters the plant through stomata and in the apoplast it breaks down into reactive oxygen species (ROS), such as superoxide and hydrogen peroxide H_2O_2 (Waszczak *et al*., 2018). Chronically, high O_3 concentrations in vegetation can cause noticeable leaf injury, lower plant yield, altered interactions among the plants, associated insects and microorganisms, eventually hampering the ecological processes like nutrient, water cycling and decomposition (Grulke and Heath, 2020). These O_3 induced alterations in physiological and biochemical traits of the crops, significantly reduces the yield (Ashrafuzzaman *et al*., 2017). The direct effect of ozone on stomata is a main role in the impairment of photosynthesis (Feng *et al*., 2015). Moreover, O_3 can also inhibit the synthesis of

[2]Ph.D. Scholar, [3]Associate Professor

photosynthetic pigments, decreasing the electron transport rate between both photosystems (Calatayud and Barreno, 2004). Deng *et al*. (2010) indicated that photosynthetic capacity is an ideal physiological activity to monitor when the health and vitality of plants is under scrutiny. Therefore, gas exchange also provides an important source of information about plant growth under O_3-enriched environment.

It has been reported that yield loss in crop from O_3 stress can be compensated by replacing tolerant cultivar selection, additional supplementation of fertilizer, application of natural or chemical ozone protectants such as EDU (ethylene di-urea), (Agathokleous *et al.*, 2016), ascorbic acid, meta-quilinol and tagetus leaves (Archambault *et al.*, 2000). Use of chemicals as fertilizer and ozone protectants, though compensate O_3 stress, but have other impacts which may be detrimental to the environment. A group of microorganisms, specifically plant growth promoting rhizobacteria (PGPR) with their potential intrinsic genetic and metabolic capabilities may contribute to alleviate abiotic stresses in the plants (Gopalakrishnan *et al.*, 2015). The role of several rhizospheric bacteria belonging to the genera *Azotobacter*, *Azospirillum*, *Rhizobium*, *Pantoea*, *Bacillus* and *Enterobacter* (Meena *et al.*, 2017) in mitigation of multiple kinds of abiotic stresses and plant growth promotion has been documented. The objective of the study is to assess the effect of plant growth promoting rhizobacteria and nutrient on gaseous exchange, and yield of chickpea under tropospheric ozone.

MATERIALS AND METHODS

Experimental site

This study was carried out at the experimental farm of Division of Environment science, ICAR-Indian Agricultural Research Institute, New Delhi (28º35'N and 77º12'E, 228.16 m above mean sea level). The mean maximum and minimum temperatures from November to March were 18.5ºC and 29.5ºC. The pot study with chickpea crop was conducted in Free Air Ozone Enrichment facility (FAOE) under two O_3 levels as Ambient (AO_3) and Elevated (EO_3) ozone. Duration of exposure was 7 hours per day (except rainy days) from 9.30 AM to 4.30 PM. The target O_3 concentration in FACE- O_3 was around 60±10 ppb duringthe entire chickpea growing season using an ozone generator. The average ambient O_3 concentration was 30±10 ppb during the crop growth season.

Treatment details

The treatments comprised of two ozone exposure (Ambient – AO_3; Elevated – EO_3) levels along with a soil application of 3 different nitrogen doses and 2 strains of PGPR (seed treatment). Three replications were taken. The details of treatment combinations used are provided in Table 1. Recommended dose of NPK for chickpea crop is 20:40:20 kg/ha. The fertilizers were mixed with sterilized soil and filled in pots, based on the treatment doses.

Table 1. Treatment combinations

Treatment	Description
T1	Absolute Control
T2	100% RDF (Recommended Dose Fertilizer)
T3	75% RDN + 100% RD PK + RPAN8
T4	50% RDN + 100% RD PK + RPAN8
T5	75% RDN + 100% RD PK + An-Rh
T6	50% RDN + 100% RD PK + An-Rh
T7	75% RDN + 100% RD PK + RPAN8 + An-Rh
T8	50% RDN + 100% RD PK + RPAN8 + An-Rh

Note: (RDN – Recommended dose of nitrogen); (RD PK - Recommended dose of phosphorous and potassium); RPAN8 - *Anabena laxa;* An-Rh - *Anabena torulosa* with *Mesorhizobium ciceri* (Chickpea Rhizobium)

Gaseous exchange

Portable Photosynthesis system LI-6400-40 infrared gas analyser (IRGA) was used for measurement of the gaseous exchange parameters of photosynthesis rate, stomatal conductance and transpiration rate at flower initiation stage of crop. Photosynthetic rate, stomatal conductance and transpiration were expressed in µmol CO_2 m^{-2} s^{-1}, mol H_2O m^{-2} s^{-1}, and mmol H_2O m^{-2} s^{-1}, respectively. Measurements were taken between the hours of 10 a.m. to 2 p.m. Indian Standard Time.

RESULTS AND DISCUSSION

Photosynthesis rate (P_N)

At the flower initiation stage,highest (25.86 µmol CO_2 m^{-2} s^{-1}) rate of photosynthesis was observed in T7 (AO_3 + 75% N+ MC1+MC2)treatment whereas lowest (14.08 µmol CO_2 m^{-2} s^{-1}) was observed in T1 (EO_3 - Absolute control) treatment (Fig. 1.). As compared to ambient condition, EO_3 reduced the photosynthesis rate by 7.9 % and 7.1 % in 1st and 2nd year respectively at flower initiation stage. Whereas an increase in photosynthesis rate was observed in AO_3 + 75% N+ MC1+MC2treatment from EO_3+100% N+US (Untreated Seed) treatment by 23.4 % and 20.9% in 1st and 2nd year respectively at flower initiation stage. The interactions among the factors were found to be significant.

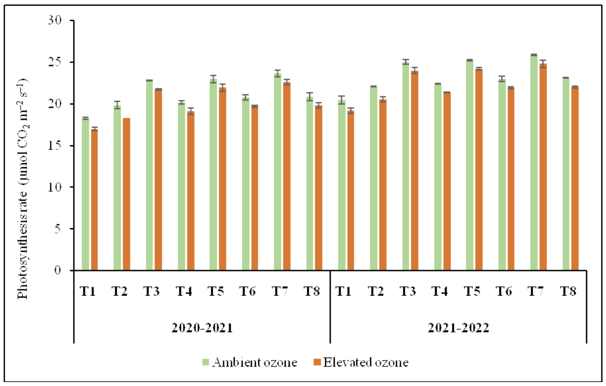

Fig. 1. Effect of O3, PGPR and nutrient on Photosynthetic rate of chickpea

Leaves exposed to O_3 in a wide range of plant species showlower gas exchange and reduced rates of carbon assimilation (Tisdale *et al*., 2021). This reduced photosynthetic capacity is mostly caused by the closing of the stomata and reduction of stomal conductivity, decreased Rubisco activity from damage on the chloroplast membrane, and reductions of photopigment contents by ozone stress (Singh *et al*., 2018). Increase in the photosynthesis rate at PGPR treatment was observed at the flowering phase which can be attributed to the increase in the Ribulose 1,5-bisphosphate (RuBP) carboxylase activity (Maheshwari *et al*., 1993) even under EO_3 at PGPR treatment. Positive effects of 75%N + MC1 +MC2 on theprocess related to photochemistry resulting in enhanced photosynthesis rate has been reported in previous studies (Fusaro *et al*., 2017).

Stomatal conductance (gs)

The stomatal conductance recorded at flower initiation stage was found to decrease under EO_3 condition. Our results showed that the highest stomatal conductance was observed in T7 (75% N+ MC1+MC2)treatment (0.45 mol $m^{-2}s^{-1}$) under ambient condition (Table 2). The stomatal conductance reduced by 36.3% in EO_3 condition as compared to ambient condition. Stomatal conductance was showed asignificance difference among all treatments with significant interactive effects. Reduction in gs would be taken as acommon leaf response to reduce the flux of O_3 into leaves (Guidi *et al*., 2001). Decrease in the stomatal conductance inO_3-treated plants commonly seems to counteract theentry of pollutant, consequently diminished the entryof CO_2 uptake which might be responsible for thereduction in photosynthetic rate of the plants (Chaudhary and Agrawal, 2015). Similar response was also reported by DeglInnocenti *et al*. (2002) on tobacco leaves after O_3 exposure. Reduction in Pn and gs was reported in O_3 exposed plants of tobacco(Pasqualini *et al*., 2002), mustard (Singh *et al*., 2009) and Qurecus (Yan *et al*., 2010).

Transpiration rate (E)

Like the stomatal conductance, when compared to AO_3conditions transpiration rate decreased in EO_3

Table 2. Effect of O_3, PGPR and nutrient on stomatal conductance (mol $m^{-2}s^{-1}$) of chickpea

Treatment	(2020-2021)		(2021-2022)	
	AO_3	EO_3	AO_3	EO_3
T1	0.33 ± 0.07	0.23 ± 0.05	0.38 ± 0.01	0.27 ± 0.04
T2	0.36 ± 0.08	0.25 ± 0.05	0.41 ± 0	0.29 ± 0.05
T3	0.38 ± 0.08	0.3 ± 0.05	0.43 ± 0.01	0.34 ± 0
T4	0.37 ± 0.06	0.28 ± 0.03	0.42 ± 0	0.32 ± 0.01
T5	0.39 ± 0.08	0.32 ± 0.04	0.44 ± 0.01	0.36 ± 0.01
T6	0.38 ± 0.06	0.29 ± 0.05	0.43 ± 0	0.33 ± 0.04
T7	0.4 ± 0.07	0.34 ± 0.05	0.45 ± 0.01	0.38 ± 0.01
T8	0.38 ± 0.06	0.3 ± 0.05	0.43 ± 0.01	0.34 ± 0.04
Mean	0.38	0.29	0.43	0.33
Factors	C.D.	SE(m)	C.D.	SE(m)
AO_3	0.004	0.002	0.006	0.002
EO_3	0.009	0.003	0.013	0.004
(AO_3 + EO_3) x (Fertilizer and PGPR)	0.015	0.005	0.018	0.006

conditions. The lowest average transpiration rate was found under T1 (EO_3+ Absolute control) (3.66 mol $m^{-2}s^{-1}$) and maximum average transpiration rate was observed under T7 in ambient condition (4.87 mol $m^{-2}s^{-1}$) at flower initiation stage (Fig. 2). Transpiration rate was showed a significance difference among the all treatments with significant interactive effects. Transpiration rate decreased by 13.6% & 13.5% in 1st and 2nd year respectively from ambient to elevated condition whereas there was an increase in transpiration rate by 11.6% & 11.4% in 1st and 2nd year respectively from AO_3+100% N+US treatment to AO_3+ 75% N+ MC1 + MC2 treatment at flowering stage. Decrease in stomatal density and guard cell length limits gs and transpiration (E), showing a shift towards minimum use of water and avoidance of O_3 infux (Hoshika *et al.*, 2015).

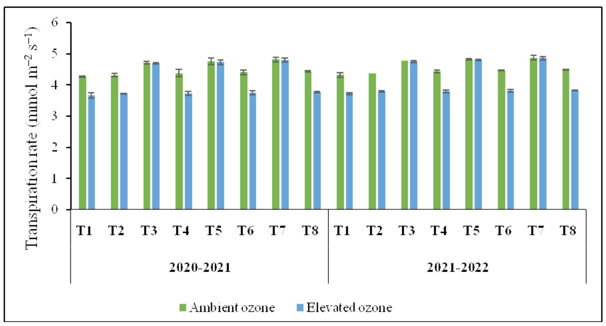

Fig. 2. Effect of O_3, PGPR and nutrient on transpiration rate of chickpea

Yield

When compared the all treatments, maximum seed weight was recorded in T7 (75% N+MC1+MC2) under AO_3 (23.96 g) & (25.19 g) and minimum seed weight was in T1 (Absolute control) (3.43 g) & (4.66 g) in 1st year and 2nd year respectively (Table 3). Similar trend was followed in EO_3 condition of seed weight per plant. There is a significant difference among all the treatments. Accordingly, the seed weight per plant in chickpea among the treatment was in the order of T_7> T_8> T_5> T_3> T_6> T_2> T_4> T_1 under AO_3 & EO_3 condition. Among elevated ozone exposure and ambient ozone treatments, the elevated ozone exposure was showed effects on the seed weight per plant. The interaction among ozone, PGPR, nitrogen on seed weight per plant was found to be significant. Moreover, the results indicate that the PGPR had a considerable influence in ameliorating O_3 induced damage to crops. During EO_3 exposure, the advancement in pod maturity by 8 and 10 days compared to AO_3 were reported (Bhatia *et al.*, 2021) and this decrease in the duration of reproductive period may have resulted in reduced seed weight and yield in our experiment. Pollen tube growth rate may decrease under stress, affecting pod and seed formation (Kaloki *et al.*, 2019). Gururani *et al.* (2013) reported that the inoculations with rhizobacteria strains 4 and 6 protected *S. tuberosum* against abiotic stress factors like water deficit, salinity, and heavy-metal toxicity.

CONCLUSION

It is concluded that elevated ozone showed

Table 3. Effect of O_3, PGPR and nutrient on seed weight/plant(g) of chickpea

Treatment	(2020-2021)		(2021-2022)	
	AO_3	EO_3	AO_3	EO_3
T1	9.74 ± 0.17^g	3.43 ± 0.07^i	10.97 ± 0.24^g	4.66 ± 0.07^i
T2	11.98 ± 0.05^f	4.83 ± 0.02^k	13.21 ± 0.15^f	6.06 ± 0.14^k
T3	15.79 ± 0.35^d	6.93 ± 0.08^i	17.02 ± 0.21^d	8.16 ± 0.14^i
T4	11.78 ± 0.17^f	4.85 ± 0.12^k	13.01 ± 0.28^f	6.08 ± 0.02^k
T5	17.46 ± 0.2^c	8.38 ± 0.06^h	18.69 ± 0.27^c	9.61 ± 0.05^h
T6	13.6 ± 0.26^e	5.63 ± 0.03^j	14.83 ± 0.14^e	6.86 ± 0.09^j
T7	23.96 ± 0.04^a	12.15 ± 0.29^f	25.19 ± 0.04^a	13.38 ± 0.09^f
T8	19.85 ± 0.15^b	10.13 ± 0.07^g	21.08 ± 0.41^b	11.36 ± 0.06^g
Mean	15.52	7.04	16.75	8.27
Factors	C.D.	SE(m)	C.D.	SE(m)
AO_3	0.16	0.05	0.18	0.06
EO_3	0.33	0.11	0.37	0.12
(AO_3 + EO_3) x (Fertilizer and PGPR)	0.47	0.16	0.52	0.18

±: Standard Error, Values followed by same letters with in columns are not significantly different at P d" 0.05; Treatment details are given under Materials and Methods.

detrimental effects on chickpea gaseous exchange and yield. When comparing the administration of the recommended dose of fertiliser to the treatments, the combination of (75% N + RPAN8 + An-Rh) ameliorates the negative effects of elevated ozone and increase the yield about 21% and 20% in both years. As seen in the present study, the gaseousexchange and yield decrease caused by elevated ozone can be mitigated by microbial interventions like PGPR by developing resistance towards abiotic stresses inside the plants. They induce novel genes, produces antioxidants which helps to scavenge ROS due to O_3 exposure. Along with nutrient, use of PGPRs can be a sustainable as well as cost effective solution to overcome the deleterious effects of rising tropospheric O_3.

REFERENCES

Agathokleous, E., Mouzaki-Paxinou, A. C., Saitanis, C. J., Paoletti, E. and Manning, W. J. 2016. The first toxicological study of the antiozonant and research tool ethylene diurea (EDU) using a *Lemna minor* L. bioassay: hints to its mode of action. *Environmental Pollution.* 213: 996-1006.

Archambault, D., Slaski, D. J. and Li, J. J. 2000. Ozone protection in plants. *The potential use of chemical protectants to measure oxidant damage in Alberta crops*. Report prepared for the Air Research Users Group. Alberta Environment, Edmonton, Alberta.

Ashrafuzzaman, M., Lubna, F. A., Holtkamp, F., Manning, W. J., Kraska, T. and Frei, M. 2017. Diagnosing ozone stress and differential tolerance in rice (*Oryza sativa* L.) with ethylenediurea (EDU). *Environmental Pollution.* 230: 339-350.

Bhatia, A., Mina, U., Kumar, V., Tomer, R., Kumar, A., Chakrabarti, B., Singh, R. N. and Singh, B. 2021. Effect of elevated ozone and carbon dioxide interaction on growth, yield, nutrient content and wilt disease severity in chickpea grown in Northern India. *Heliyon.* 7(1): e06049.

Calatayud, A. and Barreno, E. 2004. Response to ozone in two lettuce varieties on chlorophyll a fluorescence, photosynthetic pigments and lipid peroxidation. *Plant Physiology and Biochemistry.* 42(6): 549-555.

Chaudhary, N. and Agrawal, S. B. 2015. The role of elevated ozone on growth, yield and seed quality amongst six cultivars of mung bean. *Ecotoxicology and Environmental Safety.* 111: 286-294.

Degl'innocenti, E., Guidi, L. and Soldatini, G.F. 2002. Characterization of the photosynthetic response of tobacco leaves to ozone: CO_2 assimilation and chlorophyllfluorescence. *J. Plant Physiol.* 159: 1-9.

Deng, C., Zhang, G. and Zhao, K. 2010. Chlorophyll fluorescence and gas exchange responses of maize seedlings to saline-alkaline stress. *Bulgarian Journal of Agricultural Science.*

Feng, Z., Hu, E., Wang, X., Jiang, L. and Liu, X. 2015. Ground-level O_3 pollution and its impacts on food crops in China: A review. *Environmental Pollution.* 199: 42-48.

Fusaro, L., Palma, A., Salvatori, E., Basile, A., Maresca, V., Karam, E.A. and Manes, F. 2017. Functional indicators of response mechanisms to nitrogen deposition, ozone, andtheir interaction in two Mediterranean tree species. *PLoS One.* 12 (10): p.e0185836.

Gao, M., Gao, J., Zhu, B., Kumar, R., Lu, X., Song, S., Zhang, Y., Jia, B., Wang, P., Beig, G., Hu, J., Ying, Q., Zhang, H., Sherman, P. and B. McElroy, M. 2020. Ozone pollution over China and India: Seasonality and sources. *Atmospheric Chemistry and Physics.* 20(7): 4399-4414.

Gopalakrishnan, S., Sathya, A., Vijayabharathi, R., Varshney, R. K., Gowda, C. L. and Krishnamurthy, L. 2015. Plant growth promoting rhizobia: challenges and opportunities. *Biotechnology.* 5(4): 355-377.

Grulke, N. E. and Heath, R. L. 2020. Ozone effects on plants in natural ecosystems. *Plant Biology.* 22(S1): 12-37.

Guidi, L., Nali, C., Lorenzini, G., Filippi, F. and Soldatini, G.F. 2001. Effect of chronicozone fumigation on the photosynthetic process of poplar clones showing different sensitivity. *Environ. Pollut.* 113: 245-254.

Gururani, M. A., Upadhyaya, C. P., Baskar, V., Venkatesh, J., Nookaraju, A. and Park, S. W. 2013. Plant growth-promoting rhizobacteria enhance abiotic stress tolerance in *Solanum tuberosum* through inducing changes in the expression of ROS-scavenging enzymes and improved photosynthetic performance. *Journal of Plant Growth Regulation.* 32(2) : 245-258.

Hoshika, Y., Katata, G., Deushi, M., Watanabe, M., Koike, T. and Paoletti, E. 2015. Ozone-induced stomatal sluggishness changes carbon and water balance of temperate deciduous forests. *Scientific Reports.* 5(1): 1-8.

Kaloki, P., Devasirvatham, V. and Tan, D. K. Y. 2019. Chickpea abiotic stresses: Combating drought, heat and cold. *Abiotic and Biotic Stress in Plants.*

Maheshwari, M., Nair, T.V.R., Abrol, Y.P. 1993. Senescence and nitrogen remobilization.*Proc. Indian Natl. Sci. Acad.* B59 (3 and 4), 245-256.

Meena, K. K., Sorty, A. M., Bitla, U. M., Choudhary, K., Gupta, P., Pareek, A. and Singh, H. B. 2017. Abiotic stress responses and microbe-mediated mitigation in plants: the omics strategies. *Frontiers in Plant Science.* 8: 172.

Pasqualini, S., Della Torre, G., Ferranti, F., Ederli, L., Piccioni, C., Reale, L. and Antonielli, M. 2002.

Salicylic acid modulates ozone-induced hypersensitive cell death intobacco plants. *Physiol. Plant.* 115: 204-212.

Singh, A. A. and Agrawal, S. B. 2016. Tropospheric ozone pollution in India: effects on crop yield and product quality. *Environmental Science and Pollution Research.* 24(5): 4367-4382.

Singh, A.A., Fatima, A., Mishra, A.K., Chaudhary, N., Mukherjee, A., Agrawal, M. and Agrawal, S.B. 2018. Assessment of ozone toxicity among 14 Indian wheat cultivarsunder fieldconditions: growth and productivity. *Environ. Monit. Assess.* 190: 190.

Singh, P., Agrawal, M. and Agrawal, S.B. 2009. Evaluation of physiological, growth andyield responses of a tropical oil crop (*Brassica campestris* L. var. Kranti) underambient ozone pollution at varying NPK levels. *Environ. Pollut.* 157: 871-880.

Tisdale, R. H., Zentella, R. and Burkey, K. O. 2021. Impact of elevated ozone on yield and carbon-nitrogen content in soybean cultivar 'Jake.' *Plant Science.* 306: 110855.

Waszczak, C., Carmody, M. and Kangasjärvi, J. 2018. Reactive Oxygen Species in Plant Signaling. 69: 209-236. doi.org/10.1146/Annurev-Arplant-042817-040322,

Yan, K., Chen, W., He, X., Zhang, G., Xu, S. and Wang, L. 2010. Responses of photosynthesis, lipid peroxidation and antioxidant system in leaves of *Quercus mongolica* to elevated O_3. *Environ. Exp. Bot*. 69: 198-204.

.