Indian J. Applied & Pure Bio. Vol. 41(1), 331-339 (2026). **ISSN: 0970-2091**

Endophytic fungus *Fusarium* sp. enhances growth and salinity tolerance of salt-sensitive rice IR-64

R. R. Abhishek and *H. M. Kumaraswamy

Laboratory of Experimental Medicine, Department of Biotechnology,
Kuvempu University, Shankaraghatta - 577 451 (India)
*Corresponding Author Address: Dr. Kumaraswamy H M, Department of Biotechnology,
Kuvempu University, Shankaraghatta - 577 451 (India)
E-mail: drhmklab@gmail.com

Abstract

Salinity is one of the major abiotic stresses that negatively affects agricultural crop growth worldwide. Fungal endophytes are non-pathogenic, plant-associated microbes that play a significant role in conferring plant tolerance to salt stress. The exploitation of such bio stimulants and stress alleviators may benefit crop productivity and environmental sustainability. In the present study, we examined the role of a halotolerant fungal endophyte, *Fusarium* sp., for its ability to improve the growth performance of rice under salt stress. Inoculation with *Fusarium* sp., enhanced plant growth parameters under both laboratory and greenhouse conditions. Rice enriched with *Fusarium* sp., enhanced the production of chlorophyll pigments and decreased the levels of H_2O_2 and lipid peroxidation in rice under saline conditions. Conclusively, the present study suggests that the reactive oxygen species (ROS) scavenging abilities of the endophytic fungus can boost plant resistance to salinity, thereby enhancing agricultural productivity.

Key words : *Fusarium* sp., fungal endophyte, rice, salinity.

Rice (*Oryza sativa* L.) is consumed by more than half of the world's population[8]. As the global demand for rice is increasing, the growing climatic episodes have become a serious problem for its survival and have negatively impacted the plant productivity[15]. Increasing greenhouse gases, warmer temperatures, lower humidity, and more extreme rainfall events are accelerating the development of soil salinity[13]. Salinity disrupts normal plant growth by causing water stress, toxicity from excess sodium (Na^+) and chloride (Cl^-) ions creating nutritional imbalance that ultimately weaken the plant[14]. As a reflection, in the Indo-Gangetic basin, salinity stress has been reported to cause nearly 45% of reduction in rice yield, emphasizing the severity of the problem in rice-growing regions[2].

Conventional breeding and molecular

approaches have been used to develop salt-tolerant varieties, however in recent years utilization of beneficial endophytes has become an effective alternative to support normal plant growth under salinity stress. Endophytes, that reside within plant tissues without visible symptoms of infection or harm, are known to confer tolerance against biotic and abiotic stresses[5,11]. The endophytes employ multiple mechanisms such as ion homeostasis, osmotic adjustment and enhanced antioxidant activity to alleviate plant stress. They act as natural bio stimulants by producing bioactive compounds, phytohormones, and phosphate-solubilizing compounds to support healthier root development, seed germination, and plant growth[16]. Given the multifaceted benefits of endophytes it is important to identify, employ cost-effective, and sustainable solutions to improve plant growth.

Plants growing in extreme environments often harbour novel endophytes that are capable of providing sustainable benefits to crops[3]. In particular, fungal endophytes from harsh habitats such as cold deserts, saline marsh, and high-altitude possess unique physiological adaptations that can be transferred to plants to enhance their stress tolerance[4]. Therefore, harnessing these microbial communities from extreme environments offers an opportunity to identify novel functional endophytes capable of improving crop performance, including salinity.

This study investigates the potential of a Himalayan cold desert derived fungal endophyte *Fusarium* sp. to promote plant growth in the salt sensitive rice cultivar IR-64. By examining its effects on plant growth, physiological and biochemical responses under both non-stress and saline conditions we evaluate whether a cold-desert fungal endophyte can enhance salt tolerance in rice. We aim to highlight the importance of Himalayan endophytes as a natural resource for developing climate-resilient rice cultivation strategies. The findings aim to contribute to enhancing rice productivity in salt-affected regions.

Fungal inoculum preparation and rice seed inoculation :

A halotolerant *Fusarium* sp. strain was evaluated for its ability to enhance salinity tolerance in the salt-sensitive rice variety *Oryza sativa* L. cv. IR-64. Rice seeds were surface-sterilized[3] and were pre-germinated for 48 hours prior to endophyte inoculation. *Fusarium* sp. strain grown for ten days in PDA medium was used for suspension preparation by washing the mycelia with sterile deionized water. Pre-germinated rice seeds were treated with the fungal suspension (2×10^6 spores/mycelial fragments mL^{-1}) for three hours and the control group of seedlings was soaked in sterile deionized water.

Laboratory and greenhouse treatment conditions :

The endophyte treated seedlings were maintained on moistened paper towels and incubated at room temperature in the laboratory conditions. The control set of plants were treated with deionized water and the salt stressed group was treated with 150 mM of NaCl solution. The experiment was conducted in three biological replications with 25 seedlings each. Seedlings were watered regularly and seedling length was measured on 10th day. Based on the successful growth promoting effect in the laboratory conditions, *Fusarium* sp. was subsequently taken for greenhouse experiment. Further, endophyte-enriched and control seedlings (2 d old) were transferred to plastic pots (height × diameter =12 cm × 12 cm) grown under greenhouse conditions. After 30 days of plant growth, seedlings were subjected to salinity stress by 150 mM NaCl solution for a period of seven days and the control group received water. Plant growth parameters were measured, and leaf samples for biochemical assays (chlorophyll, MDA, and H_2O_2) were collected after seven days of salinity stress in the greenhouse.

Determination of chlorophyll :

Leaf samples collected from control and endophyte treated plants were used for estimating chlorophyll content. Fresh leaf tissues (0.1 g) were cut into uniform segments and incubated in a mixture of 80% acetone and DMSO (1:1, v/v). The samples were incubated at room temperature under dark conditions for 24 hours to allow total pigment extraction. Following incubation, chlorophyll content was quantified by measuring absorbance at 663 and 645 nm using a UV–visible spectrophotometer. Total chlorophyll was estimated using the formula below[10]:

Chlorophyll a = [{12.7 (A663) – 2.69 (A645)} X volume/ we ight] X 1000

Chlorophyll b = [{22.9 (A645) – 4.68 (A663)} X volume/ weight] X 1000

Total chlorophyll (mg/g fresh weight) = chlorophyll a + chlorophyll b

Determination of Malondialdehyde :

Lipid peroxidation was quantified by measuring malondialdehyde (MDA), a terminal product of the peroxidation of polyunsaturated fatty acids in cellular membrane[7]. Fresh leaf tissue (0.2 g) was homogenized in 4 mL of 0.1% (w/v) trichloroacetic acid (TCA), and the homogenate was centrifuged at 12,000 rpm for 10 min at room temperature. An equal volume of 0.5% thiobarbituric acid (TBA) prepared in 20% (w/v) TCA was added to the supernatant, and the reaction mixture was heated at 95 °C for 30 min. Samples were rapidly cooled on ice and centrifuged again at 10,000 rpm for 10 min to remove debris. The absorbance of the clarified supernatant was recorded at 532 nm and corrected for nonspecific turbidity by subtracting absorbance at 600 nm. Lipid peroxidation was expressed as the difference in absorbance (A_{532}, $-A_{600}$).

Determination of Hydrogen Peroxide (H_2O_2) :

Rice leaves were excised and were cut into 5 cm segments. Tissue bits were washed with distilled water and transferred to test tubes and immersed in DAB (3,3' - Diaminobenzidine) staining solution for the detection of H_2O_2. Test tubes were wrapped with aluminium foil and incubated for 12 hours in dark. The excess stain was drained off and chlorophyll was removed for clear visualization of the stain. Following the extraction of stained tissue, H_2O_2 content was quantified using a UV-visible spectrophotometer[12].

Statistical Analysis :

All experiments were conducted

under greenhouse conditions following a completely randomized design (CRD). For each parameter, standard errors were calculated across biological replicates. Treatment effects were analyzed using one-way ANOVA, and mean separation was performed using Tukey's test at a significance level of $p < 0.05$.

Effect of Fusarium sp. on growth parameters and biomass under salinity stress :

In the present study *Fusarium* sp. inoculation restored the rice growth facing salinity under both laboratory as well as greenhouse conditions. In the preliminary experiment, rice seedlings with fungal inoculation showed significant shoot growth compared to uninoculated seedlings. The effect of endophyte was not observed in the seedlings under non-saline condition (Fig. 1). The results indicate that the endophytic effect during saline condition is a stress-dependent phenomenon, where endophytic activity is favored during salinity in the early seedling development. However, when the experiment was conducted in greenhouse condition, the positive effect of endophyte was observed under both saline and non-saline conditions. Under greenhouse condition, the imposition of NaCl induced salt stress significantly reduced plant growth parameters (Fig. 2). In contrast, application of endophyte during salt stress improved shoot length, root length, shoot dry weight and root dry weight by 27%, 46%, 55% and 57%, respectively (Table-1). Similarly, endophyte treatment under non-stress condition improved the performance of all growth parameters. Overall, *Fusarium* sp. conferred strong growth promotion, with its greatest benefits observed under salt stress.

Effect of Fusarium sp. *on chlorophyll content of rice under salinity stress :*

Exposure of rice plants to continuous salinity hampers growth and productivity. In the current investigation, the influence of fungal endophyte on salt stressed plants was analysed by estimating chlorophyll content in leaf tissues. Inoculation of *Fusarium* sp. had positive influence for both normal and salt stressed plants. Under salt stress, endophyte application significantly enhanced chlorophyll content by 29% when compared to the control. Under normal condition, endophyte treatment followed the same trend by enhancing chlorophyll content by 9% compared to those of control (Fig. 3).

Effect of Fusarium sp. *on Lipid Peroxidation (MDA) Under Salinity Stress :*

The accumulation of Na^+ in rice plants induced by NaCl stress damages cellular homeostasis, leading to various types of membrane damage, including lipid peroxidation. Under salinity without *Fusarium* sp. MDA content was greatly increased. However, the application of endophyte in rice substantially reduced MDA up to ~32% compared untreated rice plants under stress condition. Similar data was also observed under non-stress condition where the application of endophyte decreased the MDA content. The data suggest that fungus associated with rice mitigates oxidative stress and protect cell membranes from damage (Fig. 4).

Effect of Fusarium sp. on H_2O_2 *accumulation under salinity stress :*

Rice plants exposed to NaCl stress were found to have enhanced H_2O_2 production.

Higher concentration of H_2O_2 negatively impacts cell membrane integrity. Hydrogen peroxide concentrations in the non-treated plants increased upon salt stress imposition indicating the negative impact of salinity. However, plants in the presence of fungal endophyte decreased H_2O_2 quantity by 25% compared to non-treated plants. Endophyte application to plants under non-stress conditions also diminished the levels of H_2O_2 slightly by 2%, compared to those of non-treated plants (Fig. 5).

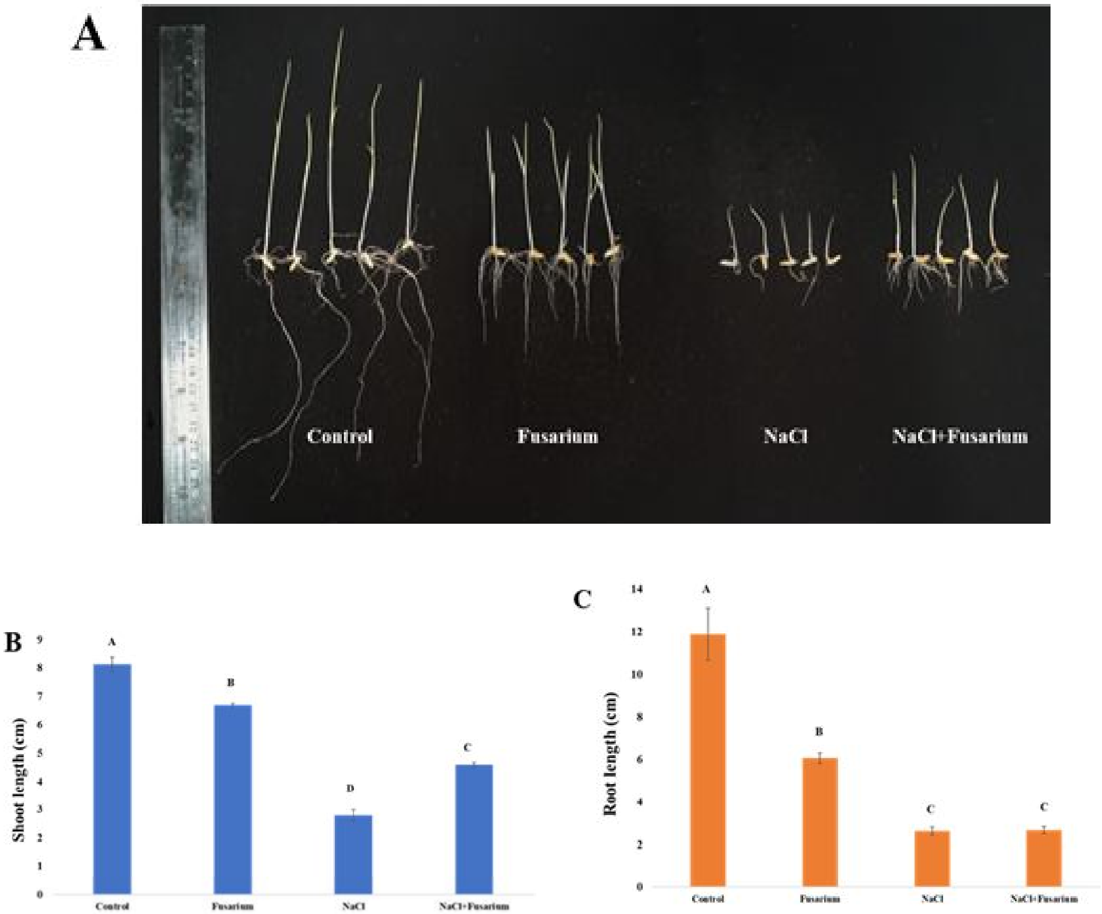

Fig. 1. Early seedling response of rice (10-day-old) to *Fusarium* sp. inoculation under salinity stress. **(A)** Phenotypic appearance of seedlings under four treatments: Control, *Fusarium,* NaCl, and NaCl + *Fusarium.* **(B)** Shoot length of 10-day-old rice seedlings under four treatments. **(C)** Root length of 10-day-old rice seedlings under four treatments. Values represent mean ± SE (n = 6). Bars with different indicate significant differences among treatments at $p < 0.05$ (Tukey's HSD).

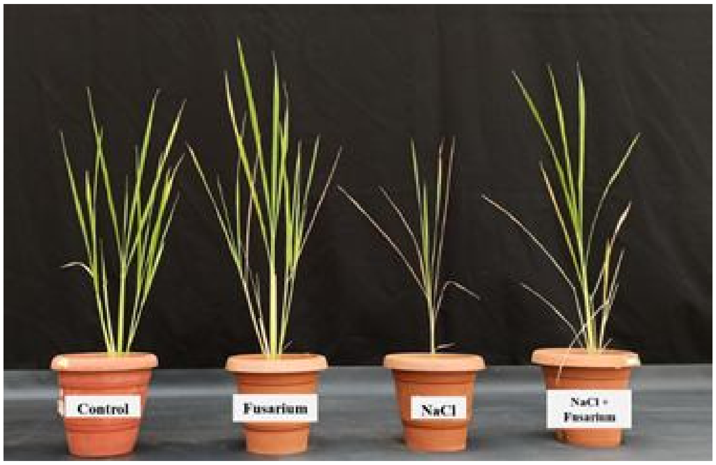

Fig. 2. Effect of fungal endophyte *Fusarium* sp. on rice plants under non-stress and salinity conditions under greenhouse conditions

Table-1. Effect of *Fusarium* sp. on the growth of rice under greenhouse conditions

Treatment	**Shoot length (cm)**	**Root length (cm)**	**Shoot DW (g)**	**Root DW (g)**
Control	35.28±1.21^{a}	22.41±0.83^{a}	1.49±0.04^{a}	1.01±0.05^{a}
Fusarium	41.68±1.00^{b}	28.30±1.03^{b}	1.81±0.05^{b}	1.27±0.04^{b}
NaCl	25.15±1.04^{c}	16.07±1.14^{c}	0.90±0.04^{c}	0.55±0.03^{c}
NaCl+Fusarium	31.82±0.94^{d}	23.51±1.32^{a}	1.39±0.04^{d}	0.86±0.03^{d}

Data presented are the means ± standard deviation of six replicates. Means with different letters are significant according to Tukey's post hoc test ($P<0.05$).

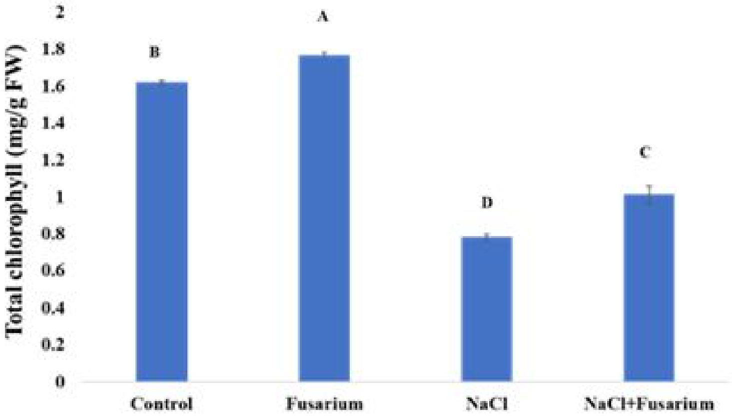

Fig. 3. Chlorophyll content of rice seedlings under four treatments. Values represent mean ± SE (n = 6). Bars with different indicate significant differences among treatments at $p < 0.05$ (Tukey's HSD).

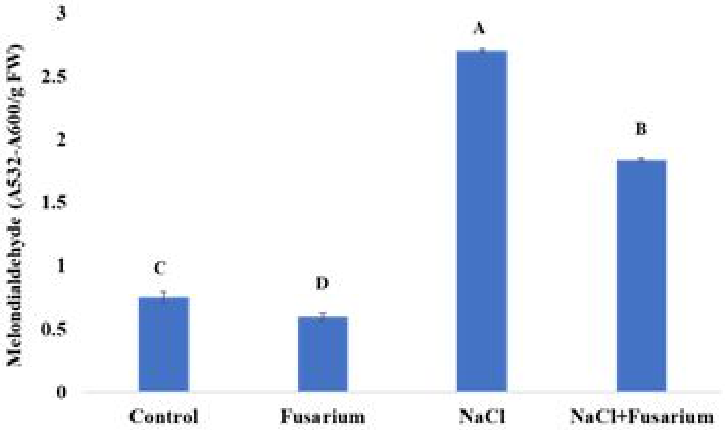

Fig. 4. MDA content of rice seedlings under four treatments. Values represent mean ± SE (n = 6). Bars with different indicate significant differences among treatments at $p < 0.05$ (Tukey's HSD).

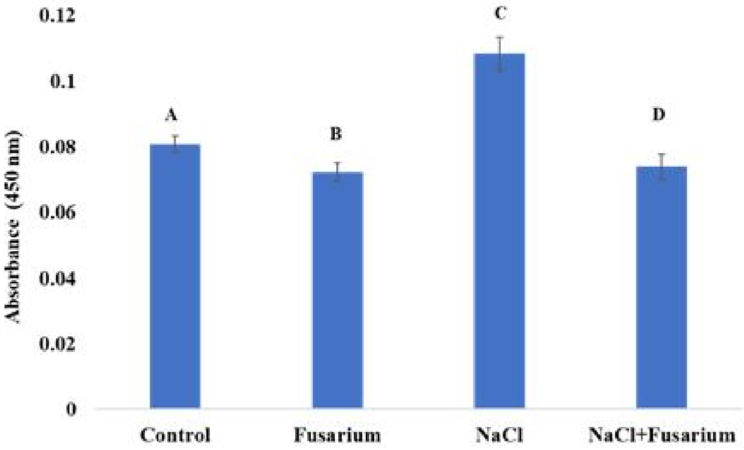

Fig. 5. DAB-staining intensity indicating hydrogen peroxide (H, O,) accumulation in rice seedlings under different treatments. Values represent mean ± SE (n = 6). Bars with different indicate significant differences among treatments at $p < 0.05$ (Tukey's HSD).

Salinity affects normal plant growth and metabolism, leading to loss of food crops. Endophytes play a major role in mitigating environmental stresses and supporting healthy plant growth. They influence the host plant and also shape the stability of the surrounding ecosystem[5]. Given this background, in this study, the halophyte *Fusarium* sp. was tested for its ability to impart salinity tolerance in the salt-sensitive rice variety IR-64 by integrating early seedling assays and greenhouse validation. Under normal conditions, the ten-day-old seedlings were observed to be the same between the endophyte-treated and un-treated, with no significant differences in their length. However, when salinity stress was introduced, the seedlings without the endophyte showed noticeable reductions in root and shoot size, which is expected under osmotic and ionic stress. In contrast, the seedlings enriched with the endophyte were observed to grow better under the same salt stress, highlighting the protective effect of the *Fusarium* sp. Previous work has demonstrated that under salt stress, endophyte-enriched plants showed improved root and shoot growth and maintained a lower Naz /Kz ratio in endophyte-enriched plants[1].

Under greenhouse conditions, the growth enhancement of plants under stress is explained by the experimental design by having controls with and without NaCl treatment, where it was evident that *Fusarium* sp. provided stress-dependent growth benefits. Furthermore, after fungal establishment in plants in the greenhouse, plant height and biomass increased. The increased biomass can be explained by the fact that the endophytic fungus helps plants cope with salt stress by improving antioxidants, increasing osmoprotectant levels, adjusting phytohormone balance, and even reducing the excessive root respiration triggered by high salinity[15].

The fungal-enriched plants showed improved chlorophyll stability under salinity stress. Decreased levels of chlorophyll in salt-stressed plants are widely recognized as a classic symptom of oxidative stress, which is attributed to the inhibition of chlorophyll biosynthesis and degradation of the enzyme chlorophyllase. Consistent with the above finding, the salt-stressed plants showed a decline in total chlorophyll levels. As expected, the endophyte-treated plants showed improved chlorophyll in both with and without salt stress conditions. Approximately 29% of chlorophyll was enhanced in endophyte-enriched, salt-stressed plants, reflecting better pigment stability and reduced cellular damage, as estimated in biochemical assay. The higher pigment levels suggest that *Fusarium* sp. helps protect chloroplasts and slows down the salt-induced breakdown of pigments.

Under normal conditions, plants maintain a balance between the production and detoxification of ROS. However, biotic and abiotic stresses create an imbalance that leads to excessive ROS accumulation[9]. To study this imbalance in the plant system, we assessed the effect of salt stress on the MDA content, which is a marker for oxidative stress, and $H_2 O_2$, an indicator of ROS levels in control plants. The increase in MDA and H_2O_2 confirms that oxidative stress is a major component of salt-induced damage. In contrast, the endophyte-treated seedlings showed lower oxidative stress, with reduced MDA levels by approximately 32% and a significant decrease in H_2O_2 absorbance, indicating the protective effect of *Fusarium* sp. against ROS accumulation. It was observed that endophyte-colonized wheat plants showed much lower oxidative damage under salt stress compared to untreated controls[6]. Considering both MDA and H_2O_2 results together, it is evident that the endophyte-colonized rice has stronger antioxidant defences, better membrane stability, and overall reduced oxidative damage.

Overall, integrating growth measurements, chlorophyll assays, and oxidative stress markers, the study demonstrates that *Fusarium* sp. plays a multifaceted role in enhancing salinity tolerance. Especially, *Fusarium* sp. shows beneficial effects on plants under saline stress, improving growth, protecting photosynthetic machinery, and minimizing oxidative damage. The combination of *in-vitro* seedling experiments, greenhouse validation, and biochemical assays provides strong methodological support for the observed physiological responses. Collectively, the results indicate that

Fusarium sp. is a promising endophyte for developing salinity-resilient rice varieties, especially in salt-affected agricultural systems.

References :

1. Ali, R., H. Gul, M. Hamayun, M. Rauf, A. Iqbal, A. Hussain, and I. J. Lee, (2022). *Frontiers in Plant Science, 13:* 890565. https://doi.org/10.3389/fpls.2022.890565
2. Al-Tamimi, N.C., H. Brien, B. Oakey, S. Berger, Y. Saade, S. Ho, S.M., Schmockel, S.M., Tester and S. Negrao (2016). *Nature communications*, *7*(1): p.13342. https://doi.org/10.1038/ncomms13342
3. Arnold, A. E., Z. Maynard, G.S. Gilbert, P. D. Coley and T.A. Kursar (2000). *Ecology Letters, 3*(4): 267-274. https://doi.org/10.1046/j.1461-0248.2000.00159.x
4. Ballesteros, G. I., K.K., Newsham, I.S., Acuna-Rodriguez, C. Atala, C.Torres-Díaz and M.A. Molina-Montenegro (2024). Plants, People, Planet, 6. https://doi.org/10.1002/ppp3.10415
5. Cui, J., F. Nie, Y. Zhao, D. Zhang, D, Zhou, J. Wu, and L. Liu (2024). *Environmental Pollutants and Bioavailability, 36:* https://doi.org/10.1080/26395940.2024.2323123
6. Dargiri, S.A., S. Naeimi and M.K. Nekouei (2025). *BMC Plant Biology, 25*(1): 354. https://doi.org/10.1186/s12870-025-04354-0
7. Dhindsa, R. S., P. Plumb-Dhindsa and T.A. Thorpe (1981). *Journal of Experimental Botany, 32*(1): 93-101. https://doi.org/10.1093/jxb/32.1.93
8. Ganie, S. A., J.A. Bhat and A. Devoto (2022). *Plant Molecular Biology, 109:* https://doi.org/10.1007/s11103-021-01219-8
9. Hasanuzzaman, M. and M. Fujita (2022). *Plants, 11*(9): 1185. https://doi.org 10.3390/plants11091185
10. Hiscox, J. D. and G.F. Israelstam (1979). *Canadian Journal of Botany, 57*(12): 1332-1334. https://doi.org/10.1139/b79-163
11. Khalmuratova, I., D.H. Choi, J.R. Woo, M.J. Jeong, Y. Oh, Y.G Kim and J.G. Kim (2020). *Journal of Microbiology and Biotechnology, 30:* https://doi.org/10.4014/jmb.2006.06050
12. Mostofa, M. G., D. Saegusa, M. Fujita and L.S.P. Tran (2015). *Frontiers in Plant Science, 6:* 1055. https://doi.org/10.3389/fpls.2015.01055
13. Mukhopadhyay, R., B. Sarkar, H. S. Jat, P.C. Sharma and N.S. Bolan (2021). *Journal of Environmental Management, 280:* https://doi.org/10.1016/j.jenvman.2020.111736
14. Sahu, P. K., Z. Shafi, S. Singh, K. Ojha, K. Jayalakshmi, J. Tilgam and A.K. Srivastava (2023). *Frontiers in Microbiology, 14:* https://doi.org/10.3389/fmicb.2023.1226149
15. Siddiqui, Z. S., X. Wei, M. Umar, Z. Abideen, F. Zulfiqar, J. Chen and R. Yasmeen (2022). *Frontiers in Plant Science, 12:* 770084. https://doi.org/10.3389/fpls.2021.770084
16. Verma, A., N. Shameem, H.S. Jatav, E. Sathyanarayana, J.A. Parray, P. Poczai and R.Z. Sayyed (2022). *Frontiers in Plant Science, 13:* https://doi.org/10.3389/fpls.2022.953836