Original Research Article

Effects of plant growth-promoting rhizobacteria (PGPR) and arbuscularmycorrhizal fungi (AMF) on morpho-physiological parameters of strawberry cv. Chandler under different moisture levels

Abstract:

Drought stress is one of the challenges that can affect the growth and quality of strawberries. The present study aims to determine the effect of PGPR and AMF on the growth of the strawberry cultivar Chandler under different moisture levels. This study was conducted under polyhouse at the Horticulture Research Center, GBPUA&T, Pantnagar, during 2021-2022 and 2022-23. The experiment was arranged in a factorial CRD design (FCRD) with three replications.The experiment included moisture levels (100% field capacity (FC), 75% FC and 50% FC)and bioinoculants: control (without PGPR or AMF), PGPR,AMF and combination of PGPR+AMF. The results showed that water stress significantly reduced all the growth and biochemical characteristics of leaves. Plants treated with PGPR and AMF either alone or in combination showed higher survival rate of plants as compared to control.The numbers of leaves,petiole length, chlorophyll a, chlorophyllb, total chlorophyll and carotenoids were found to increase in all the treatments where bioinoculants were incorporatedin the potting media.The increase in chlorophyll content suggests improved photosynthetic efficiency which eventually enhanced stress tolerance of the plants. The use of PGPR and AMF provides a ray of hope for sustainable agricultural cultivation of strawberry particularly in regions affected by water scarcity.

Keywords: Plant growth-promoting rhizobacteria, arbuscularmycorrhizal fungi,drought stress, strawberry, bioinoculants.

Introduction:

Strawberry (*Fragaria* × *ananassa*Duch.) is one of the most widely known fruit and is favorite among people worldwide. The popularity of strawberries can be attributed to their attractive color, shape, and unique taste, as well as their nutritional content, which helps in the prevention of various infections (**Nezhadahmadi*et al.*, 2015**).Strawberries require large amounts of water and are very much sensitive to drought, affecting its production and yield (**Chaves *et al.*, 2009**). The drought condition significantly brings changes in plant morphology, physiology and biochemistry.

Under water stress conditions, stomata are closed and thereby theleaf conductance is decreased. Further, the CO_2availability is reduced in plants due to which thenet photosynthesis under mild and moderate water stress conditions decreases. Besides, thisreduced leaf gas exchange under drought conditions is drastically declining carbon assimilation in plants, ultimately influencing the photoassimilates supply. Once the carbon assimilation yield declines, the development of the vegetative phase and the reproductive organs is also inhibited**(Yenni*et al.*, 2021).** The vegetative stage of strawberry plants is highly sensitive to drought stress as plant growth rate, leaf area, leaf area index, biomass and production can be adversely affected (**Pandey *et al.*, 2000).** The negative effect of drought stress on leaf area, leaf number, chlorophyll content and chlorophyll stability index has previously been observed in strawberry plants (**Nezhadahmadi*et al.*, 2015**). Microbes such as PGPR and AMF help in the amelioration of different types of plant stresses such as drought and other biotic stress. Plant growth-promoting rhizobacteria (PGPR) are a group of beneficial soil bacteria that colonize the roots of plants and promote plant growth and development. PGPR adopts various mechanisms to overcome drought stress in plants. These mechanisms include alteration in root morphology, production of osmolytes, antioxidants, phytohormones, extracellular polymeric substances (EPS), volatile organic compounds (VOCs), siderophores and 1-aminocyclopropane-1-carboxylate (ACC) deaminase (**Kaushal*et al.*, 2016., Ahmad *et al.*, 2022**). The use of PGPRs can minimize crop losses under limited water conditions and promote sustainable crop production in drylands (**Chakrabarti*et al.*, 2022**).Arbuscularmycorrhizal fungi (AMF) are symbiotic fungi that predominate in the root and soil of agricultural and horticultural crops. Arbuscularmycorrhizal fungi penetrate the roots of plants and form a mutualistic symbiotic relationship. AMFs can change the water relation of their host plants and influence the metabolism, protective adaptation and morphology of host plants in water deficit stress conditions (**Mickan*et al.*, 2016**). In addition, these fungi can enhance drought tolerance of host plants through mechanisms such as improvement of soil properties inthe rhizosphere, enlargement of root areas, enhancement of water use efficiency and enhance uptake of P and other nutrient elements, quick activation ofthe defense system and protection against oxidative damages created by drought **(Auge, 2001).** In this study, it was tried to evaluate the impact of AMF and PGPR on survival percentage and leaf traits of strawberry cv. Chandler under different moisture levels.

Materials and Methods:

Plant Material

The runners of strawberry cv. Chandler wasprocured from NBPGR, regional station Bhowali, Uttarakhand and transplanted in plastic polybags (2 L capacity). The pots were filled with a mixture of soil, farmyard manure (FYM) and sand in a ratio of 2:1:1. One runner per pot was planted and the plants were kept in a polyhouse.

Inoculation with PGPR and AMF

The bioinoculants (PGPR and AMF) were collected from Rhizosphere Laboratory, Department of Biological Sciences, College of Basic Sciences and Humanities, G. B. Pant University of Agriculture and Technology, Pantnagar. The strain of PGPR used was*Pseudomonas palleroniana* (DPB16) and that of AMF was *Rhizophagusintraradices.*The inoculation of AMF was performed by adding 1g of AMF (70 spores/g) to the soil around the plant roots while the inoculation of PGPR, the roots of plants were dipped in the PGPR formulation containing $45x10^7$ cells per ml.

Application of Moisture Stress

After 45 days of planting (DAP), once the symbiotic relationshipof roots with bioinoculants was established, the plants were subjected to moisture stress. Three different regimes of soil moisture level were applied, namely 100 % field Capacity (FC)/Well watered, 75% FC and 50 % FC. After 45 DAP, pots under the well-watered treatment, were maintained at 100% FC. Pots under the mild and severe water stress treatments, were however left to lose water, until they were slightly below their required FC levels. When the soil moisture content went below either 75% or 50% FC, they were re-watered and brought to their required moisture levels. The pots continued to be maintained at their required soil moisture levels, with daily replacements of lost water, throughout the rest of the water stress period. Digital Moisture meter (Sanco, SAN-ST-124)was used to determine the Relative Water Content (RWC) daily.

Survival Percentage, Vegetative Parameters and photosynthetic pigments

The survival percentage, vegetative parameters (number of compound leaves, petiole length) andphotosynthetic pigments (chlorophyll a, chlorophyll b, total chlorophyll and total carotenoid) of the plants were recorded at 60 DAP. The petiole length was measured using a scale and the photosynthetic pigments were measured using spectrophotometric standard methods as described by **Lichtenthaler and Wellburn (1983).** The absorbance of the pigment extract was measuredat wavelengthsof 470 nm, 646 nm and 663 nm.

Statistical Analysis

The statistical analysis of variance (ANOVA) was conducted using a Factorial Completely Randomized Design (CRD) for the pooled data obtained from the experiments. IBM SPSS software (version 16) was used to assess significant differences among the treatmentsand Duncan's Multiple Range Test was applied at a significance level of 5%.

Results and Discussion

The survival percentage, vegetative parameters (number of leaves, petiole length) and photosynthetic pigments(chlorophyll *a*, chlorophyll *b*, total chlorophyll and total carotenoid) in the leavesof strawberry cv. Chandler(Table 1) was significantly influenced ($p \leq 0.05$) by the application of bioinoculants and moisture regimes. Moreover, survival percentage and vegetative parameters arealso significantly influenced ($p \leq 0.05$) by the interaction effect ofbioinoculants and moisture levels. The plants treated with a combination of PGPR and AMF displayed highest survival rate, number of leaves, petiole length, chlorophyll *a*, chlorophyll *b*, total chlorophyll and total carotenoid content and the results wereclosely followed by the treatments whereAMF and PGPR were applied solely.In the treatment where PGPR was applied along with AMF,moresurvival% (16.77%), number of leaves (52.37%), petiole length(57.94%), chlorophyll *a*(7.1%), chlorophyll b(16.26%,), total chlorophyll (11.01%) and total carotenoidcontent (10.23%) was obtained as compared to non-inoculated plants (control). The results revealed a gradual reduction in all leaf traits as the field capacity was decreased from 100% to 50%. The plants with 100% FC obtainedmore survival% (8.51%), number of leaves (21.29%), petiole length (127.78%), chlorophyll *a* (31.09%), chlorophyll b(30.33%), total chlorophyll (74.06%) and total carotenoid content (4.85%) as compared to 50 % FC (severe moisture stress). The findings of our study strongly suggest that water deficit can drastically limit the growth of strawberry plants.Ininteraction (bioinoculants × moisture levels), PGPR+AMF and sole application of AMF resulted in 100% survival of the plants on the 60th day after planting under 100% and 75% field capacity conditions. However, under 50% field capacity, the combined application of PGPR+AMF outperformed sole application of AMF and PGPR in terms of survival percentage. The plants in control exhibited lowest survival percentage across all moisture levels. In case of petiole length, number of leaves, chlorophyll *a*, chlorophyll *b*, total chlorophyll and total carotenoid content, the treatment where PGPR+AMF was given outperformed the results of AMF and PGPR alone under all 3 moisture levels. The maximum value for vegetative parameters and

photosynthetic pigments were obtained in plants inoculated with PGPR+AMF at 100% FC, while the lowest was found in the non-inoculated plants at 50% FC.

Water stress remarkably reduced the survival rate, vegetative parameters and photosynthetic pigments. Water deficit could limit the growth of the plants, which is evident through reduced petiole length and leaf number. The findings of our research areunderaccordance with the study of **Nezhadahmadi*et al.*, (2015)** in strawberry and **Azizi*et al.*, (2021)** in common myrtle, where thenegative effect of drought stress on leaf area, leaf number, petiole length, chlorophyll content, chlorophyll stability index, leaf pigment was observed. Vegetative stage of strawberry plants is very sensitive to drought stress as plant growth rate, leaf area, leaf area index, biomass and production are adversely affected (**Pandey *et al.*, 2000).**During water stress the plant cell's turgor and water potential decreases, which can enhance theconcentration of the solutes in cytosol situated outside cell matrices.This can modify how water moves over the plasma membrane of the plant, as well as alter physiological and biochemical traits**(Hsiao, 1973; Lisar*et al.*, 2012).**Usually, under increased oxidative stress due to drought, the chloroplast and thylakoid structures are injured, leading to decrease in chlorophyll and carotenoid contents**(Asrar and Elhindi, 2011).**

The results of this study indicate that, regardless of the moisture regimes, the use of PGPR and AMF showed better growth and development than the non-inoculated plants.The synergistic effect of the consortium (PGPR+AMF) remarkably reduced the harmful effects of water stress.Our findings are in harmony with**Azizi*et al.* (2021),**who observed that dual inoculation of PGPR +AMF significantly alleviated negative impact of drought on biomass and morpho-physiological traits of common myrtle.Additionally, **Quintana *et al.*(2022)**also observedthat plant survival is increased in strawberry under drought conditions mainly due to the presence of fungal endophytes. Mycorrhizal association enhances the uptake of water and nutrients by the host plants largely by expanding the mycelia network in the rhizospheric region, which eventually facilitates enhanced growth and biomass accumulation (**Begum*et al.* 2019).** AMF hasthe ability to change water relation of their host plants and influence metabolism, protective adaptation and morphology of host plants in water deficit mediated stress conditions (**Mickan*et al.*, 2016**).The production of plant growth hormones such as auxins, gibberellins, cytokinins and ethylene stimulates young sapling growth through PGPR application (**Kloepper, 1996).**PGPR adopts various mechanisms including alteration in root morphology, production of osmolytes, antioxidants, phytohormones, extracellular polymeric substances (EPS), volatile organic compounds (VOCs), production of

siderophores and 1-aminocyclopropane-1-carboxylate (ACC) deaminase to overcome drought stress in plants (**Kaushal*et al.*, 2016., Ahmad *et al.*, 2022**). These mechanisms help plants to counteract osmotic and oxidative damage and ensure plant survival under drought stress (**Kaushal*et al.*, 2016).** PGPR can also increase chlorophyll and total carotenoid content by increasing stomatal conductivity, photosynthesis and water & nutrient uptake (**Marius *et al.*, 2005**).

Conclusion

The current study showed that water deficit significantly influenced the growth of strawberry plants. Water stress remarkably reduced survival rate, growth parameters (petiole length, number of leaves) and biochemical parameters (chlorophyll *a*, chlorophyll *b*, total chlorophyll and total carotenoid) of strawberry plants. Based on the experimental results obtained, it can be concluded that the bioinoculants played a significant role in increasing survival, growth and photosynthetic pigments of strawberry cv. Chandler over non-inoculated plants under moisture stress conditions. This study highlights the synergistic effects of PGPR+AMF inoculation on strawberry growth parameters and photosynthetic pigments over non-inoculated plants. The present findings indicate that PGPR+AMF treatment can be used as a cost - effective method in boosting strawberry production by imparting drought resistance and can be recommended as a part of standard nursery practice in drought- prone areas.

Table 1. Effect of bioinoculants on leaf parameters of strawberry cv. Chandler under different moisture levels at 60 DAP.

Treatments		Survival %	Number of trifoliate leaves/plant	Petiole length (cm)	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total Chlorophyll (mg/g)	Total Carotenoid (mg/g)
Bioinoculants								
Control		84.343^{c}	4.967^{d}	4.931^{a}	1.787^{c}	1.334^{c}	3.122^{c}	.420^{c}
PGPR		93.939^{b}	5.900^{c}	7.169^{b}	1.857^{b}	1.490^{b}	3.348^{b}	.442^{b}
AMF		97.980^{a}	6.632^{b}	6.888^{c}	1.877^{ab}	1.514^{b}	3.392^{b}	.457^{a}
PGPR + AMF		98.484^{a}	7.568^{a}	7.788^{a}	1.914^{a}	1.551^{a}	3.466^{a}	.463^{a}
SEm±		0.357	.042	0.032	0.015	.009	0.020	0.004
CD at 5%		1.049	0.123	0.095	0.044	0.028	0.058	0.012
Moisture levels								
100 % FC		96.591^{a}	6.824^{a}	7.400^{a}	2.312^{a}	1.607^{a}	3.918^{a}	.454^{a}
75% FC		95.454^{b}	6.350^{b}	7.037^{b}	2.249^{b}	1.578^{b}	3.827^{b}	.449^{a}
50% FC		89.015^{c}	5.626^{c}	5.645^{c}	1.015^{c}	1.233^{c}	2.251^{c}	.433^{b}
SEm±		0.309	0.036	0.028	0.013	0.008	0.017	0.004
CD at 5%		0.908	0.106	0.082	0.038	0.024	0.051	0.010
Interaction (bioinoculants × moisture levels)								
	Control	89.393^{d}	5.497^{g}	5.697^{i}	2.250	1.457	3.707	.433
	PGPR	96.970^{b}	6.400^{e}	7.800^{c}	2.303	1.620	3.923	.447
100 % FC	AMF	100^{a}	7.097^{c}	7.503^{d}	2.330	1.653	3.983	.467
	PGPR + AMF	100^{a}	8.303^{a}	8.600^{a}	2.363	1.697	4.060	.470
	Control	86.363^{e}	5.100^{h}	5.197^{j}	2.170	1.423	3.593	.427
	PGPR	95.453^{bc}	6.100^{f}	7.543^{d}	2.267	1.607	3.873	.447
75% FC	AMF	100^{a}	6.700^{d}	7.180^{e}	2.270	1.623	3.893	.457
	PGPR + AMF	100^{a}	7.500^{b}	8.227^{b}	2.290	1.657	3.947	.467
	Control	77.273^{f}	4.303^{i}	3.900^{k}	.940	1.123	2.067	.400
	PGPR	89.393^{d}	5.200^{h}	6.163^{g}	1.000	1.243	2.247	.433
50% FC	AMF	93.940^{c}	6.100^{f}	5.980^{h}	1.030	1.267	2.300	.447
	PGPR + AMF	95.453^{bc}	6.900^{cd}	6.537^{f}	1.090	1.300	2.390	.453
SEm±		0.618	0.072	0.056	.026	.016	0.034	0.007
CD at 5%		1.816	0.213	0.165	NS	NS	NS	NS

References:

Ahmad, H. M., Fiaz, S., Hafeez, S., Zahra, S., Shah, A. N., Gul, B., Aziz, O., Fakhar, A., Rafique, M., Chen, Y., Yang, S. H. and Wang, X. 2022. Plant growth-promoting rhizobacteria eliminate the effect of drought stress in plants: A Review. *Front. Plant Sci*., *13*, 875774. doi:10.3389/fpls.2022.875774.

Auge, R.M., 2001. Water relations, drought and vesicular-arbuscularmycorrhizal symbiosis. *Mycorrhiza*., 11, 3–42.

Asrar, A.A. andElhindi, K.M., 2011. Alleviation of drought stress of marigold (*Tageteserecta*) plants by using arbuscularmycorrhizal fungi. *Saudi J. Biol. Sci*. 18, 93–98.

Azizi, S., TabariKouchaksaraei, M., Hadian, J., FallahNosrat Abad, A. R., ModarresSanavi, S. A. M., Ammer, C. and Bader, M. K. F. 2021. Dual inoculations of arbuscularmycorrhizal fungi and plant growth-promoting rhizobacteria boost drought resistance and essential oil yield of common myrtle. *Forest Ecology and Management, 497, 119478.* doi: 10.1016/j.foreco.2021.119478

Begum, N., Qin, C., Ahanger, M. A., Raza, S., Khan, M. I., Ashraf, M., Ahmed, N. and Zhang, L. 2019. Role of ArbuscularMycorrhizal Fungi in Plant Growth Regulation: Implications in Abiotic Stress Tolerance. *Front. Plant Sci*., 10, 466052. doi:10.3389/fpls.2019.01068.

Chakraborti, S., Bera, K., Sadhukhan, S. and Dutta, P. 2022. Bio-priming of seeds: Plant stress management and its underlying cellular, biochemical and molecular mechanisms. *Plant Stress*, *3*, 100052. doi: 10.1016/j.stress.2021.100052

Chaves, M. M., Flexas, J. andPinheiro, C. 2009. Photosynthesis under drought and salt stress: Regulation mechanisms from whole plant to cell. *Ann. Bot*., 103: 551–560. doi:10.1093/aob/mcn125

Hsiao, T. C. 1973. Plant responses to water stress. *Annu Rev Plant Physiol*24: 519–70. doi:10.1146/annurev.pp.24.060173.002511

Kaushal, M. andWani, S.P. 2016. Plant-growth-promoting rhizobacteria: drought stress alleviators to ameliorate crop production in drylands. *Ann Microbiol*., 66, 35–42. doi: 10.1007/s13213-015-1112-3.

Kloepper, J. W. 1996. Biological control agents vary in specificity for host, pathogen control, ecological habitat and environmental conditions. *Bio. Sci*.,46:406–9.

Lichtenthaler, H. K. and Wellburn, A. R. 1983. Determinations of total carotenoidsand chlorophylls a and b of leaf extracts in different solvents. *Biochem.Soc. Trans.*, 603: 591–592.

Lisar, S.Y.S., Motafakkerazad. R., Hossain, M. M. and Rahman, I.M.M. (2012). Water Stress in plants: Causes, effects and responses. In: Rahman IMM, Hasegawa H, (Eds.), *Water Stress*, Croatia: InTech, 1–14. doi:10.5772/39363.

Marius, S., Octavita, A., Eugen, U. and Vlad, A., 2005. Study of a microbial inoculation on several biochemical indices in sunflower (*Helianthus anuus*L.). *Genet. Mol. Biol*., 12, 11–17.

Mickan, B.S., Abbott, L.K., Stefanova, K. and Solaiman, Z.M., 2016. Interactions between biochar and mycorrhizal fungi in a water-stressed agricultural soil. *Mycorrhiza.*, 1–10.

Nezhadahmadi, A., Faruq, G. and Rashid, K. 2015. Influence of drought stress on leaf traits of different strawberry (*Fragariaananassa* l.) Varieties in natural environment.*Commun.Soil Sci.*, 46(10),1249–1262. doi:10.1080/00103624.2015.1033536.

Pandey, R. K., J. W. Maranville and M. M. Chetima. 2000. Deficit irrigation and nitrogen effects on maize in a Sahelian environment, II: Shoot growth. *Agric. Water Manag.*,46: 15–27. doi:10.1016/S0378-3774(00)00074-3.

Quintana, L. M., Moya, M., Moya, R. S., Ariza, A. C., Rabert, C., Pollmann, S. and Ramos, P. 2022. Improvement in the physiological and biochemical performance of strawberries under drought stress through symbiosis with Antarctic fungal endophytes. *Front.Microbiol.*, 13, 939955. doi: 10.3389/fmicb.2022.939955.

Yenni, Ibrahim, M. H., Nulit,R. and Sakimin, S. Z. 2021.Influence of drought stress on growth, biochemical changes and leaf gas exchange of strawberry (*Fragaria x ananassa* Dutch.) in Indonesia. *AIMS Agric. Food.,* 7(1), 37-60.