Biological Forum – An International Journal **15(10): 1017-1021(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Evaluating Plant Growth Promoting Abilities of *Rhizobium* under Moisture Deficit Stress in Horse Gram (*Macrotyloma uniflorum* Lam. [Verdc.])

Priyadharshini Aaradhana[1], Shobana Narayanasamy[2] and Sivakumar Uthandi[3]*
[1]Post-graduate Scholar, Biocatalysts Laboratory, Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore (Tamil Nadu), India.
[2]Research Associate, Biocatalysts Laboratory, Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore (Tamil Nadu), India.
[3]Professor and head, Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore (Tamil Nadu), India.

(Corresponding author: Sivakumar Uthandi)*
(Received: 19 August 2023; Revised: 07 September 2023; Accepted: 30 September 2023; Published: 15 October 2023)
(Published by Research Trend)

ABSTRACT: The horse gram crop is an unexplored legume cultivated in arid and semi-arid regions of India and is well known for its potential to withstand environmental adversities. But it is considered a poor man's crop and is grossly underutilized owing to lower yields. Rhizobial cultures isolated from horse gram with plant-growth-promoting potential possess inherent drought tolerance capacity. They can be exploited as effective bio inoculants for horse gram growth and yield enhancement under varied climatic conditions.
Five rhizobia isolates were obtained from the Biocatalysts Laboratory of the Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore. The isolates were tested for their inherent ability to grow under different levels of moisture deficit stress in PEG 6000. Their ability to promote growth under moisture-stress conditions was assessed through germination plate assay. The length of the seedlings, germination percentage, and proline content were estimated.
The isolates were able to withstand and exhibit growth under lower osmotic potential of upto -1.17MPa. The germination percentage of the seeds treated with cultures also significantly increased over the control. The growth parameters were also enhanced compared to the untreated seeds. This study thus explores the five isolates in question and their ability to enhance drought stress tolerance and plant health promotion in horse gram. This can be used effectively for furthering sustainable cultivation systems of horse gram.

Keywords: Horse gram, drought tolerance, *Rhizobium*, plant growth.

INTRODUCTION

Horse gram is a notable unexploited legume species largely cultivated as a rain-fed crop in the Indian sub-continent during the kharif season (Sahoo *et al*., 2020). It is mostly preferred by marginalized farming communities, due to their minimal input requirements (Ramani *et al*., 2020; Bhartiya *et al*., 2017). Horse gram is a natural drought-hardy minor legume that can tolerate varied climatic conditions in addition to flourishing in dual cropping systems (Bhartiya *et al*., 2015). It has been identified as one of the promising legumes that can become an important nutrient source by the US National Academy of Sciences (Sreerama *et al*., 2008). It is also called an orphan crop due to very limited scientific proceedings towards crop improvement and production of the crop. It does not give a yield as high as other legumes but promises a minimum yield that is scantily affected by climate. This puts the crop as a potentially farmer-friendly legume that can be cultivated as an easily accessible nutrient source. The use of bio-inoculants to promote the growth of horse gram can be adopted (Tonial *et al*., 2020). Native microbial endophytes of crops serve as better plant growth promoters and are more suitable than non-specific bio-inoculants (Long *et al*., 2008). Thus, rhizobia isolates that can promote plant growth in horse gram were isolated from horse gram root nodules (unpublished) and were chosen for this study. These rhizobia isolates exhibited multi-functional plant growth-promoting traits including indole acetic acid (IAA), exopolysaccharides (EPS), 1-Amino cyclopropane carboxylate deaminase (ACCd) production, and nutrient solubilization. These isolates may also possess drought stress tolerance, due to the growing habitat and conditions of the crop (Vandarkuzhali *et al*., 2015). It was hypothesized that the isolates could possess the capacity to alleviate stress in addition to plant growth-promoting abilities. Hence, the present study aimed to evaluate the effect of moisture stress on the selected rhizobia isolates and the plant growth-promoting potential under moisture-stress conditions in horse gram. This could be useful for developing a newer bio inoculum for improved plant growth and drought stress alleviation in horse gram.

MATERIALS AND METHODS

Isolates, media, and maintenance. Five plant growth-promoting potential rhizobial isolates (HR1, HR2, HR3, HR4, and HR5), isolated from horse gram root nodules were obtained from Biocatalysts Lab, Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore(unpublished). The isolates were maintained in Yeast Extract Mannitol Agar media in slants at 4°C. They were sub-cultured in Yeast Extract Mannitol Agar (YEMA) broth and used for seed biotization.

Estimation of growth curve under induced moisture stress. The isolates were assessed for their ability to tolerate and grow under laboratory-induced moisture stress conditions. YEM broth was amended with different concentrations of PEG 6000 pertaining to water potentials of -0.14, -0.45, -1.0, and -1.7 MPa respectively and their optical density at 600 nm was recorded using a spectrophotometer (Spectramax I3X) at 600 nm.

Germination plate assay. Horse gram seeds were surface sterilized by soaking in 0.1% mercuric chloride for 2 min followed by soaking for 30 S in 70% ethanol. The seeds were then soaked in 24h-old cell cultures (10^8CFU/mL) for 30 min before placing them in the petri plates for germination. The seeds were placed on sterile filter paper in petri plates, moistened with sterile water, containing various levels of moisture deficit stress corresponding to 10% PEG, 20% PEG, 25% PEG, and 30% PEG. The plant growth parameters like root length, shoot length, and seed vigour index were measured from the seeds germinated in the plates (Khalaki *et al*., 2019).

Growth parameters. The germination percentage, root length, shoot length, and seed vigour index of the respective treatments were measured. The seeds that showed the emergence of radicle/plumule were considered germinated (Shah *et al.*, 2021).

Germination percentage (%) = (Number of germinated seeds/ Number of seeds sown) × 100

The length of the plumule and radicle were measured using a scale corresponding to shoot length and root length respectively. The total of the root and shoot length gave the seedling length, which was used to calculate the seed vigour index (Hyder *et al.*, 2021)

Vigour Index = (Total length of the germinated seedling) × Germination percentage

Proline content. The proline content of the germinated seedlings was estimated using the method described (Abraham *et al.*, 2010). The germinated seedlings were weighed and proline was extracted using sulfosalicylic acid and ninhydrin. The proline content was estimated colorimetrically at 520 nm.

RESULTS AND DISCUSSION

The isolates were grown in YEM broth for 72 h and optical density was observed periodically. All the isolates were able to withstand and grow at -0.14 MPa and exhibited very similar growth to that of isolates grown under normal conditions (Fig. 1). At lower osmotic potential (-0.45 MPa), HR2 and HR5 were able to grow significantly than the other isolates up to 24 h and showed a slight decline in growth at 48 h. Similarly, HR5 exhibited a higher growth pattern at both -1.0 MPa and -1.7 MPa, but HR2 was unable to grow at -1.7 MPa. At -0.45 MPa, HR4 showed a significant growth although it was lesser than the growth recorded by HR2 and HR5. The other 2 isolates, HR1 and HR3 were not able to grow under -1.7 MPa. These growth patterns indicate a potential drought tolerance by the selected plant growth-promoting rhizobial isolates.

The germination percentage of the seeds at different water potentials was recorded after 3 days of placing the treated seeds in the Petri dishes (Table 1). Seeds treated with HR5 showed the highest germination percentage of53.3% at -0.14 MPa registering a 75% increase in germination over control. This is followed by isolate HR4 with germination of 36.6% recoding 64% increase over control at -0.14 MPa. At -0.45 MPa, HR5 and HR4 showed 27% and 20% germination, respectively whereas no germination was found in control seeds, exhibiting its significant role in plant growth under drought stress. The seeds treated with HR5 and HR4 recorded 13.3% and 7% germination, respectively at -0.69 MPa, showing their significant ability to promote plant growth even at increased levels of drought stress. Although HR2 exhibits better tolerance to induced moisture deficit stress, it is not able to promote plant growth at a moisture deficit stress of -0.45 MPa. This can be due to the inherent plant growth-promoting traits that are not as significant as those observed in HR5 and HR4, despite their ability to tolerate drought. Germination is a critical stage of plant growth that can decide the establishment of the crops in later stages. The ability to withstand drought at such stages and promote germination is a valuable trait that can promote plant establishment and subsequently promote their health and fitness (Muscolo *et al.,* 2015). The effects of moisture stress tolerance were also studied in lentils, showing seed germination when exposed to 21% PEG. The isolates used in this study performed better by showing germination even under 25% PEG, pertaining to -0.64 MPa.

The highest root and shoot length was recorded for the seeds treated with HR5. At - 0.14MPa, seeds treated with HR5 showed a 69% and 32% increase in root and shoot length respectively over control. In the case of the isolate HR4, 43.4% and 20% increases in root and shoot lengths, respectively were observed at -0.14 MPa. The isolate HR5 showed germination at -0.45 MPa, exhibiting a significant root and shoot growth of 1.1 and 6.3 cm, respectively (Fig. 2a,b). Additionally, isolate HR4 was also able to show moderate germination and registering a root length and shoot length of 0.8 and 4.0 cm, respectively at -0.45 MPa. The increase in shoot and root length and germination percentage was reflected in a consequent increase in the seed vigour index. At -0.14 MPa, the seed vigour index of HR5-treated seeds was 549 which was 84.2% higher than the control (86.7). At a water potential of -0.45 MPa, HR5 exhibited a seed vigour index of 197.3 as opposed to no seed germination in the control (Fig. 2c). The record of an increase in the growth parameters and seed vigour can be attributed to the isolates that were able to promote plant growth and alleviate moisture deficit stress. Similarly, an increase in the improvement of plant growth parameters was also recorded when maize seeds were subjected to moisture deficit stress in plate assay (Chukwuneme *et al.*, 2020). This study also demonstrated the effectiveness of seed treatment using microbes in promoting plant growth under stress conditions, which can be attributed to nitrogen-fixing ability and plant growth hormone production.

Accumulation of proline in plant parts is an indication of the ability of the plants to withstand drought stress. The accumulation of proline in germinated seedlings treated with the five isolates was assessed. At -0.14 MPa there was a 41% increase in proline content in seeds treated with HR5 over control. When the stress was increased to -0.45 MPa, the proline content also increased to 62.1 μg g^{-1} of plant fresh weight in seeds treated with HR5 (Fig. 3). The seeds treated with HR5 recorded the highest accumulation of proline accumulation ranging upto 75.3μg g^{-1} of plant fresh weight at -1.0 MPa, whereas no germination was found in control seeds. In the case of isolate HR4, The proline content increased from 49.3 μg g^{-1} of plant fresh weight at -0.45 MPa to 55 μg g^{-1} of plant fresh weight at -0.64 MPa. A very similar pattern of increase in the proline content of plant parts was observed with an increase in moisture deficit stress in the case of certain cultivars of chickpea (Mafakheri *et al.*, 2010). Several mechanisms are adopted by the microbes to alleviate drought stress in plants *viz.*, production of exopolysaccharides, ACC deaminase, and accumulation of osmolytes, antioxidants, and root structure modifications (Vurukonda *et al.*, 2016). These results support the fact that proline accumulation helps in drought stress and is one of the main mechanisms that is adopted by the isolates used in this study for drought stress alleviation.

Table 1: Germination percentage of horse gram seeds treated with different rhizobial isolates at different concentrations of PEG 6000.

Sr. No.	Treatments	Germination %				
		0 Mpa	-0.14 Mpa	-0.45 Mpa	-0.69 Mpa	-1 Mpa
1.	T1	100 ± 0	16.6 ± 0.33^{b}	0.0	0.0^{c}	0.0
2.	T2	100 ± 0	23.3 ± 0.58^{b}	10± 0.33	0.0^{bc}	0.0
3.	T3	100 ± 0	16.6 ± 0.33^{b}	6.6 ± 0.33	0.0^{bc}	0.0
4.	T4	100 ± 0	36.6 ± 0.88^{b}	20 ± 0.33	6.6 ± 0.33^{ab}	0.0
5.	T5	100 ± 0	53.3 ± 0.33^{a}	26.6 ± 0.33	13.3 ± 0.33^{a}	0.0
6.	T6	100 ± 0	13.3 ± 0.33^{b}	0.0	0.0^{c}	0.0

10% PEG, 20% PEG, 25% PEG, and 30% PEG pertaining to water potentials of -0.14, -0.45, -1.0, and -1.7 Mpa respectively; T1 – HR1; T2 – HR2; T3 – HR3; T4 – HR4; T5 – HR5; T6 – absolute control without treatment. (HR1, HR2, HR3, HR4, HR5 – Nodule-associated rhizobial isolates). The values are the mean standard deviation of the respective treatment replicates (n=3). The different letters indicate the treatments in order of their significant difference. The significant difference was arrived at by DMRT (P≤0.05).

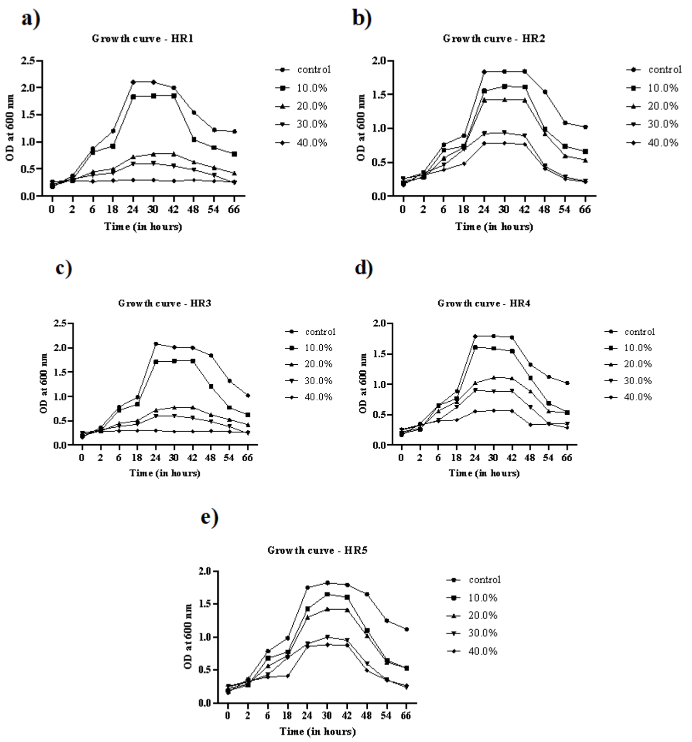

Fig. 1. Growth of the individual isolates under varied conditions of moisture stress induced by PEG 6000. 10% PEG, 20% PEG, 25% PEG, and 30% PEGpertaining to water potentials of -0.14, -0.45, -1.0, and -1.7 Mpa respectively.

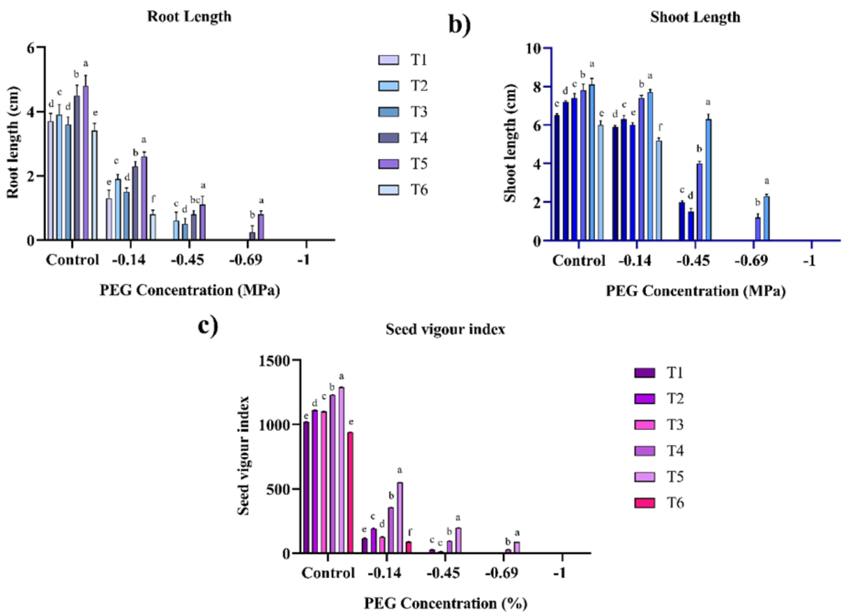

Fig. 2 (a) Root length, b) Shoot length, and c) Seed Vigour index of horse gram after treatment with individual rhizobial isolates under moisture stress. The values are the mean standard deviation of the respective treatment replicates (n=3). The different letters indicate the treatments in order of their significant difference. The significant difference was arrived at by DMRT (P≤0.05).

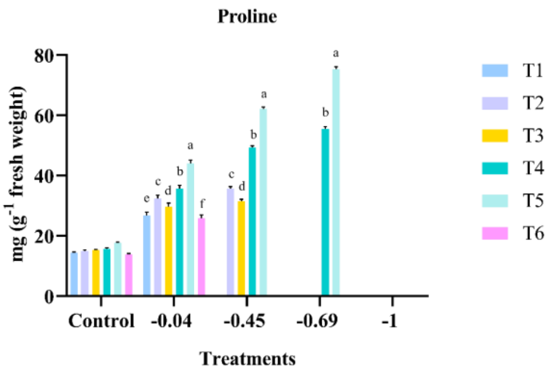

Fig. 3. Proline content in the leaves of horse gram after treatment with individual rhizobial isolates. The values are the mean standard deviation of the respective treatment replicates (n=3). The different letters indicate the treatments in order of their significant difference. The significant difference was arrived at by DMRT (P≤0.05).

CONCLUSIONS

The five isolates were tested for their ability to tolerate and grow at lower osmotic potential, and out of the five, HR5 had the highest tolerance to induced drought stress under *in vitro* conditions. Furthermore, the isolate also exhibited an increase in plant growth parameters in horse gram seeds under varied moisture deficit stress. The isolate was observed to promote drought stress alleviation by facilitating an increase in the accumulation of proline. Thus the isolate HR5 (*Rhizobium* sp.) can be selected to alleviate drought stress and promote plant growth in horse gram. It has the potential to be a drought-alleviating PGP microbe for horse gram cultivation for exploitation in the future.

FUTURE SCOPE

The isolates are potential drought-resilient PGPsthat can also provide insight into the molecular mechanisms involved with drought resilience and plant interaction. The isolates can be further evaluated under field conditions for effective drought stress tolerance and plant growth promotion.

Author contributions. The work plan was devised by SU. All experiments and statistical analyses were carried out by PA. The initial draft of the manuscript was prepared by PA. SN and SU guided all the experiments. Corrections and review of the manuscript was also carried out by SN and SU.

Acknowledgments. The Department of Science and Technology, Government of India, granted funding through GOI-SERB (CRG/2020/004932), awarded to SU, for facilitating this research.

Conflict of Interest. None.

REFERENCES

Abraham, E., Hourton-Cabassa, C., Erdei, L., & Szabados, L. (2010). Methods for determination of proline in plants. *Plant stress tolerance: methods and protocols*, 317-331.

Abbasi Khalaki, M., Ghorbani, A., & Dadjou, F. (2019). Influence of nano-priming on *Festuca ovina* seed germination and early seedling traits under drought stress, in laboratory condition. *Ecopersia*, *7*(3), 133-139.

Bhartiya, A., Aditya, J. P., Pal, R. S., & Bajeli, J. (2017). Agromorphological, nutritional and antioxidant properties in horse gram [*Macrotyloma uniflorum* (Lam.) Verdc.]. *Germplasm Collection from Diverse Altitudinal Range of North Western Himalayan Hills of India. Vegetos 30*, 1.

Bhartiya, A., Aditya, J. P., & Kant, L. (2015). Nutritional and remedial potential of an underutilized food legume horsegram (Macrotyloma uniflorum): a review. *JAPS: Journal of Animal & Plant Sciences*, *25*(4).

Chukwuneme, C. F., Babalola, O. O., Kutu, F. R., & Ojuederie, O. B. (2020). Characterization of actinomycetes isolates for plant growth promoting traits and their effects on drought tolerance in maize. *Journal of Plant Interactions*, *15*(1), 93-105.

Hyder, S., Gondal, A. S., Rizvi, Z. F., Atiq, R., Haider, M. I. S., Fatima, N., & Inam-ul-Haq, M. (2021). Biological control of chili damping-off disease, caused by *Pythium myriotylum*. *Frontiers in Microbiology*, *12*, 587431.

Long, H. H., Schmidt, D. D., & Baldwin, I. T. (2008). Native bacterial endophytes promote host growth in a species-specific manner; phytohormone manipulations do not result in common growth responses. *PloS one*, *3*(7), e2702.

Muscolo, A., Junker, A., Klukas, C., Weigelt-Fischer, K., Riewe, D., & Altmann, T. (2015). Phenotypic and metabolic responses to drought and salinity of 2four contrasting lentil accessions. *Journal of experimental botany*, *66*(18), 5467-5480.

Mafakheri, A., Siosemardeh, A. F., Bahramnejad, B., Struik, P. C., & Sohrabi, Y. (2010). Effect of drought stress on yield, proline and chlorophyll contents in three chickpea cultivars. *Australian journal of crop science*, *4*(8), 580-585.

Ramani, K., Latha, K. R., Tamilselvan, N., & Sivakumar, R. (2020). Influence of sowing time and varieties on growth and yield of horsegram (*Macrotyloma uniflorum* Lam.) under rainfed condition. *Int J Curr Microbiol App Sci*., *9*(11), 2214-2221.

Shah, S., Ullah, S., Ali, S., Khan, A., Ali, M., & Hassan, S. (2021). Using mathematical models to evaluate germination rate and seedlings length of chickpea seed (*Cicer arietinum* L.) to osmotic stress at cardinal temperatures. *PLoS One*, *16*(12), e0260990.

Sahoo, S. C., & Mohanty, M. (2020). Horsegram (*Macrotyloma uniflorum*) production technology: A review. *IJCS*, *8*(6), 2723-2726.

Sreerama, Y. N., Sasikala, V. B., & Pratape, V. M. (2008). Nutritional implications and flour functionality of popped/expanded horse gram. *Food Chemistry*, *108*(3), 891-899.

Tonial, F., Nava, F., Gayger, A., & Mar, T. (2020). Endophyte potential use in crop production. *Sustainable crop production*.

Vandarkuzhali, P., & Narayanasamy, S. (2015). Horse Gram-A prospective functional food. *The Indian Journal of Nutrition and Dietetics*, 226-243.

Vurukonda, S. S. K. P., Vardharajula, S., Shrivastava, M., & SkZ, A. (2016). Enhancement of drought stress tolerance in crops by plant growth promoting rhizobacteria. *Microbiological research*, *184*, 13-24.

How to cite this article: Priyadharshini Aaradhana, Shobana Narayanasamy and Sivakumar Uthandi (2023). Evaluating Plant Growth Promoting Abilities of *Rhizobium* under Moisture Deficit Stress in Horse Gram (*Macrotyloma uniflorum* Lam. [Verdc.]). *Biological Forum – An International Journal, 15*(10): 1017-1021.