Assessment of Stress Tolerance Properties of Chickpea Actinomycetes

Kavita Rani*, Dhinu Yadav, Atul Parashar and Leela Wati
Department of Microbiology CCS Haryana Agricultural University, Hisar-125004 (Haryana), India
*Corresponding Author E-mail: kavita260892@gmail.com
Received: 12.02.2020 | Revised: 23.03.2020 | Accepted: 29.03.2020

ABSTRACT
Chickpea (Cicer arietinum L.) is the second most widely grown food legume crop after common bean. However, the productivity of chickpea has decreased to a significant extent since last two decades due to stressful conditions arising from unpredictable climatic conditions as well as saline nature of soil. Since last few years, studies on beneficial traits of actinomycetes regarding plant growth promotion and stress tolerance activities had opened new avenues for their applications in sustainable agriculture. In the present investigation, total 40 (AK1-AK40) actinomycete isolates were retrieved from different soil samples and chickpea nodules collected from CCS Haryana Agricultural University, Hisar farms and assessed for their stress tolerance abilities by determining salt tolerance potential and ACC utilization. Actinomycete isolates, AK3, AK6 (showing good growth upto 6% NaCl concentrations), AK11 and AK34 (showing good growth upto 5% NaCl concentrations) were observed to show good growth on ACC supplemented plates depicting their ability to utilize ACC which results in diminishing the ethylene levels and may play an important role in mitigating the stress conditions and improved productivity of chickpea.

Keywords: Actinomycetes, Climatic Conditions, Productivity, Stress Tolerance

INTRODUCTION
Legumes belonging to Leguminosae family comprise 800 genera and 20,000 species denoting 3rd largest family among flowering plants (Stagnari et al., 2017). Legumes, being a major source of protein in human and animal nutrition, are mainly grown for their edible seeds and crop rotation. In crop rotation, legumes play an important role in improving soil fertility and reducing the occurrence of weeds, diseases and pests. Among legumes, chickpea (Cicer arietinum L.) is a food legume grown globally on a very wide area (Sreevidya et al., 2016). It is usually consumed as seed food, however, it is also eaten as green vegetable and cooked young chickpea leaves in certain parts of the world, mainly in malnourished populations.

Because of various stress conditions arising due to salinity and harsh climatic conditions, the production of chickpea has declined to a very large extent.

Cite this article: Rani, K., Yadav, D., Parashar, A., & Wati, L. (2020). Assessment of Stress Tolerance Properties of Chickpea Actinomycetes, Ind. J. Pure App. Biosci. 8(4), 639-646. doi: http://dx.doi.org/10.18782/2582-2845.7954
Ethylene, for all the simplicity of its structure (C₂H₄), regulates many aspects of plant growth and development under normal conditions (Schaller, 2012). However, under stressful conditions, plants produce higher levels of ethylene which has detrimental effects on normal plant growth and developmental processes. Furthermore, soil salinity is a major constraint to modern agriculture. For most trees and crop plants that are salt sensitive, elevated Na⁺ imposes toxic effects by perturbing potassium (K⁺)-dependent processes, inducing deleterious protein conformations and causing osmotic stress that causes growth inhibition and ultimately, cell death (Chinnusamy et al., 2006). Also, salinity creates stress conditions which lead to increased concentration of ethylene resulting in premature senescence of different plant parts, thereby affecting productivity. The high salt concentrations also influence the efficiency of plant growth promoting rhizobacteria.

For salt-stressed plants, restricting Na⁺ uptake and shoot Na⁺ accumulation is critical for minimizing salt phytotoxicity. Therefore, it is important to investigate and reveal such techniques which can impart the potential to tolerate high salt concentrations. Although many technologies have been implicated in the improvement of salt tolerance in plants, only PGPR-elicited plant tolerance against salt stress has been previously studied. Numerous soil dwelling microorganisms have a beneficial property of salinity stress tolerance and can help the plants to promote growth by various means under salt stress conditions. Amid several PGPR, actinomycetes have also been reported to alleviate salt stress, for instance, Streptomyces sp. strain PGPA39 isolated from agricultural soil has been stated to alleviate salt stress in Tomato plants (‘Micro Tom’ tomato) under gnotobiotic condition (Palaniyandi et al., 2014). Moreover, some actinomycetes also have the ability to lower ethylene levels by using 1-aminocyclopropane-1-carboxylic acid (ACC) which acts as a precursor molecule for the biosynthesis of ethylene in the plants by the secretion of ACC deaminase enzyme responsible for hydrolysis of ACC into ammonia and alpha-ketoglutarate (El-Tarabily, 2008) and prevent the creation of stress conditions. Keeping in view the enormous applications of actinomycetes in alleviation of stress conditions, the present work was directed to isolate, characterize and assess stress tolerance efficacy of actinomycetes associated with nodules and rhizosphere of chickpea.

**MATERIALS AND METHODS**

**Location of Investigation and Collection of Samples**
The present investigation was carried out in the “Plant-Microbial Interactions Laboratory”, Department of Microbiology, College of Basic Sciences and Humanities, CCS Haryana Agricultural University, Hisar, during the sessions 2017-2019. For isolation of actinomycetes, chickpea nodules were collected from chickpea crop raised at CCS Haryana Agricultural University Farm, Hisar. Soil samples were collected from different locations of CCS Haryana Agricultural University Farm and nearby farmer’s field, Hisar, Haryana, India.

**Isolation and Characterization of Actinomycetes**
Actinomycete isolates were retrieved from nodules of chickpea plants according to Vincent (1970) and from soil samples by using serial dilution and spread plate technique. To isolate actinomycetes from nodules, the nodules were first surface sterilized with 70% ethanol, followed by immersing in 0.1% mercuric chloride (HgCl₂) solution for 1 minute. After HgCl₂ treatment, the nodules were washed with sterilized water 3 to 5 times to get rid of the sterilizing agent. The surface sterilized nodules were crushed in sterilized plates and suspension was streaked on Kenknight and Munaire’s medium (KMM) plates (MacCartney, 1989). To isolate actinomycetes from soil samples, an aliquot of 0.1ml of 10⁻⁴ -10⁻⁶ dilutions was spread over KMM plates and the plates were incubated at 28±2°C for 7 to 10 days. Isolated colonies were further purified on KMM plates and purified colonies of actinomycete isolates were
transferred on KMM slants and stored in refrigerator at 4±1ºC for further studies. All the actinomycete isolates were subjected to identification on the basis of morphological characteristics.

**Characterization of Actinomycete Isolates for Stress Tolerance**

**Salt tolerance**

The ability of all the actinomycete isolates to grow at different concentrations of sodium chloride (1-8% w/v), was monitored on KMM medium agar plates each containing 20mM HEPES (N-2-hydroxyethane-sulphonic acid) buffer (Marsudi et al., 1999). The plates were spotted with 10µl of freshly grown cultures of different actinomycete isolates and incubated at 28±2ºC for 7 days. The growth of actinomycete isolates was recorded as positive or negative and plates with 0.01% NaCl concentration in basal medium were used as control.

**Utilization of 1-amino cyclopropane-1-carboxylate**

The ability of all the actinomycete isolates to utilize ACC was determined as per the method described by (Penrose & Glick, 2003). The minimal medium plates (Dworkin & Foster, 1958) supplemented with 2mM ACC were prepared and spotted with 3µl of log phase grown cultures of actinomycete isolates. The plates were incubated at 28±2ºC in a BOD incubator. Growth of actinomycete isolates on ACC supplemented medium plates was recorded after 7-10 days of incubation. The actinomycete isolates showing good growth on ACC supplemented medium plates as an indication of high efficiency of ACC utilization as nitrogen source were selected for further studies. Minimal medium plates containing ammonium sulfate were used as control for comparison of growth of different actinomycete isolates.

**RESULTS AND DISCUSSION**

**Isolation and Characterization of Actinomycetes**

Actinomycetes are copiously dispersed in soil with an average of $10^4$-$10^6$ propagules or spores (CFU) g$^{-1}$ soil in cultivated lands (Mareckova & Kopecky, 2012) and flourish in the rhizosphere as well as colonize the plant roots and nodules in agricultural lands. Actinomycetes have been isolated by various researchers for numerous purposes. Singh and Gaur (2016) isolated 68 actinomycetes from various medicinal plants and evaluated for plant growth promotion in chickpea after screening for various plant growth promoting attributes. In current investigation, total forty isolates (AK1-AK40) were retrieved from different nodules and soil samples including two isolates (AK1 and AK2) from nodules of chickpea and thirty eight isolates (AK3-AK40) from different soil samples (Table 1) depending upon different morphological characteristics (Table 2 and Fig. 1) to examine their ability to impart stress tolerance. All the isolates were found to be Gram- positive in nature with filamentous and coccoid colony morphology. Their colour varied from pale green to brown, cream, grey and violet.
coastal region of Gujarat, India which was found to grow up to 11% salt concentration.

**Utilization of 1-amino cyclopropane-1-carboxylate**

Ethylene is an imperative plant hormone which in minor concentrations enhances plant growth, however, at toxic concentrations acts as an inhibitor of nodulation in legumes (Grobbelaar et al., 1971). Several bacteria in the rhizosphere have the ability to utilize ACC, thereby reducing the levels of ethylene in the plants. In present study, all the actinomycete isolates were assessed for their ability to utilize ACC on minimal medium plates supplemented with 2mM ACC and total 82.5% actinomycete isolates were observed to show growth on ACC supplemented plates depicting their ability of ACC utilization. Out of forty isolates, nineteen isolates (47.5%) were showing good growth, five isolates (12.5%) were showing moderate growth, nine isolates (22.5) were showing less growth and seven isolates (17.5%) were not showing growth on ACC supplemented plates indicating high, moderate, less and no utilization of ACC, respectively (Table 4). The utilization of ACC by actinomycetes has also been supported in other studies illustrating enhancement of plant growth by reducing the levels of ethylene (Penrose et al., 2001; Mayak et al., 2004). El-Tarabily (2008) isolated 64 actinomycetes from soil samples collected from rhizosphere of tomato and reported that 26% of these isolates were showing growth on ACC supplemented plates signifying as potent ACC-deaminase producers, an enzyme responsible for utilization of ACC.

### Table 1: Actinomycete isolates retrieved from roots, nodules and soil samples

| Sample          | Actinomycete isolates                      |
|-----------------|-------------------------------------------|
| Nodules of chickpea | AK1, AK2 (2)                             |
| Roots of chickpea | -                                         |
| S1              | AK3, AK4, AK5, AK6, AK7, AK8, AK9, AK10, AK11, AK12, AK13 (11) |
| S2              | AK14, AK15, AK16, AK17 (4)                |
| S3              | AK18, AK19, AK20, AK21 (4)                |
| S4              | AK22, AK23, AK24, AK25, AK26, AK27, AK28 (7) |
| S5              | AK29, AK30, AK31, AK32, AK33 (5)         |
| S6              | AK34, AK35, AK36, AK37 (4)               |
| S7              | AK38, AK39, AK40 (3)                     |
| Total           | 40                                        |

S1-S7 = Soil samples

### Table 2: Morphological characteristics of actinomycete isolates

| Isolate | Colony colour | Colony morphology | Isolate | Colony colour | Colony  |
|---------|---------------|-------------------|---------|---------------|---------|
| AK1     | Pale grey     | Filamentous       | AK21    | Pale white    | Filamentous |
| AK2     | Pale grey     | Filamentous       | AK22    | Pale white    | Coccoid |
| AK3     | White         | Filamentous       | AK23    | Brown         | Filamentous |
| AK4     | Brown         | Filamentous       | AK24    | Cream         | Filamentous |
| AK5     | Brown         | Filamentous       | AK25    | Brown         | Filamentous |
| AK6     | Pale white    | Filamentous       | AK26    | Whitish yellow| Filamentous |
| AK7     | Pale white    | Filamentous       | AK27    | Grey          | Filamentous |
| AK8     | Pale grey     | Filamentous       | AK28    | Brownish-grey | Filamentous |
| AK9     | White         | Filamentous       | AK29    | Brown         | Filamentous |
| AK10    | Grey          | Filamentous       | AK30    | Cream         | Filamentous |
| AK11    | Creamy-white  | Filamentous       | AK31    | Whitish-cream | Filamentous |
| AK12    | Brown         | Coccoid           | AK32    | Pale white    | Filamentous |
Table 3: Growth of actinomycete isolates at different salt concentrations

| Isolate | Sodium chloride concentration (%) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---------|-----------------------------------|---|---|---|---|---|---|---|---|
| AK1     | +                                 | + | + | - | - | - | - | - | - |
| AK2     | +                                 | - | - | - | - | - | - | - | - |
| AK3     | +                                 | - | - | - | - | - | - | - | - |
| AK4     | -                                 | - | - | - | - | - | - | - | - |
| AK5     | +                                 | + | + | + | - | - | - | - | - |
| AK6     | +                                 | + | + | + | + | - | - | - | - |
| AK7     | +                                 | + | + | + | - | - | - | - | - |
| AK8     | +                                 | + | + | + | + | - | - | - | - |
| AK9     | +                                 | + | + | - | - | - | - | - | - |
| AK10    | +                                 | + | + | + | + | - | - | - | - |
| AK11    | +                                 | + | + | + | + | - | - | - | - |
| AK12    | +                                 | + | + | + | + | - | - | - | - |
| AK13    | +                                 | + | + | + | + | - | - | - | - |
| AK14    | +                                 | + | + | + | + | - | - | - | - |
| AK15    | +                                 | + | + | + | + | - | - | - | - |
| AK16    | +                                 | + | + | + | + | - | - | - | - |
| AK17    | +                                 | + | + | + | + | - | - | - | - |
| AK18    | +                                 | + | + | + | + | - | - | - | - |
| AK19    | +                                 | + | + | + | + | - | - | - | - |
| AK20    | +                                 | + | + | + | + | - | - | - | - |

Copyright © July-August, 2020; IJPAB
Table 4: Growth of different actinomycete isolates on ACC supplemented plates

| Actinomycete isolate | ACC utilization | Actinomycete isolate | ACC utilization |
|---------------------|-----------------|---------------------|-----------------|
| AK1                 | +               | AK 21               | +++             |
| AK 2                | +               | AK 22               | +++             |
| AK 3                | +++             | AK 23               | +++             |
| AK 4                | +++             | AK 24               | +++             |
| AK 5                | -               | AK 25               | +++             |
| AK 6                | +++             | AK 26               | -               |
| AK 7                | ++              | AK 27               | +++             |
| AK 8                | +               | AK 28               | +++             |
| AK 9                | ++              | AK 29               | +               |
| AK 10               | -               | AK 30               | +++             |
| AK 11               | +++             | AK 31               | +               |
| AK 12               | -               | AK 32               | ++              |
| AK 13               | +               | AK 33               | ++              |
| AK 14               | ++              | AK 34               | +++             |
| AK 15               | -               | AK 35               | +++             |
| AK 16               | +++             | AK 36               | +               |
| AK 17               | +++             | AK 37               | -               |
| AK 18               | +++             | AK 38               | +++             |
| AK 19               | +               | AK 39               | +++             |
| AK 20               | -               | AK 40               | +               |

+++ (Good growth), ++ (Moderate growth), + (Low growth), - (No growth)

Fig. 1: Actinomycete isolates with different colony morphology
CONCLUSION

Global yields of chickpea have been relatively stagnant for the last two decades regardless of the use of various conventional and molecular breeding approaches due to inconsistent climatic changes and stress conditions. Also the problem of food security arises along with the increase in world population. Therefore, there is a big challenge faced by the world to feed the increasing population with little available food. Microorganisms in soil are critical for the maintenance of soil function in both natural and managed agricultural soils because of their contribution in key processes such as soil structure formation, decomposition of organic matter, detoxification and nutrient recycling resulting in stress tolerance and improved plant growth. Actinomycetes are especially significant because they can survive in soils of various types such as in saline soil also under stressed conditions. In present investigation, actinomycete isolates showing good growth on high salt concentrations (AK3, AK6, AK11 and AK34) were also observed to show good growth on ACC supplemented plates and thereby, it is concluded that the triumphant formulation and exploitation of these actinomycete isolates may be suggested for their application to diminish stress conditions in chickpea after screening for numerous plant growth promoting traits additionally.

Acknowledgement

I am sincerely thankful to CCS Haryana Agricultural University Hisar, for providing merit scholarship and necessary facilities to complete the work.

REFERENCES

Chinnusamy, V., Zhu, J., & Zhu, J.K. (2006). Salt stress signaling and mechanisms of plant salt tolerance. In:Setlow, J.K. (Eds.) Genetic Engineering: Principles and Methods, Springer, Boston, MA, pp.141-177.

Dworkin, M., & Foster, J. (1958). Experiments with some microorganisms which utilize ethane and hydrogen. Journal of Bacteriology, 75, 592-601.

El-Tarabily, K.A. (2008). Promotion of tomato (Lycopersicon esculentum Mill.) plant growth by rhizosphere competent L-aminocyclopropane-1-carboxylic acid deaminase-producing streptomyete
actinomycetes. *Plant and Soil, 308*(1-2), 161-174.

Grobbeelaar, N., Clarke, B., & Hough, M.C. (1971). The nodulation and nitrogen fixation of isolated roots of *Phaseolus vulgaris* L. *Plant and Soil, 35*(1), 203-214.

MacCartney, A.J. (1989). *FEMS Microbiological Reviews, 46*, 145-163.

Mareckova, M.S., & Kopecky, J. (2012). Actinobacteria: relationship to soil environment. In: Lal, R. (Ed.) *Encyclopaedia of Soil Sciences, (2nd edn.),* Taylor and Francis press, London, pp1-4.

Marsudi, N.D.S., Gelinn, A.R., & Dilworth, M.J. (1999). Identification and characterization of fast and slow growing root nodule bacteria from South-Western Australian soils able to nodulate *Acacia saligna.* *Soil Biology and Biochemistry, 31*, 1229-1238.

Mayak, S., Tiros, T., & Glick, B.R. (2004). Plant growth-promoting bacteria confer resistance in tomato plants to salt stress. *Plant physiology and Biochemistry, 42*(6), 565–572.

Palaniyandi, S.A., Damodharan, K., Yang, S.H., & Suh, J.W. (2014). *Streptomyces* sp. strain PGPA39 alleviates salt stress and promotes growth of “Micro Tom” tomato plants. *Journal of Applied Microbiology, 117*(3), 766-773.

Penrose, D.M., & Glick, B.R. (2003). Methods for isolating and characterizing ACC deaminase containing plant growth-promoting rhizobacteria. *Plant Physiology, 118*, 10-23.

Penrose, D.M., Moffatt, B.A., & Glick, B.R. (2001). Determination of 1-aminocyclopropane-1-carboxylic acid (ACC) to assess the effects of ACC deaminase-containing bacteria on roots of canola seedlings. *Canadian Journal of Microbiology, 47*, 77-80.

Schaller, G.E. (2012). Ethylene and the regulation of plant development. *BMC Biology, 10*(9), 1-3.

Singh, S.P., & Gaur, R. (2016). Evaluation of antagonistic and plant growth promoting activities of chitinolytic endophytic actinomycetes associated with medicinal plants against *Sclerotium rolfsii* in chickpea. *Journal of Applied Microbiology, 121*(2), 506-518.

Sreevidya, M., Gopalakrishnan, S., Kudapa, H., & Varshney, R.K. (2016). Exploring plant growth-promotion actinomycetes from vermicompost and rhizosphere soil for yield enhancement in chickpea. *Brazilian Journal of Microbiology, 47*(1), 85-95.

Stagnari, F., Maggio, A., Galieni, A., & Pisante, M. (2017). Multiple benefits of legumes for agriculture sustainability: An overview. *Chemical and Biological Technologies in Agriculture, 4*(2), 1-13.

Thumar, J.T., & Singh, S.P. (2009). Organic solvent tolerance of an alkaline protease from salt-tolerant alkaliphilic *Streptomyces clavuligerus* strain Mit-1. *Journal of Industrial Microbiology and Biotechnology, 36*(2), 211.

Vasavada, S.H., Thumar, J.T., & Singh, S.P. (2006). Secretion of a potent antibiotic by salt-tolerant and alkaliphilic actinomycete *Streptomyces sannanensis* strain RJT-1. *Current Science, 91*(10), 1393-1397.

Vincent, J.M. (1970). A manual for the practical study of root nodule bacteria. Blackwell, Oxford.