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Research Article

Assessment of Stress Tolerance Properties of Chickpea Actinomycetes

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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.

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Ethylene, for all the simplicity of its structure (C_2H_4), 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 ($HgCl_2$) solution for 1 minute. After $HgCl_2$ 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.

Characterization of Actinomycete Isolates for Stress Tolerance

Salt tolerance

Actinomycetes have been noticed to tolerate high salt concentrations; therefore, it is essential to examine native actinomycetes for their potential to tolerate different concentrations of salt which could be beneficial for plants growth under salt stressed conditions. In present study, all the actinomycete isolates were tested for their ability to tolerate different salt concentrations and it was found that out of 40 isolates, only 7.5% and 5.0% isolates were able to grow up to 5% and 6% of sodium chloride concentration, respectively (Table 3, Fig. 2). Actinomycetes are mainly alkaliphilic and different researchers have reported various degree of salt tolerance of actinomycete isolates. Vasavada et al. (2006) studied salt tolerance in *Streptomyces sannanensis* retrieved from the soil of Saurashtra University Campus, Rajkot which could show optimum growth up to 5% salt concentration, showing potential of *Streptomyces* sp. to tolerate high salt concentration. Likewise, Thumar and Singh (2009) isolated a salt-tolerant alkaliphilic actinomycete, *Streptomyces clavuligerus* from Mithapur, 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

AK13	Cream	Filamentous	AK33	Brown	Filamentous
AK14	Reddish grey	Filamentous	AK34	Cream	Coccoid
AK15	Grey	Filamentous	AK35	Grey	Filamentous
AK16	Pale white	Filamentous	AK36	Blackish-grey	Coccoid
AK17	Grey	Filamentous	AK37	Greenish-grey	Coccoid
AK18	Pinkish-violet	Filamentous	AK38	Grey	Filamentous
AK19	Cream	Filamentous	AK39	Grey	Coccoid
AK20	White	Coccoid	AK40	Dark brown	Coccoid

Table 3: Growth of actinomycete isolates at different salt concentrations

Isolate	Sodium chloride concentration (%)								Isolate	Sodium chloride concentration (%)							
	1	2	3	4	5	6	7	8		1	2	3	4	5	6	7	8
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	+	+	+	+	-	-	-	-

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)			

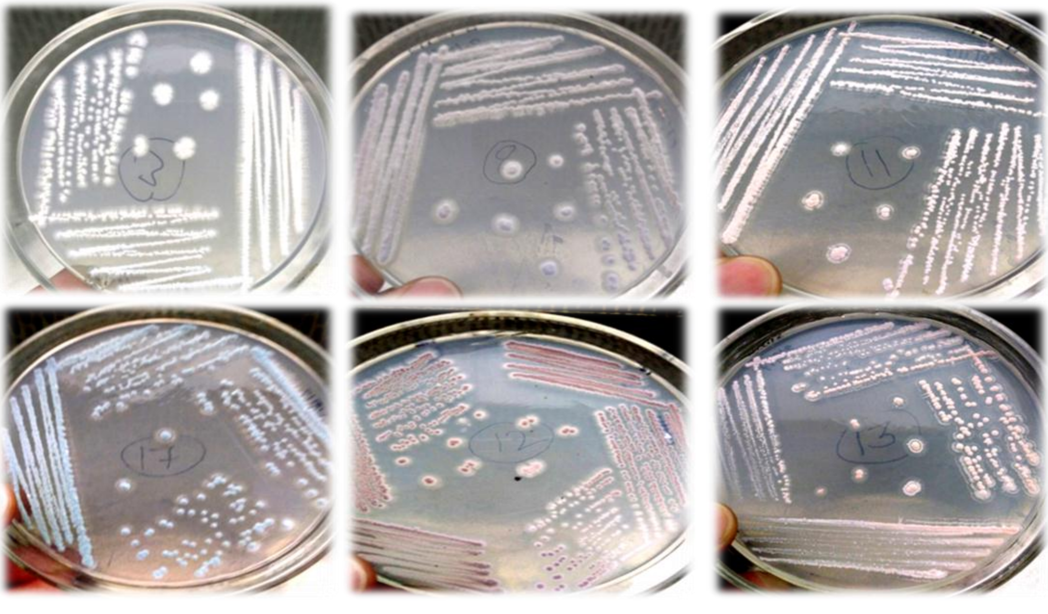

Fig. 1: Actinomycete isolates with different colony morphology

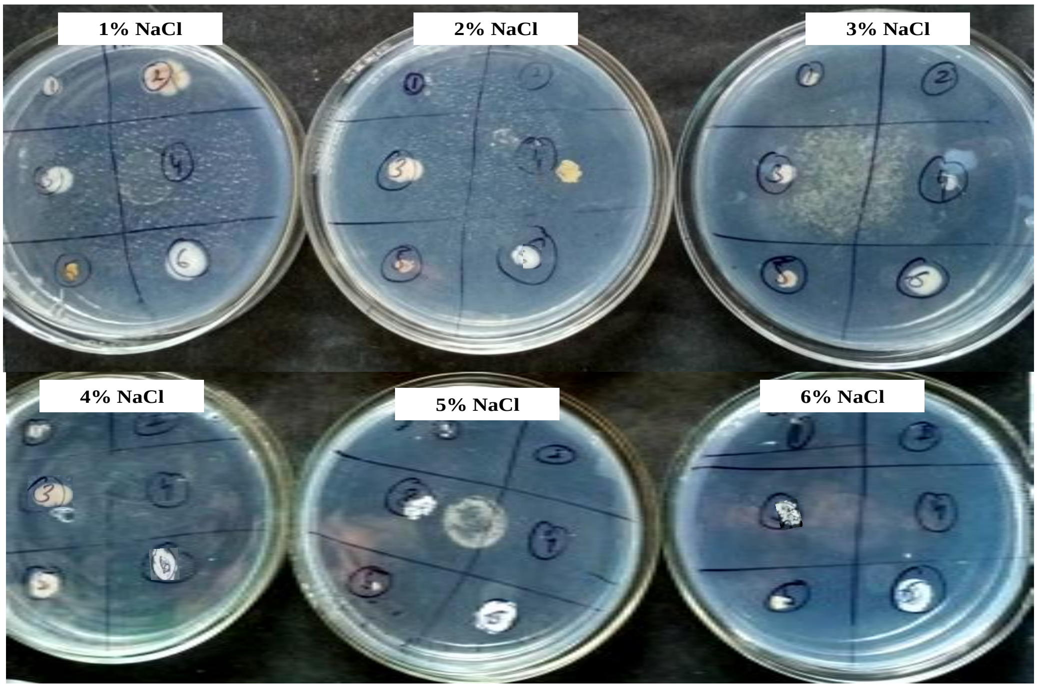

Fig. 2: Growth of actinobacterial isolates at different salt concentrations

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.

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