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*Management of Fungal Diseases of Chickpea (*Cicer arietinum *L.) through Plant Growth Promoting Actinobacteria and Their Secondary Metabolites*

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CONTENTS

10.1 Introduction

Chickpea (*Cicer arietinum* L.) is an important pulse crop and ranks third in overall production after bean and pea on a world basis. It is grown in 33 countries over an area of about 11.5 million hectares (Bidyarani et al. 2016). Chickpea is mainly used as food because of its high protein (12–31%) and carbohydrate (52–71%) contents (Mergaand Haji 2019). Global yields of chickpea (968 kg ha^{-1}) have been stagnant for the past five decades in spite of using various conventional and molecular breeding approaches and extensive use of synthetic fertilizers and pesticides (FAOSTAT 2014). Productivity of chickpea may be considerably improved if the adverse effects of biotic stresses (such as *Ascochyta* blight, dry root rot, *Fusarium* wilt, collar rot, and *Botrytis* gray mold) are addressed. Management of fungal diseases of chickpea is difficult, as no single control measure is fully effective. Some of the control measures such as advanced sowing date, solarization of soil, use of pathogen-free seed and fungicide-treated seed are usually employed to control the diseases, but with limited success. The use of resistant cultivar is the most efficient control measure but the effectiveness of disease resistance is restricted by the occurrence of several races of the pathogen. Hence, there is a need to use biological options to manage plant pathogens.

Biocontrol is a natural method to manage plant pathogens and it usually occurs by one or more distinct mechanisms such as parasitism, antibiotics production, induced systemic resistance, and competition for nutrients (Bach et al. 2016). Biocontrol agents are widely reported to manage plant pathogens of important crops including chickpea. Of which, *Bacillus*, *Pseudomonas*, and *Trichoderma* were widely reported. In recent years, actinobacteria and their secondary metabolites are exploited for controlling plant pathogens as they are the most prolific source for the production of bioactive metabolites (Bach et al. 2016; Gopalakrishnan and Srinivas 2019). This study explores how actinobacteria and their metabolites are exploited for the management of the fungal diseases of chickpea.

10.2 Actinobacteria and Their Metabolites

Actinobacteria are Gram-positive, aerobic, spore-forming, filamentous bacteria belong to the order Actinomycetales. All members of the order Actinomycetales consist of high G+C content (> 50%) in their genomic DNA (Ventura et al. 2007). Actinobacteria produce secondary metabolites of agricultural interest. There is a growing interest in the use of secondary metabolites, such as toxins, proteins, hormones, amino acids, and antibiotics from actinobacteria for the control of plant pathogens as these are readily degradable, highly specific, and less toxic to nature (Doumbou et al. 2001). Actinobacteria are commonly found in soil, compost, and fresh and marine water ecosystems.

Among the actinobacteria, *Streptomyces* is the predominant genus and principal contributors of secondary metabolites followed by *Micromonospora*, *Nocardia*, *Frankia*, *Nonomurea*, *Actinomadura*, *Microbispora*, *Mycobacterium*, *Actinoplanes*, *Saccharopolyspora*, and *Verrucosispora* (Martinez-Hidalgo et al. 2014). Such metabolites hold fungicidal, bactericidal, insecticidal, and PGP traits and can fill the need for biological agents.

10.3 Mechanisms Involved in Antagonistic Traits of Actinobacteria

Actinobacteria facilitate plant growth either by direct stimulation (such as fixed nitrogen, soluble phosphate, chelate iron and produce phytohormones and cell wall degrading enzymes) or by indirect stimulation (by inhibiting phytopathogens). Some of the important PGP and biocontrol traits of importance are as described in the following sections.

10.3.1 Siderophore Production

Siderophores are produced by many actinobacteria, which bind Fe^{3+} from the environment and make it available for plant growth. Actinobacteria compete with other rhizosphere plant pathogens for iron, hence competition for iron is also a possible mechanism to control the phytopathogens. Siderophore production was estimated as per the protocol of Schwyn and Neilands (1987). Numerous species of actinobacteria including *Streptomyces* and *Amycolatopsis* have been reported to produce siderophores and inhibit phytopathogens (Wang et al. 2014; Alekhya and Gopalakrishnan 2016).

10.3.2 Cellulase Production

Cellulase degrades cellulose on the plant cell wall, thus destroying the adhesion of the pathogen to the root surface of the plant. Cellulase-producing actinobacteria are reported to have nutrient mineralization, organic matter decomposition, and biocontrol traits on cellulose cell wall-bearing pathogens such as *Pythium* and *Phytophthora* (Lima et al. 1998). Actinobacteria including *Streptomyces* are known to produce cellulase (Gopalakrishnan et al. 2011; 2014) and inhibit pathogens of chickpea. The protocol of Hendricks et al. (1995) was used to determine the cellulase production.

10.3.3 Protease and Lipase Production

Protease and lipase production confers antibiosis to antagonistic microbes by degrading the proteins and lipids, respectively. Protease and lipase producing bacteria act as biocontrol agents on protein/lipid cell wall-bearing plant pathogens (Lima et al. 1998). Actinobacteria are widely demonstrated to produce protease and lipase and observed to inhibit both fungal pathogens and insect pests (Yandigeri et al. 2015). The protease and lipase production was measured as per Bhattachaya et al. (2009).

10.3.4 Hydrocyanic Acid (HCN) Production

HCN is reported as an anti-fungal secondary metabolite produced by many PGP microorganisms including actinobacteria (*Streptomyces* in particular) and play an important role in disease suppression (Bhosale and Kadam 2015). HCN was estimated qualitatively by the sulfocyanate colorimetric method (Gopalakrishnan et al. 2011).

10.3.5 Chitinase Production

Chitin, a linear polymer of beta-1, 4-linked N-acetyl glucosamine, is synthesized in all major groups of organisms including animals, plants, and fungi. The pathogenic fungal cell wall is composed of chitin. The actinobacteria capable of producing chitinase can degrade pathogenic cell wall and thus inhibit those (Yandigeri et al. 2015). Several chitinolytic enzymes have been identified in various *Streptomyces* species and a correlation between chitinolysis and production of bioactive compounds has also been reported (Yandigeri et al. 2015). Chitinase production by actinobacteria was estimated as per the protocols of Hirano and Nagao (1988).

10.3.6 β-1,3-glucanase Production

β-1,3-glucan is present in the cell walls of fungal pathogens. The lysis of β-1,3-glucan by β-1,3-glucanase producing bacteria leads to leakage of cell contents and thus inhibiting the pathogen invasion (Singh et al. 1999; Gopalakrishnan et al. 2014). β-1,3-glucanase production in actinobacteria was done as per the protocols of Singh et al. (1999) and Gopalakrishnan et al. (2014). Actinobacteria including *Streptomyces* and *Amycolatopsis* are reported to produce β-1,3-glucanase and play a role in disease suppression in chickpea (Alekhya and Gopalakrishnan 2016).

10.3.7 Indole Acetic Acid (IAA) Production

IAA is the common product of L-tryptophan metabolism produced by several bacteria including actinobacteria. IAA producing bacteria are known to promote root elongation and plant growth. It was measured as per the protocols of Patten and Glick (1996). Actinomycetes including *Streptomyces*, *Amycolatopsis*, and *Nocardiopsis*are widely reported to produce IAA and promote plant growth (Shutsrirung et al. 2013; Alekhya and Gopalakrishnan et al. 2016).

10.3.8 Other Cell Wall Degrading Enzymes

The other important cell wall degrading enzymes that play a role in the mechanism of pathogen inhibition include nucleases, xylanases, amylases, peptidases, peroxidases, ligninases, pectinase, hemicellulase, and keratinase. These enzymes also contribute to inhibiting phytopathogens because the cell walls of most fungal and bacterial pathogens consist of polymers like chitin, glucan, cellulose, proteins, and lipids (Yandigeri et al. 2015).

10.4 Actinobacteria and Their Role in Biocontrol of Fungal Diseases of Chickpea

Streptomyces is the most prominent and largest genus of actinobacteria. *Streptomyces* protect plants from plant pathogens

by various mechanisms including antibiosis (Soltanzadeh et al. 2016), competition for nutrients and space (Li et al. 2010), production of siderophores (Sadeghi et al. 2012), induction of systemic resistance (Singh and Gaur 2017), production of extracellular enzymes (Gopalakrishnan et al. 2011; Vijayabharathi et al. 2018).

Five strains of *Streptomyces* (CAI-24, CAI-121, CAI-127, KAI-32, and KAI-90) isolated from vermicompost, were reported to have antagonistic potential against *Fusarium* wilt in chickpea under wilt sick field conditions (Gopalakrishnan et al. 2011). Amini et al. (2016) reported efficient control of *Fusarium* wilt in chickpea by five strains of *Streptomyces* in Iran. Soltanzadeh et al. (2016) reported *Streptomyces antibioticus* S3, *Streptomyces antibioticus* S12, and *Streptomyces peruviensis* S40, having antagonistic potential against black root rot of chickpea caused by *Fusarium solani* f. sp. *pisi*. Singh and Gaur (2017) demonstrated six endophytic *Streptomyces* viz., *S. diasataticus*, *S. fradiae*, *S.olivochromogenes*, *S. collinus*, *S. ossamyceticus*, and *S. griseus* to control collar rot of chickpea which led to an induced resistance against *S. rolfsii*. Misk and Franco (2011) reported a total of 11 *Streptomyces* having antagonistic potential against *Botrytis* gray mold disease in chickpea. The *Streptomyces* strains were also found to have antagonistic potential against *Phytophthora* root rot disease and PGP traits in chickpea when co-inoculated with *Mesorhizobium ciceri* WSM1666 (Misk and Franco 2011).

Streptomyces were reported to have broad-spectrum biocontrol and PGP potentials. *Streptomyces* with broad-spectrum properties offer effective novel strategies not only for controlling plant pathogens and insect pests that attack crops but also for crop growth and yield. Alekhya and Gopalakrishnan (2014) reported a total of eight strains of *Streptomyces* (BCA-657, BCA-671, BCA-679, BCA-687, BCA-690, CAI-67, CAI-70, and CAI-98) having broad-spectrum antagonistic potential against important fungal diseases of chickpea such as *Fusarium* wilt, dry root rot, and *Botrytis* gray mold. Nine strains of *Streptomyces* (SAI-13, SAI-29, VAI-7, VAI-30, AC-5, AC-6, AC-10, AC-18, and AC-19) were reported to have a broad spectrum of antagonistic potential against fungal diseases of chickpea including *Fusarium* wilt, dry root rot, and collar rot (Sreevidya et al. 2016; Anusha et al. 2019).

PGP potential of *Streptomyces* was reported extensively in chickpea (Gopalakrishnan et al. 2015). However, PGP by other members of the actinomycete family is rarely reported. PGP potentials of *Amycolatopsis* was demonstrated in chickpea under field conditions (Alekhya and Gopalakrishnan 2016).

Secondary metabolites from *Streptomyces* are reported to control *Helicoverpa armigera*, one of the important polyphagous insect pests of many legume crops including chickpea, causing yield losses of up to 100% (Vijayabharathi et al. 2014). An insecticidal compound, diketopiperazine, cyclo (Trp-Phe), was purified from the extracellular extract of *Streptomyces griseoplanus* SAI-25 against *H. armigera* of chickpea (Sathya et al. 2016). One more metabolite was also purified from the culture filtrate of *Streptomyces* sp. CAI-155 as *N*-(1-(2,2-dimethyl-5-undecyl-1, 3-dioxolan-4-yl)-2-hydroxyethyl)stearamide (Gopalakrishnan et al. 2016) against *H. armigera* of chickpea. Among all the actinobacteria which produce secondary metabolites of commercial interest. Streptomycetes have the ability to produce enormous varieties of metabolites.

10.5 Conclusion

The agricultural sector is in need of newer biocontrol agents and their significant secondary metabolites. Biocontrol often appears promising in specialized environments, especially where disappointing results were frequently observed in the field as several factors determine the survival and delivery of the antagonist. Therefore, the strategy to combat the disease should be to integrate different methods of pest control including disease-resistant cultivars, biocontrol using actinobacteria in specific and safe cultural practices. There is a need to also evaluate these PGP actinobacteria under different field conditions (multi-location trials) for commercialization and to improve sustainability in agricultural production, especially in chickpea.

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