Bioefficacy of Cyclic Depsipeptide Producing Fluorescent Pseudomonads Against Leaf Blight Pathogens of Maize (Zea Mays)

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Abstract*- Cyclic lipopeptides (CLPs) with antibiotic, biosurfactant producing Fluorescent Pseudomonads were isolated from sugar beet, maize intercropped in sandy loam soils at Maize Research Station, Vagarai, TNAU. The impact of CLP producing flurorescent Pseudomonads strains on maize pathogens were tested under in vivo conditions during Kharif & Rabi seasons 2015-2016. Among the treatments implied during Rabi 2015, Seed treatment and Foliar spraying with Pf VMD1 recorded lower Turcicum Leaf Bight incidence (30.31%), Maydis Leaf Blight (11.40%) followed by Pf VMD2,Pf VMD3 & Bavistin TLB (43%) & MLB (12-13%), compared with control TLB (63.32%) & MLB (18.30). Among the treatments implied during kharif 2015, Seed treatment and Foliar spraying with PfVMD1 recorded lower Turcicum Leaf Bight incidence (10%), Maydis Leaf Blight (6.4%) as effective as Bavistin TLB (8%) & MLB (6.4%), compared with control TLB (21.2%) & MLB (16.8%). observed. Among the treatments implied during kharif 2016, Seed treatment and Foliar spraying with Pf VMD1 recorded lower Turcicum Leaf Bight incidence (10%), Maydis Leaf Blight (6.4%) as effective as Ridomil TLB (8%) & MLB (6.4%), compared with control TLB (21.2%) & MLB (16.8%). There were no soil-borne diseases observed in all the seasons. . Pf-VMD strains were found to be as effective as systemic fungicides in controlling leaf blight pathogens under field conditions in terms of systemic protection with growth promotion*.

Keywords- Maize leaf blights, Helminthosporium turcicum (Pass), Bipolaris maydis (Nisik.and Miyake) Cyclic lipopeptides, Fluorescent Pseudomonads, systemic fungicides

I. INTRODUCTION

Among the various diseases damaging the maize crop, the turcicum leaf blight *Helminthosporium turcicum* (Pass) and maydis leaf blight *Bipolaris maydis (*Nisik. and Miyake*)* are the most important diseases in maize growing areas. Turcicum leaf blight also called as Northern leaf blight caused by Exserohilum turcicum (Pass.) Leonard and Suggs. (Syn. *Helminthosporium turcicum* Pass.) is of worldwide importance (Carlos, 1997). In India, the disease is prevalent in almost all the maize growing areas. Severe losses in grain yield due to epiphytotics have been reported in various parts of India and these losses vary from 25 to 90 per cent depending upon the severity of the disease (Chenulu and Hora, 1962; Jha, 1993). The disease is responsible for the untimely death of blighted leaves and results in substantial yield reductions. Turcicum leaf blight of maize is considered to be one of the most devastating disease, Earlier the disease was considered as minor, but now it has assumed the status of major disease in world as its occurrence and incidence assumes greater significance resulting in reduction of grain yield by 28 to 91 per cent (Kachapur, 1988 and Harlapur *et al*., 2000). Southern Corn Leaf Blight is one among the most important maize diseases and caused by the fungus *Bipolaris maydis*. MLB is reported from most maize growing regions in the world including India. Under severe conditions depending upon the susceptibility of the variety, MLB may cause significant grain yield losses (Thompson and Bergquest, 1984), up to 70% (Kumar *et al.,* 2009). Infected tissue is extensively covered with spots and chlorosis rendering them non productive. It is found to have a higher saprophytic ability (Blanco and Nelson, 1972) and hence high primary inoculums level will be likely to be found in areas with high disease occurrence. In South East Asia it is reported to cause heavy losses in Pakistan, India, Nepal, Kampuchea, Philippines, Indonesia, Vietnam and China. 'Maydis Leaf Blight' is serious disease in India particularly in J & K, Himachal Pradesh, Sikkim, Meghalaya, Punjab, Haryana, Rajasthan, and Delhi, UP, Uttrakhand, Bihar, MP, Gujarat, Maharashtra, Andhra Pradesh, Karnataka and Tamil Nadu. Turcicum blight caused the yield loss of 50% (Shurtleff, 1980) and Maydis blight caused the yield loss of 70%. Chandrashekara *et al*. (2014) Turcicum leaf blight (TLB) and Maydis leaf blight (MLB) are the important diseases of maize grown in the North Western Himalayas. To reduce the yield loss in maize, effective management practices for the above diseases must be evolved. In 2007, use of fungicides increased in corn and now a day's corn crop is typically produced with higher fungicide inputs compared to last five years (Wise and Mueller, 2011). Foliar fungicides have seen the most drastic increase in use over the past 10 years compared to other pesticides in corn (Gianessi and Reigner, 2006). Increased corn residues can serve as a source of primary inoculums for several important foliar diseases (Wise and Mueller, 2011). Hence, in recent years the need for disease management in corn is augmented and crucial.

II.OBJECTIVE

Development of new biocide molecules is very essential to manage the leaf blights for low disease severity with increase in yield. Extensive use of chemicals to control plant diseases has disturbed the ecological balance of microbes inhabiting soil leading to development of drug resistant strains, groundwater contamination and obvious health risks to humans (Ongena *et al*., 2008). Demand of lipopeptides is also surging due to their utility in human welfare, these lipopeptides have projected peak annual revenue and their use has been approved in more than 70 countries. Hence an attempt was made to evaluate CLP producing pseudomonads against the maize pathogens to manage the disease and they are found to be as effective as systemic fungicides being practiced for corn in India.

III.METHODOLOGY

A. Testing the efficacy of CLP strains under field conditions:

The maize crop was raised with all standard and recommended packages of agronomic practices at the Maize Research Station, Vagarai during Kharif & Rabi seasons. The test Pf VMD strains (Data not shown) were applied as seed treatment (10g/kg) and foliar spray (0.1%) with knap sack sprayer fitted with hollow cone nozzle along with test fungicides. About1.25 liters of water was mixed with each test fungicides and sprayed over the crop in each plot measuring 12 m^2; first spraying was done at the appearance of visible symptom (45 days after sowing) and second spray was given at 20 leaf stage (Taselling & Silking). Untreated check (Positive control) was recommended with no Pf VMD/fungicide application. Ten plants were marked in each plot for recording percent disease severity following 0-5 rating scale. At flowering time, the length and width of each lesion, and its corresponding position according to the top ear were recorded. With regard to no-lesion resistance of *Ht/Hm*.

B. Disease Score

The scale consists of five broad categories designated by numerals from 1 to 5. Intermediate ratings between two numerals (1.5, 2.5, 3.5 etc.) have also been given, thereby providing for a total of nine classes or categories. Wherever possible, observations on lesion types can also be made, such as large sporulating wilt type or small chlorotic, non-sporulating type. Data was recorded 30-35 days after inoculation, then on flowering and finally just before dough stage. The disease scoring (Payak and Sharma, 1985) was done as per symptoms mentioned below:

Very slight to slight infection, one or two to few scattered lesions on lower leaves. Light infection, moderate number of lesions on lower leaves only. Moderate infection, abundant lesions are on lower leaves, few on middle leaves. Heavy infection, lesions are abundant on lower and middle leaves, extending to upper leaves. Very heavy infection, lesions abundant on almost all leaves plants prematurely dry or killed by the disease.

The plants were kept under close vigil to protect them from the ravages pests' attack. The intensity of the disease was recorded just before each spraying. Disease intensity on leaves was graded in 0-5 rating scale (Payak and Sharma, 1983). The per cent disease intensity (PDI) was calculated by the following formula.

$$\text{PDI} = \frac{\text{Sum of all numerical ratings}}{\text{Total plants (leaves) observed} \times \text{Maximum rating scale used}} \times 100$$

The details of materials used and method employed in the three experiments were as follows.

I^st Experiment

In this experiment during Rabi 2015 in RBD with three replication and plot size of 4.0 m x 3.0 m each with CoHM6 hybrid were employed with the following treatment schedules

Sl.No	Treatments (Rabi 2015)
1	Seed treatment with PfVMD1 and Foliar spraying at 45DAS
2	Seed treatment with PfVMD2 and Folar spraying at 45DAS
3	Seed treatment with PfVMD3 and Foliar spraying at 45DAS
4	Seed treatment with Pf1 and Foliar spraying of Pf1 broth culture at 45DAS
5	Seed treatment with Carbendazim (0.1%) and Folar spraying at 45DAS
6	Untreated Control

II^nd Experiment

In this experiment during Kharif 2015 in RBD with three replication and plot size of 4.0 m x 3.0 m each with CoHM6 hybrid were employed with the following treatment schedules

Sl.No	Treatments (Kharif 2015)
1	Foliar spraying Ridomil Gold (0.1%) at 45DAS
2	Folar spraying Carbendazim(0.1%) at 45DAS
3	Foliar spraying Tricyclazole (0.1%) at 45DAS
4	Foliar spraying Chlorothalonil (0.1%) at 45DAS
5	Seed treatment with Pf VMD1 and Folar spraying at 45DAS
6	Seed treatment with Pf1 TNAU and Folar spraying at 45DAS
7	Untreated Control

III[nd] Experiment

In this experiment during Rabi 2015 in RBD with three replication and plot size of 4.0 m x 3.0 m each with CoHM6 hybrid were employed with the following treatment schedules

Sl.No	Treatments (Rabi 2015)
1	Seed treatment with Pf1 and Foliar spraying at 45DAS
2	Seed treatment with PfVMD1 and Foliar spraying at 45DAS
3	Seed treatment with PfVMD2 and Foliar spraying at 45DAS
4	Seed treatment with PfVMD3 and Foliar spraying at 45DAS
5	Seed treatment with Metalaxyl (0.1%) and Folar spraying at 45DAS
6	Seed treatment with Ridomil (0.1%) and Folar spraying at 45DAS
7	Untreated Control

IV.RESULTS AND DISCUSSIONS

During Rabi 2015, Seed treatment and Foliar spraying with *PfVMD1* recorded lower Turcicum Leaf Bight incidence (30.31%), Maydis Leaf Blight (11.40%) followed by *Pf VMD2 (*43.22 , 12.40) ,*Pf VMD3(*43.25, 12.53) & Bavistin TLB (43%) & MLB (12-13%), compared with control TLB (63.32%) & MLB (18.30). During kharif 2015, Seed treatment and Foliar spraying with *PfVMD1* recorded lower Turcicum Leaf Bight incidence (10%), Maydis Leaf Blight (6.4%) as effective as Bavistin TLB (8%) & MLB (6.4%), compared with control TLB (21.2%) & MLB (16.8%). observed. During kharif 2016, Seed treatment and Foliar spraying with *Pf VMD1* recorded lower Turcicum Leaf Bight incidence (10%), Maydis Leaf Blight (6.4%) as effective as Ridomil TLB (8%) & MLB (6.4%), compared with control TLB (21.2%) & MLB (16.8%). There were no soil-borne diseases observed in all the seasons.

Pf VMD1 treated plots recorded highest yield of 9106 kg/ha followed by *PfVMD2* (8988 kg/ha) during Rabi 2015. *Pf VMD1* treated plots recorded highest yield of 9025 kg/ha during Kharif 2015 compared with the untreated control plots (5025 kg/ha during Rabi 2015 and 5030 kg/ha during Kharif 2015). During Kharif 2016 *PfVMD1* plots recorded 9095 kg/ha compared with untreated control 5030

Table: I. In vivo evaluation of VMD producing Pf strains against Northern Maize blight Helminthosporium turcicum (TLB) during Rabi 2015

S.No	Treatment	Grade (0-5)	PDI (%)	Leison Length(cm)	Leison width(cm)	Yield (kg/ha)
T1	**Pf-VMD1**	**2.0**	**30.31**	**2.75**	**0.25**	**9106[a]**
T2	Pf-VMD2	2.1	43.22	5.43	0.35	8988[b]
T3	Pf-VMD3	2.2	43.25	5.45	0.34	8503[c]
T4	Pf1(TNAU)	2.1	43.56	5.47	0.36	8515[d]
T5	Bavistin	2.1	43.52	5.63	0.33	7250[e]
T6	Untreated control	5.0	63.32	8.0	0.47	5025[f]
	CD(0.05)		0.298	0.186	0.015	20.45

Table: II. In vivo evaluation of VMD producing Pf strains against Southern Maize blight Bipolaris maydis (MLB) during Rabi 2015

Sl. No	Treatment	Grade (0-5)	PDI (%)	Leison Length(cm)	Leison Width(cm)	Yield Kg/ha
T1	**Pf-VMD1**	**2.0**	**11.40**	**0.87**	**0.16**	**9106**
T2	Pf-VMD2	2.1	12.40	1.03	0.17	8988
T3	Pf-VMD3	2.1	12.53	1.16	0.26	8503
T4	Pf1(TNAU)	2.0	13.56	2.33	0.40	8515
T5	Bavistin	2.0	13.53	2.52	0.42	7250
T6	Untreated control	4.0	18.30	2.86	1.15	5025
CD (0.05%)			0.375	0.303	0.020	23.16

Table: III. In vivo evaluation of VMD producing Pf strains against Southern Maize blight Bipolaris maydis (MLB) during Kharif 2015

S.No	Treatment	Grade (0-5)	PDI (%)	Leison Length (cm)	Leison width (cm)	Yield (kg/ha)
T1	Ridomil Gold	2.46	14.8	2.25	0.37	7263
T2	Carbendazim	1.06	6.4	1.18	0.32	8850
T3	Tricyclazole	2.46	14.8	2.33	0.40	8605
T4	Chlorothalonil	1.93	11.6	1.16	0.36	8515
T5	Pf-VMD1	1.06	6.4	0.87	0.17	9025
T6	Pf-TNAU	2.26	13.6	1.03	0.76	7205
T7	Untreated control	3.0	16.8	3.56	1.15	5030
	CD(0.05)		0.475	0.305	0.023	20.36

Table: IV. In vivo evaluation of VMD producing Pf strains against Northern Maize blight Helminthosporium turcicum (TLB) during Kharif 2015

S.No	Treatment	Grade (0-5)	PDI (%)	Leison Length (cm)	Leison width (cm)	Yield (kg/ha)
T1	Ridomil Gold	2.86	17.2	5.40	0.35	7263
T2	Carbendazim	1.33	8.0	2.75	0.25	8850
T3	Tricyclazole	2.46	14.8	2.33	0.40	8605
T4	Chlorothalonil	2.33	14.4	3.68	0.30	8515
T5	Pf-VMD1	1.66	10.0	2.50	0.25	9025
T6	Pf-TNAU	2.43	14.6	4.32	0.35	7205
T7	Untreated control	4.23	21.2	8.0	0.47	5030
	CD(0.05)		0.302	0.192	0.016	20.36

Table: V. In vivo evaluation of VMD producing Pf strains against Southern Maize blight Bipolaris maydis (MLB) during Kharif 2016

S.No	Treatment	Grade (0-5)	PDI (%)	Leison Length (cm)	Leison width (cm)	Yield (kg/ha)
T1	Pf1(TNAU)	2.26	13.6	1.03	0.76	8852
T2	Pf-VMD1	1.06	6.4	0.87	0.17	**9095**
T3	Pf-VMD2	1.93	11.6	1.16	0.36	8713
T4	Pf-VMD3	2.46	14.8	2.33	0.40	8615
T5	Metalaxyl (0.1%)	2.46	14.8	2.25	0.37	8305
T6	Ridomil (0.1%)	1.06	6.4	1.18	0.32	8660
T7	Untreated control	3.0	16.8	3.56	1.15	5030
	CD(0.05)		0.298	0.186	0.015	20.45

Table: VI. In vivo evaluation of VMD producing Pf strains against Northern Maize blight Helminthosporium turcicum (TLB) during Kharif 2016

S.No	Treatment	Grade (0-5)	PDI (%)	Leison Length (cm)	Leison width (cm)	Yield (kg/ha)
T1	Pf1(TNAU)	2.43	14.6	4.32	0.35	8852
T2	Pf-VMD1	1.66	10.0	2.50	0.25	**9095**
T3	Pf-VMD2	2.33	14.4	3.68	0.30	8713
T4	Pf-VMD3	2.63	17.6	4.50	0.32	8615
T5	Metalaxyl (0.2%)	2.86	17.2	5.40	0.35	8305
T6	Ridomil (0.2%)	1.33	8.0	2.75	0.25	8660
T7	Untreated control	4.23	21.2	8.0	0.47	5030
	CD(0.05)		0.375	0.303	0.020	23.16

In order to reduce the higher fungicide inputs, present investigation was carried out to find the efficacy of CLP producing PGPR strains with systemic fungicides, which are being practiced by the farmers. Among the strains tested under field conditions, *P. fluorescens* strains including *Pf* VMD1 decreases the disease severity and increased the yield of maize crop in both the seasons. *Pf* VMD1 strain is the potent producer of visocinamide, which is an antibiotic and biosurfactant (Data not shown). Rhizosphere-inhabiting *P. fluorescens* strains producing CLPs perform fungal antagonism and reduction of surface tension detected *in vitro* (Nielson *et al*., 2002). Based on these *in vivo* results, it may be speculated that the *in situ* production of CLPs on maize seeds in soil is advantageous to the *P. fluorescens* inoculants during their growth activity in the rhizosphere and phyllosphere.

Lipopeptides surfactants are environmental ecofriendly alternatives to synthetic surfactants. Several CLP producers are widely used to control plant diseases. Iturin/Fengycin from *Bacillus subtilis* M4 strain controlled damping off disease of bean (Ongena *et al*.,2005), Fengycin from *Bacillus subtilis* controlled gray mold disease of apple and surfactin from *Bacillus subtilis* 6051 strain 96.578 controlled *R.solani* in sugarbeet, chromobactomycin controlled rice blast (*Magnaporthe grisea*) from chromobacterium sp C61 (Ongena *et al*.,2005; Basis *et al*.,2004 ; Liu *et al*.,2005; Nielson *et al*.,2000 & Alvarez *et al*., 2012; Kim *et al*.,2014).

Recently, a new lipopeptide referred to as “Kinnorin” isolated from *Bacillus cereus* has been found to exhibit good antifungal activity (Gordillo.A and Maldonado.M.C.2012; Ajesh *et al*., 2013; Deleu *et al*., 2003). Viscosinamide producing PGPR strains are a novel class of potent versatile weapons to deal with a variety of phytopathogens. These lipopeptides seem to be promising biopesticides in agriculture practices for replacing harmful chemical pesticide and thus they can be considered as potent alternative tools to overcome increasing chemical resistance of phytopathogens. Lipopeptide produced by PGPRs are nontoxic, biodegradable, highly stable, eco friendly and nonpolluting biomolecules. Moreover, their ability to induce systemic resistance in plants and their use in the spreading of the bacterial cells leading to rhizosphere & phyllosphere colonization could open new fields of applications for their use as promising phytochemical products.

V. CONCLUSION

Cyclic lipopeptides (CLPs) with antibiotic, biosurfactant producing Fluorescent *Pseudomonads* were isolated and enumerated from maize rhizosphere (Data not shown). The impact of flurorescent *Pseudomonads* strain (CLP) on the zoospores of Downy mildew pathogen of maize was studied by direct microscopy and encysted zoospores were observed (Data not shown) and field experiments were conducted for the performance of *Pf*-VMD strain against fungal pathogens of maize. *Pf*-VMD strains were found to be as effective as systemic fungicides in controlling leaf blight pathogens under field conditions in terms of systemic protection with growth promotion.

VI. ACKNOWLEDGMENT

This study was funded by the Women Scientist Scheme (A), Department of Science and Technology, Government of India, New Delhi-110016. We thank Dr.R.Viswanathan, Director (i/c), Sugarcane Breeding Institute, Coimbatore-641007, Tamil Nadu, India for making use of the HPLC facility.

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