Plant growth stimulation and biocontrol potential of fluorescent pseudomonads under saline conditions

Presented by
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Introduction

- **Agriculture is considered to be amongst the most vulnerable sectors, often exposed to a plethora of stress conditions**

- **Abiotic stresses especially salinity have resulted in dipping of average yields of major crops (Mahajan and Tuteja 2006)**

Introduction

- **For centuries, agriculture, particularly in arid and semiarid environments has faced salinity stress**

- **Salinity is one of the major abiotic stress factor limiting plant growth and productivity (Khan and Panda 2008)**

- **The total salt-affected land worldwide was estimated to be 900 million ha, 6% of the total global land mass in 2004 and the area under saline stress is increasing day by day (Flowers 2004)**

Saline soils

Salt affected areas of the world

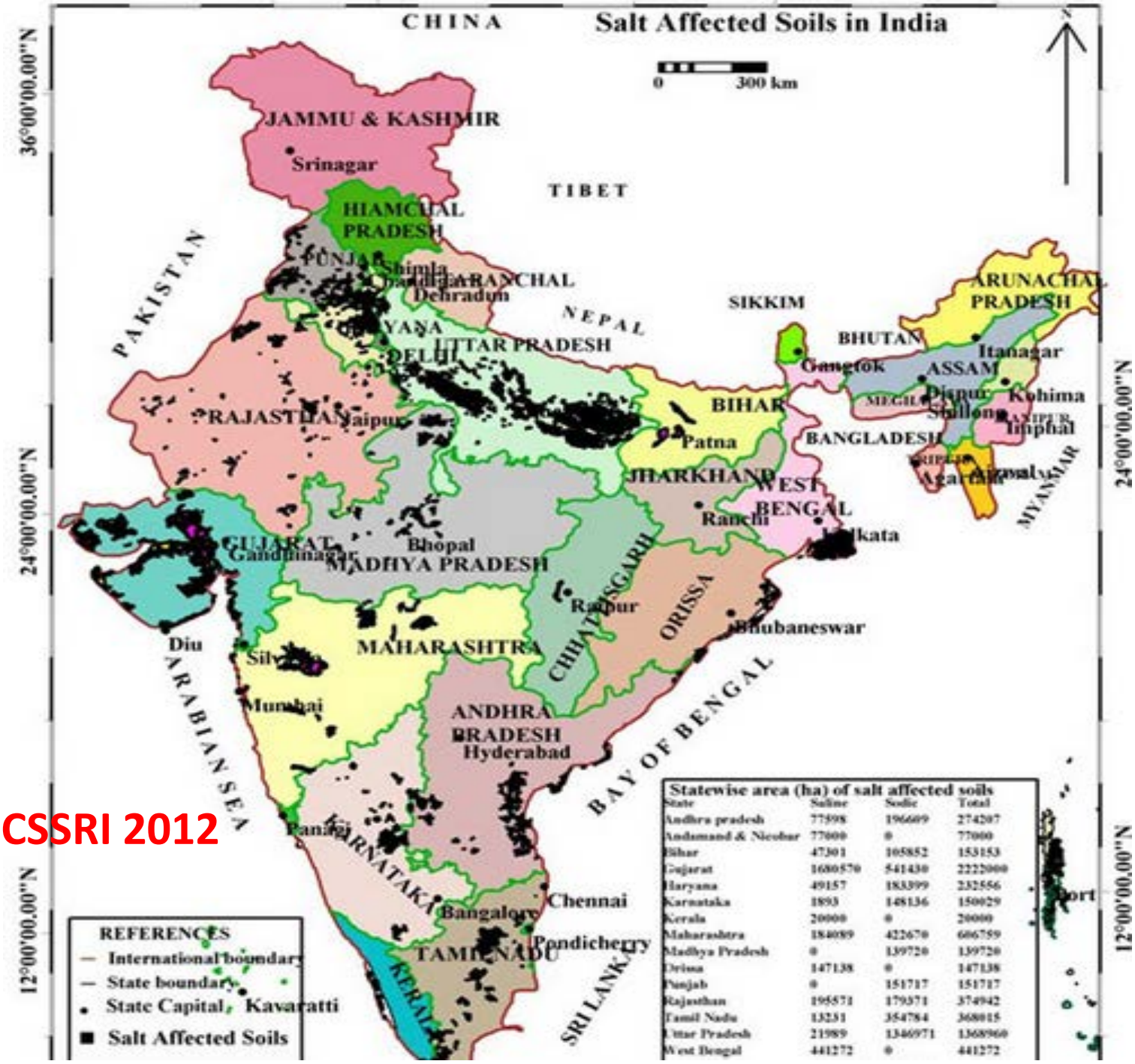
Region	Area (10^6 ha)
North America	16
Argentina	86
Paraguay	22
Ethiopia	11
India	24
Iran	27
Pakistan	10
China	37
(Former) USSR	171
Indonesia	13
Australia	357

Source : Melbourne Engineering Research Institute , MERIT;
(Pathak et al. 2013)

Salt affected areas in India

State	Saline soils (ha)
Bihar	47301
Gujarat	1218255
Haryana	49157
Karnataka	1307
Maharashtra	177093
Rajasthan	195571
Uttar Pradesh	21989

Source: Central Soil Salinity Research Institute, India, 2012 http://www.cssri.org/index.



Source: CSSRI 2012

Constraints associated with salinity

- Salinity prevents plants from taking up water, exposing them to drought stress

- Salinity stress have an adverse effect on plants, hampering their growth and finally production

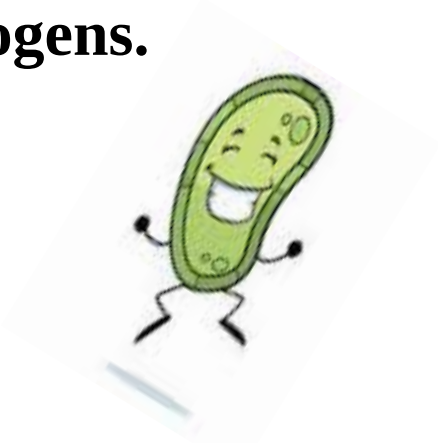
- According to Food and Agricultural Organization (FAO), if corrective measures are not taken then salinity stress may result in 30% land loss in next 25 years and 50% loss by 2050 (Munns 2002)

- Use of **Plant Growth Promoting Rhizobacteria (PGPR)** can be helpful to enhance productivity and remediate the saline soils

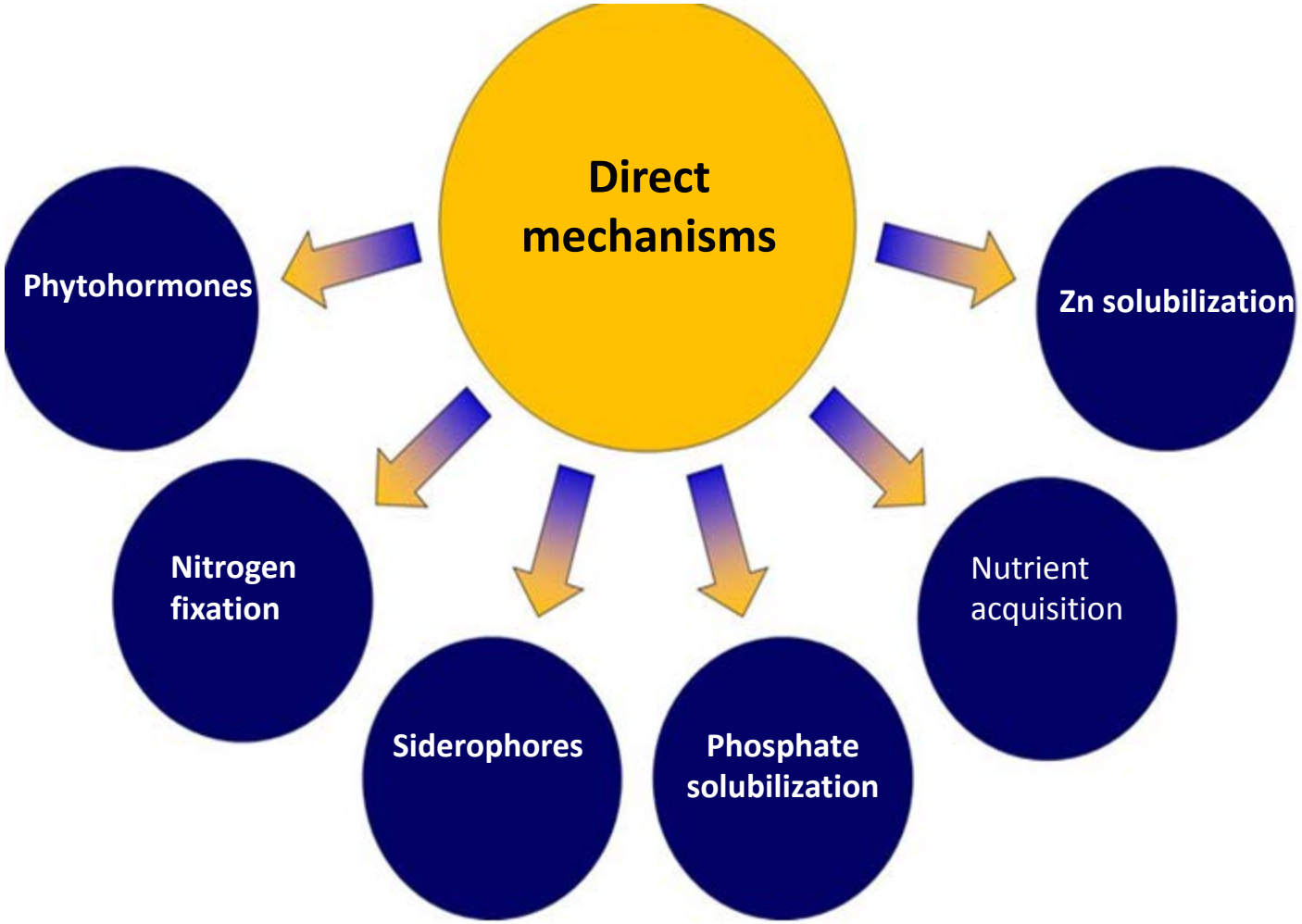
PGPR

- **Kloepper and Schroth in 1978 coined the term plant growth promoting rhizobacteria (PGPR). PGPRs are in mutualistic relationship with the plant.**

- **PGPR perform diverse roles by direct and indirect mechanisms (Kloepper and Schroth 1978; Kloepper et al. 1980; Glick 1995). These are plant growth promotion and control of phytopathogens.**

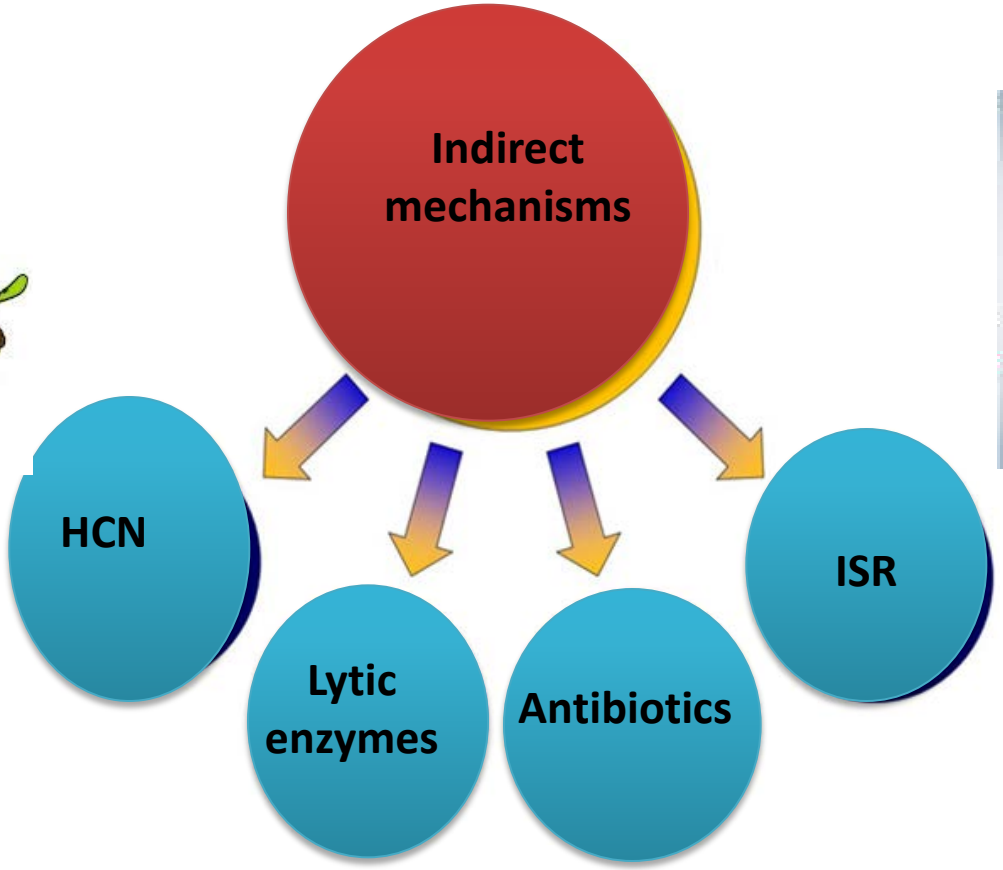


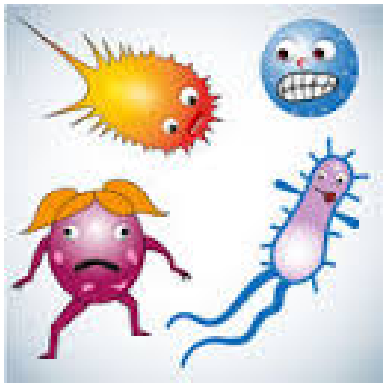
Direct roles of PGPR



Indirect Mechanisms

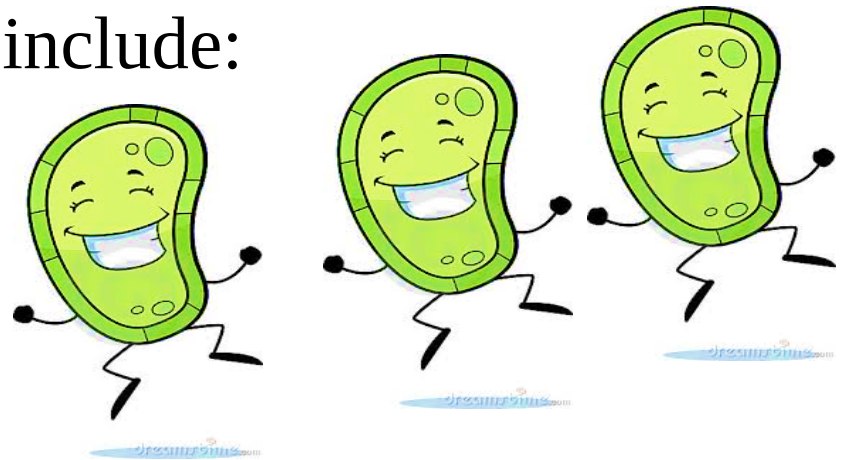
- Indirect mechanisms are basically related to biocontrol of phytopathogens





Other Mechanisms of PGPR

However, PGPRs are now also known to perform and utilized for other roles which include:

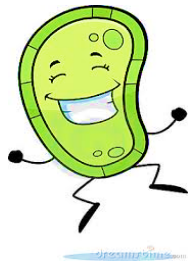


- Bioremediation
- Bioaccumulation
- Biodegradation
- Stress management
- Combating climate change

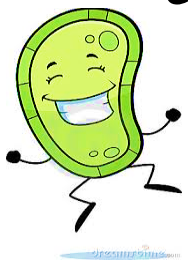
(Arora et al. 2011; Tewari and Arora 2013; Arora et al. 2013; Tewari and Arora 2014)

PGPR in stress management

- **The role of PGPRs under non-stress conditions is very well known**



- **However, scarce information is available regarding the utility of microbes in plant growth promotion and disease suppression under soil stress conditions such as salinity**



Fluorescent pseudomonads

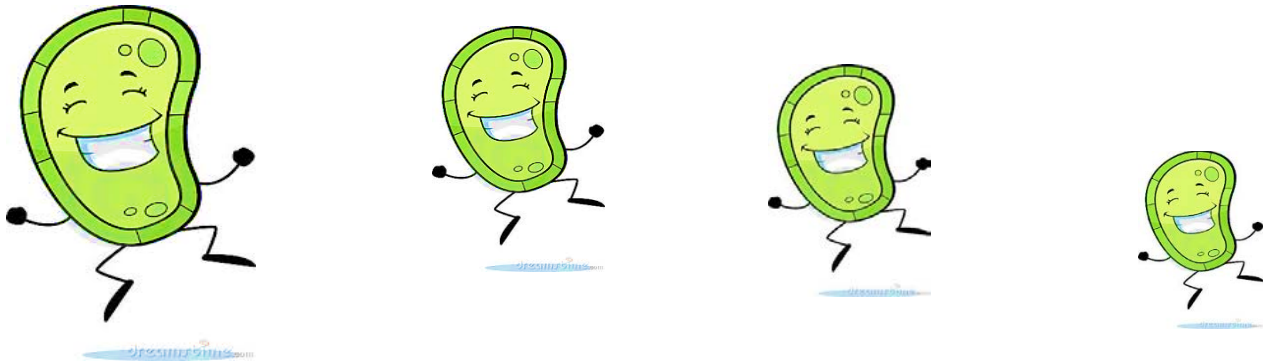
- **Soil-borne bacteria known as fluorescent pseudomonads have received particular attention as PGPRs due to:**


Pseudomonas aeruginosa

- Catabolic versatility
- Excellent root colonizing ability
- Capacity to produce a wide range of metabolites
- Favor the plant to withstand under varied biotic and abiotic stresses
- Bioremediation (Rhizoremediation), Biodegradation

Fluorescent pseudomonads as efficient PGPRs

- **Role of fluorescent pseudomonads has been widely explored in enhancing production of various crops but their role under salinity-stress conditions is still at initials and needs to be explored further**

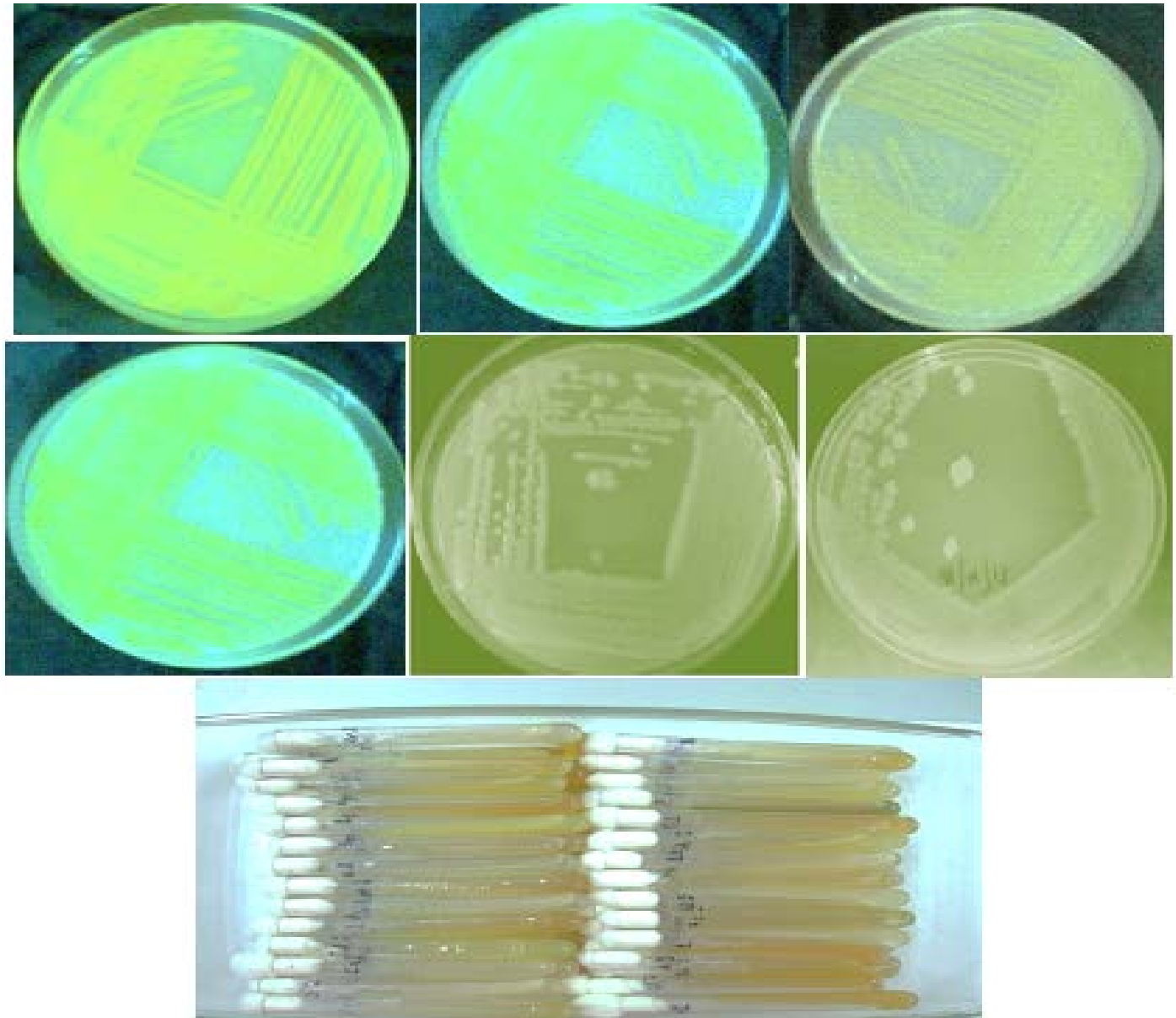


Isolation of Fluorescent pseudomonads

- **Fluorescent pseudomonads were isolated from the rhizosphere of various crop plants growing under semi-arid regions of Kanpur (Uttar Pradesh, India)**

- **Isolates were characterized morphologically, biochemically and by molecular techniques, most of them being *Pseudomonas fluorescence* and *P. aeruginosa***

Fluorescent pseudomonad isolates



Characterization of Fluorescent pseudomonads

- **Some of the isolates particularly those from arid regions displayed high salt tolerance up to 2000 mM NaCl** (1)

- **These isolates also maintained PGP and biocontrol traits up to 500 mM salinity** (2)

[1]Khare et al. 2011, Curr Microbiol, vol 62

[2]Tewari and Arora 2014, Curr Microbiol, vol 69

Effect of salinity on PGP and Biocontrol metabolites

Production of PGP metabolites under saline stress

- ***Pseudomonas*** **strain EK1 was found to be capable of producing IAA (plant growth hormone), siderophore (iron chelator) and HCN up to 400 mM NaCl concentration**[(1)]

- **PF23 was capable of producing exopolysaccharides (EPS) and solubilized insoluble phosphates up to 500 mM NaCl salinity**[(2)]

[1]Khare et al. 2011, Curr Microbiol, vol 62

[2]Tewari and Arora 2014, Curr Microbiol, vol 69

Antagonistic potential of fluorescent Pseudomonads under saline conditions

- Antagonistic activity of *Pseudomonas* EK1 and PF23 was checked by dual culture technique against *Macrophomina phaseolina* under saline conditions

- *M. phaseolina* is a fungal opportunist that likes to take advantage of salinity stressed plant and causes 70% reduction in crop production and yield in saline soils infested with it (Ullah et al. 2011)

Effect of salinity and *M. phaseolina* on sunflower and chickpea

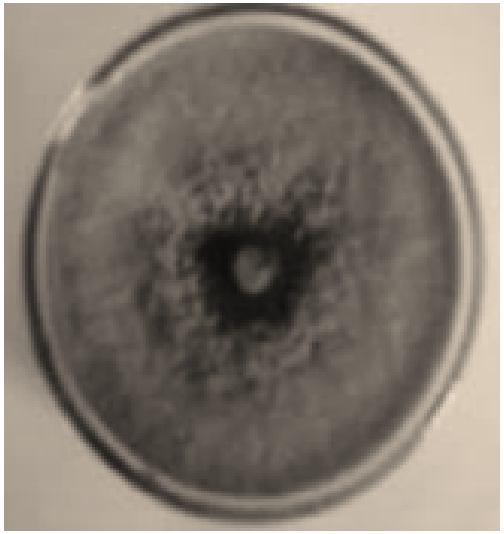
Sunflower

Chickpea

Macrophomina phaseolina

- ***Macrophomina phaseolina* (Tassi) Goid. is a soil-borne fungus that causes –**

  - Charcoal rot disease
  - Root rot disease
  - Head rot disease

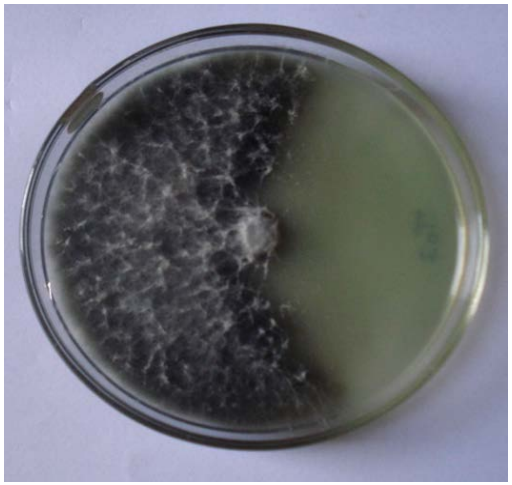


M.phaseolina

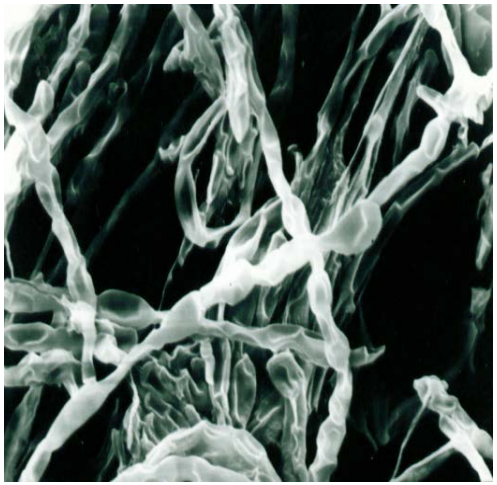
- ***M. phaseolina* causes destruction of numerous crops including sunflower, ground nut, chickpea, soybean, alfa alfa, beans etc**

In vitro inhibition of *M. phaseolina* by fluorescent *Pseudomonas*

- *Pseudomonas* strain EK1 and PF23 showed 93 % and 86 % inhibition of *M. phaseolina* by dual culture technique[1,2]



a



b

a : Inhibitory activity of *Pseudomonas* against *M. phaseolina*

b : Deformities appeared in the hyphae of *M. phaseolina*

Khare et al. 2011, Curr Microbiol, vol 62

Tewari and Arora 2014, Curr Microbiol, vol 69

Biocontrol potential of isolates under saline conditions

0 mM

100 mM

200 mM

300 mM

400 mM

0 mM

100 mM

200 mM

300 mM

500 mM

Biocontrol potential of fluorescent Pseudomonads under saline conditions

- EK1 and PF23 showed significant inhibition of *M. phaseolina* up to 500 mM NaCl concentration [1,2]

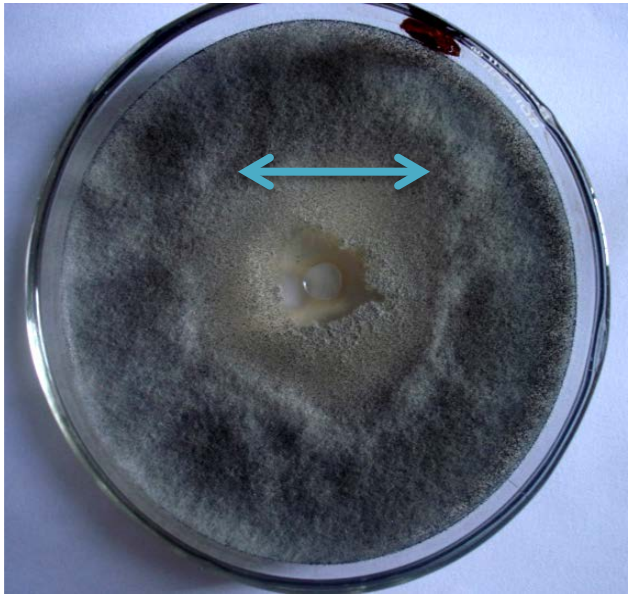
- There was 76% and 74% suppression of phytopathogen, *M. phaseolina*, at 500 mM and 400 mM concentration by purified EPS and pyocyanin respectively, obtained from PF23 and EK1

[1]Khare et al. 2011, Curr Microbiol, vol 62

[2]Tewari and Arora 2014, Curr Microbiol, vol 69

Biocontrol metabolites working under saline stress

Purified EPS from PF23 showing inhibition of *M. phaseolina* at 500 mM NaCl



Purified pyocyanin from EK1 inhibiting *M. phaseolina* at 400 mM NaCl

Biocontrol metabolites working under saline stress

- **Chemical Mutagenesis was done for developing EPS defective mutant ($PF23^{EPS-)}$ and pyocyanin defective mutants ($EK1^{Pyo-}$) for checking the role of biocontrol metabolites under saline conditions** (1,2,3)

- **Mutants $PF23^{EPS-}$ and $EK1^{Pyo-}$ showed reduction of 86% and 88% in production of EPS and pyocyanin in comparison with wild strains PF23 and EK1**

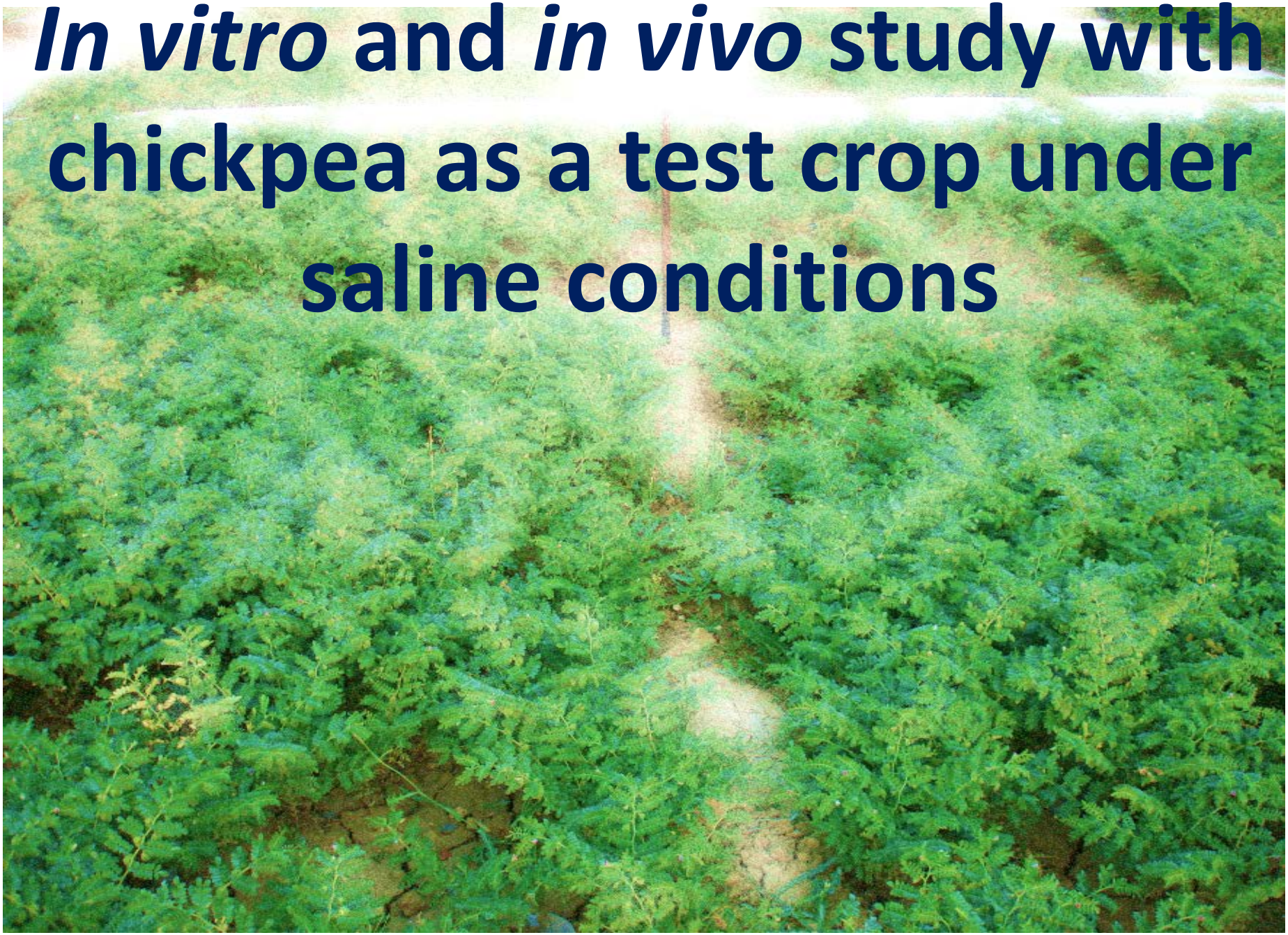
- **Mutant strains showed reduced antagonism and growth promotion under saline conditions**

[1]Khare et al. 2011, Curr Microbiol, vol 62

[2]Tewari and Arora 2014, Curr Microbiol, vol 69

[3]Khare and Arora 2011, Can J Microbiol vol 57

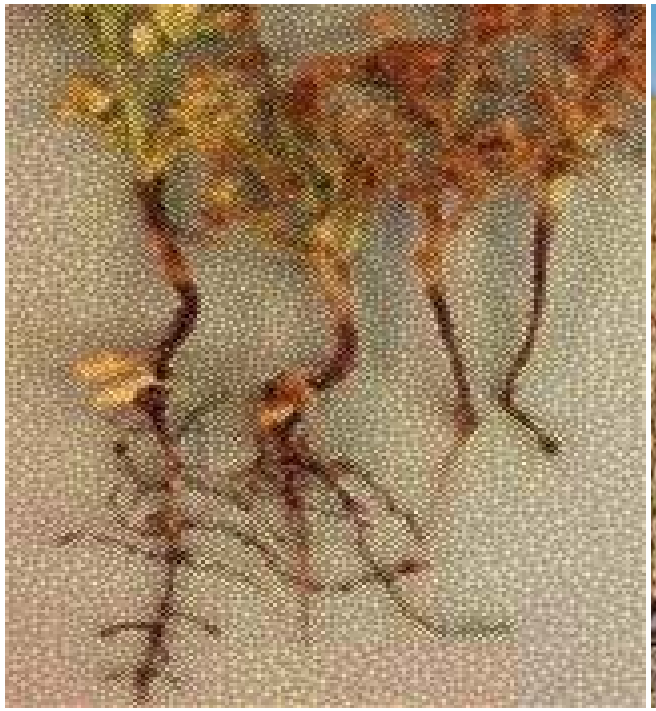
In vitro and *in vivo* study with chickpea as a test crop under saline conditions



Chickpea

- Chickpea is the third most important food legume and second most important pulse crop of the world (Singh, 1997)
- Uttar Pradesh accounts for 6.85% production of chickpea (http://zpdk.org.in/districtprofile)
- It is considered a salt resistant crop and is therefore, mostly grown in arid and semi arid areas where salinity is a common problem (Saxena, 1984; Nitin et al. 2007).
- Annual salt induced losses in chickpea yields are between 8–10% globally (Jan et al. 2013).

Chickpea crop affected



Loss in chickpea yields due to salinity and *M. phaseolina*

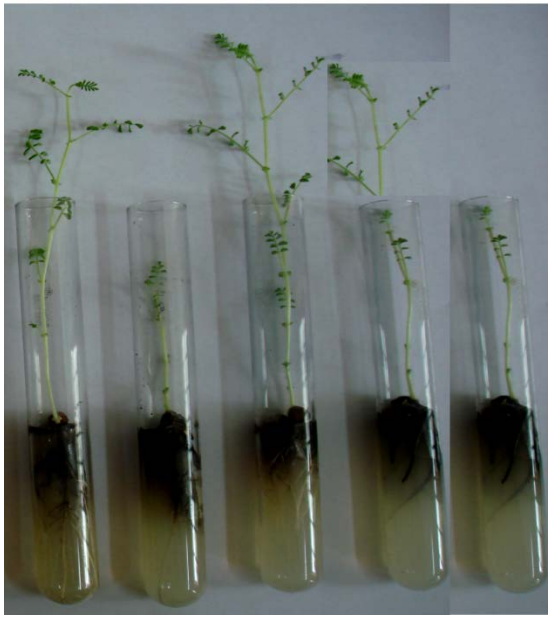
In vitro and *in vivo* study with chickpea under saline conditions

- PGP and charcoal rot suppression ability of strain EK1 was determined taking chickpea as the test crop under *in vitro* and *in vivo* conditions

- The tolerance of the chickpea seedlings to saline stress was determined on water agar plates and germination was recorded up to 150 mM salinity. Hence, further study was conducted at this saline state in tubes and field.

In vitro study taking chickpea

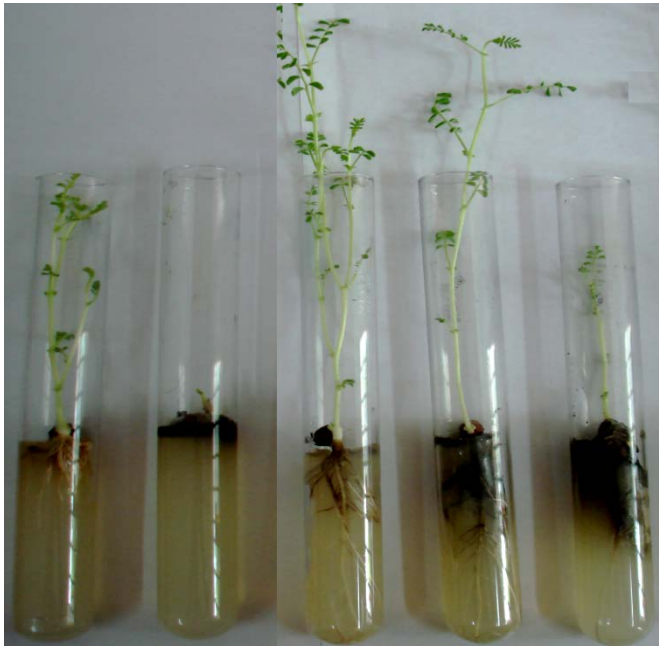
Non- saline conditions (0 mM)



a b c d e

a: control (untreated seeds)
b: *M. phaseolina*
c: EK1
d: EK1 + *M. phaseolina*
e : EK1^{pyo-}

Saline conditions (15O mM)



a b c d e

a: control (untreated seeds)
b: *M. phaseolina*
c: EK1
d: EK1+ *M. phaseolina*
e : EK1^{pyo-}

In field study taking chickpea

- *M. phaseolina* caused 75% and 79% incidence of disease in non-saline and saline conditions. Treatment of seeds with *Pseudomonas* EK1 resulted in 68 and 58% reduction of disease incidence in comparison to non-bacterized control under non- saline and saline conditions respectively.

- In presence of *M. phaseolina*, EK1 caused 50% and 43% enhancement of dry weight and seed yield respectively, in comparison to non-bacterized control under saline conditions.

In field study taking chickpea

- Pyocyanin defective mutant EK1^{pyo-} showed reduced biocontrol and plant growth promoting activity under field conditions

- Results showed that pyocyanin defective mutant was less effective in controlling charcoal rot disease of chickpea (both in saline and non-saline conditions), which supports the theory that microbial pyocyanin could be involved in the suppression of root rot disease in saline conditions [1,2]

[1]Khare et al. 2011, Curr Microbiol, vol 62

[2]Khare and Arora 2011, Can J Microbiol vol 57

Field study chickpea

Control

Treatment

In vitro and *in vivo* study with sunflower under saline conditions

Sunflower

- Sunflower is one of the fastest growing oilseed crop grown in India
- More than 60 % loss in the production of sunflower is due to salinization (Khan 2007)

M. phaseolina causing disease in sunflower

- Unfortunately apart from soil salinity, sunflower is also attacked by variety of fungal pathogens which affect its yield and oil quality

- *M. phaseolina* in salinized regions causes up to 70 % reduction in sunflower oil production (Ijaz et al. 2013)

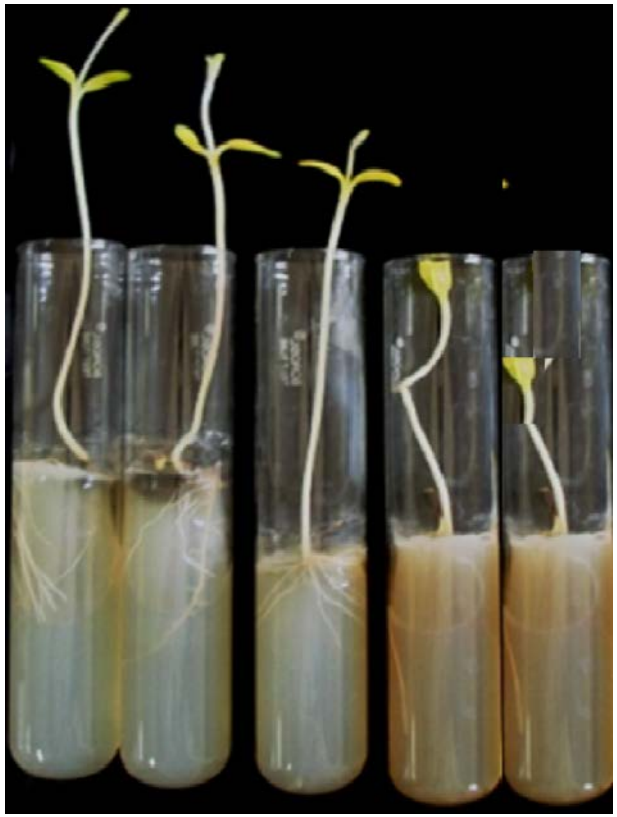
In vitro and *in vivo* study with sunflower under saline conditions

- The study was conducted to detect PGP response and charcoal rot suppression ability of strain PF23 taking sunflower

- The tolerance of the sunflower seedlings to saline stress was determined on water agar plates and germination was recorded up to 180 mM salinity. Hence further study was conducted at this saline state in tubes and fields.

In vitro study taking sunflower as test crop

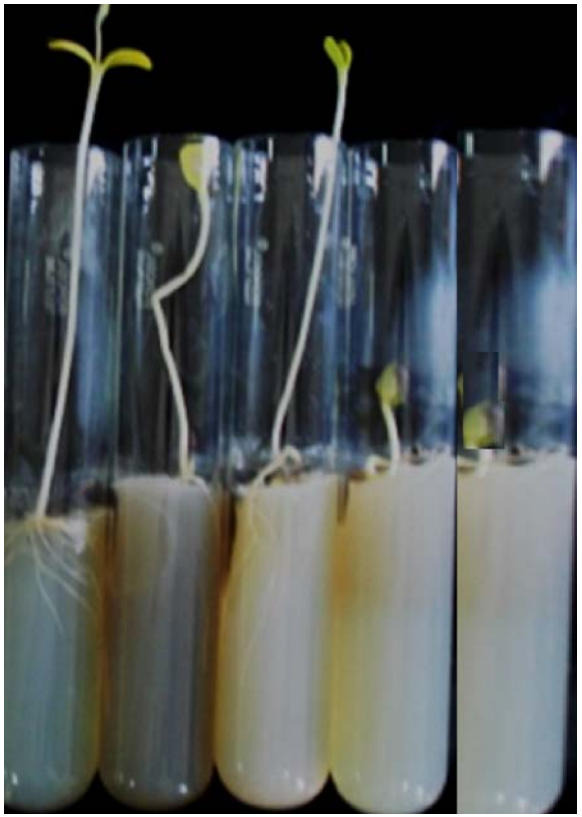
Non-saline conditions (0 mM NaCl)



(a) (b) (c) (d) (e)

(a): seeds + PF23
(b): Untreated seeds
(c) : seeds + PF23 + *M. phaseolina*
(d) : seeds + *M. phaseolina*
(e): seeds + $PF23^{EPS-}$

Saline conditions (180 mM NaCl)



(a) (b) (c) (d) (e)

(a): seeds + PF23
(b): Untreated seeds
(c) : seeds + PF23 + *M. phaseolina*
(d) : seeds + *M. phaseolina*
(e): seeds + $PF23^{EPS-}$

Field Study

Saline Field Where Sunflower Crop was Grown

Field Study

- Treatment of seeds with PF23 brought 25% and 50% increment in germination % in comparison to control under non-saline and saline conditions respectively (2,3)

- Treatment with PF23 brought 79 and 70% reduction of disease incidence in non-saline and saline conditions respectively, in comparison to untreated seeds

- **Treatment of seeds with PF23 brought increment in fresh weight, dry weight and seed yield by 59%, 65%, and 82% respectively, in comparison to control (untreated seeds) under saline conditions**

[2]Tewari and Arora 2014, Curr Microbiol, vol 69

[3]Tewari and Arora 2014, Cell Mol Bio, vol 60

In Field Study

- EPS-defective strain PF23^{EPS-} brought reduction of disease incidence by only 33% under non-saline conditions

- However, the mutant was ineffective in controlling the pathogen and enhancing plant growth parameters under saline conditions

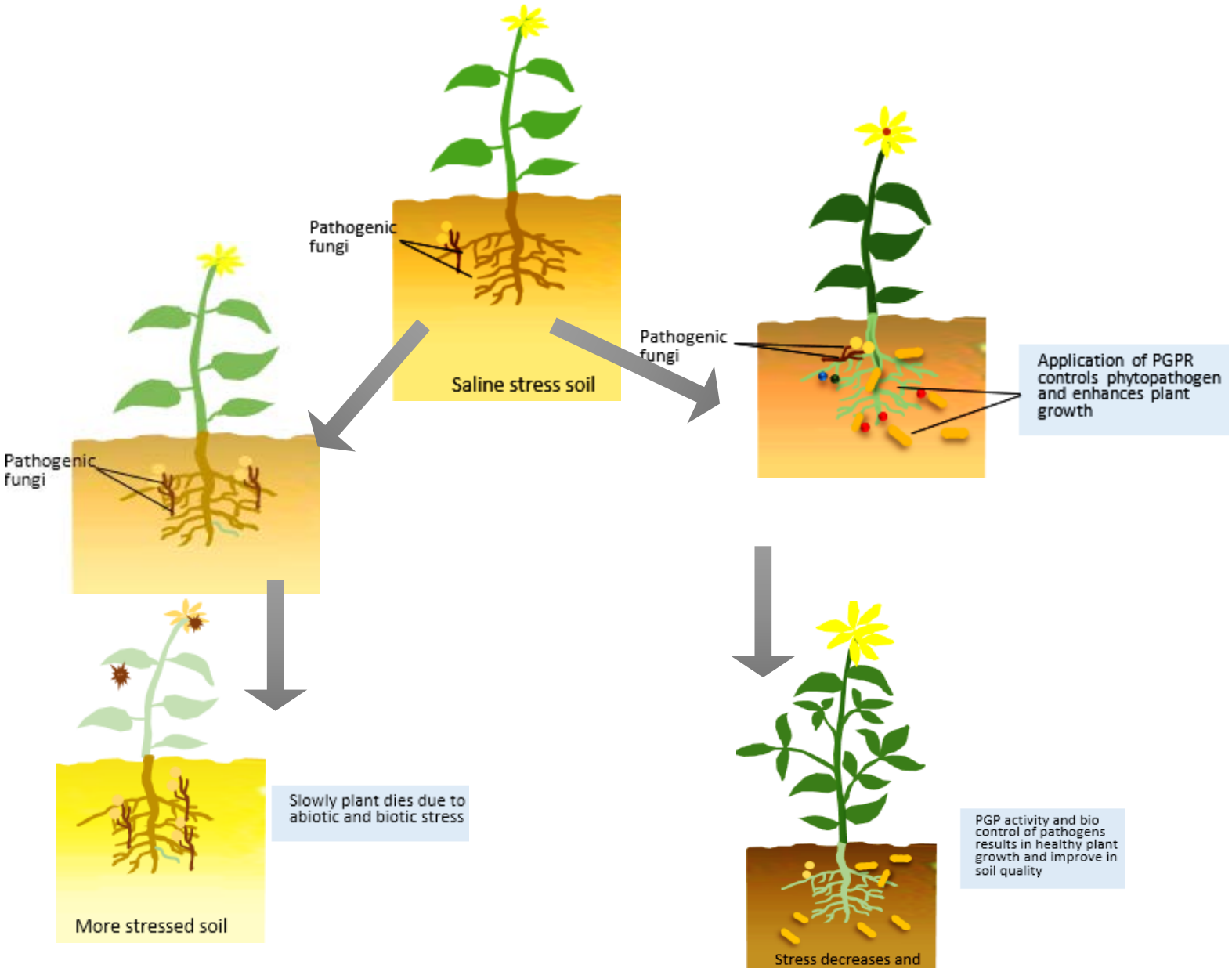
Field trials in salinized regions

Arid soil (180 mM salinity)

Untreated

Bacterial treatment

Effect of PGPR on plant growth and soil quality



Conclusion

- Results suggest the potency and efficacy of utilizing multi-stress tolerating PGPR bacteria **EK1 and PF23 in reclamation of arid and semiarid regions**

- While EK1^{Pyo-} showed drastic reduction in capacity to control *M. phaseolina* in saline condition, PF23^{EPS-} isolate lost the ability to promote the plant growth and protect the plant in saline conditions, suggesting the roles of both the metabolites

In presence of abiotic (high salinity) and biotic (phytopathogen) stresses EK1 and PF23 effectively enhanced growth and production of chickpea and sunflower [1,2,3,4]

- Strain EK1 and PF23 can be considered as commercially important microbes for renovation of stressed sites. Such strains can be used in reclamation of arid and semiarid regions, realizing **enhanced food production and food security**

- Microbial inoculation to alleviate stresses in plants could be a more cost effective and environmental friendly option to enhance crop yields under stress conditions

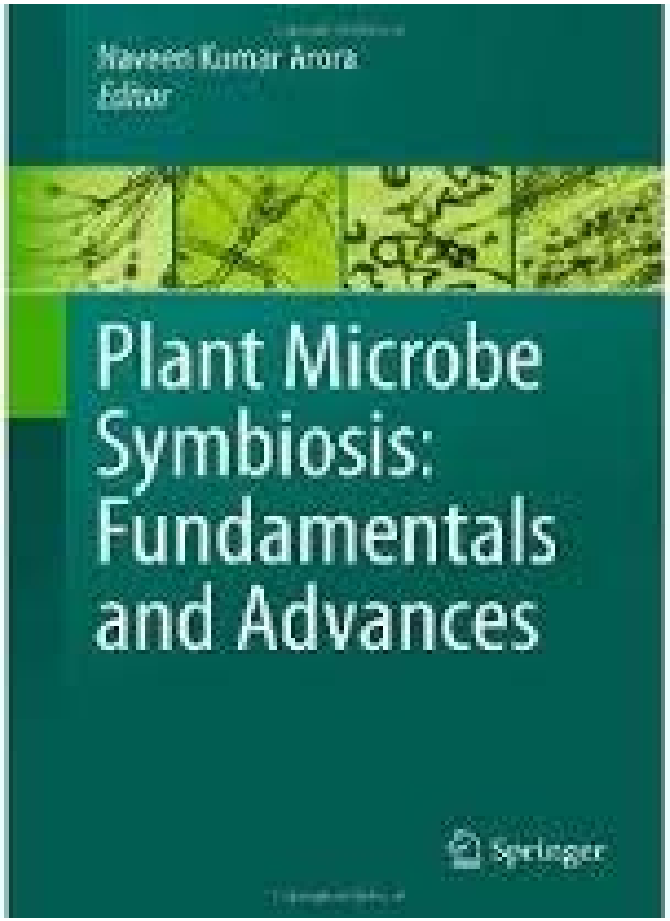
[1]Khare et al. 2011, Curr Microbiol, vol 62

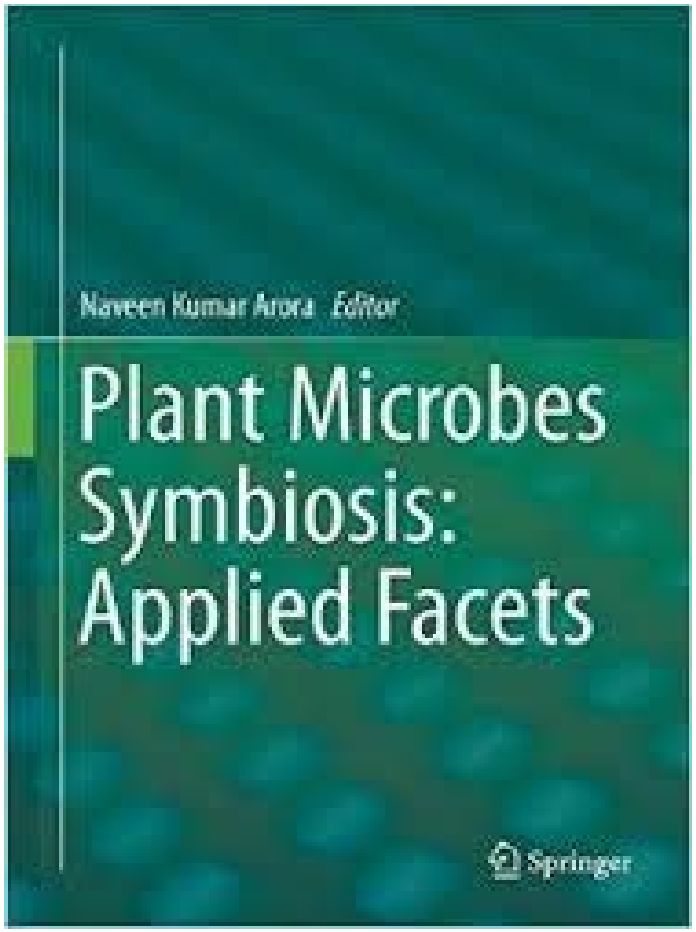
[2]Tewari and Arora 2014, Curr Microbiol, vol 69

[3]Khare and Arora 2011, Can J Microbiol vol 57

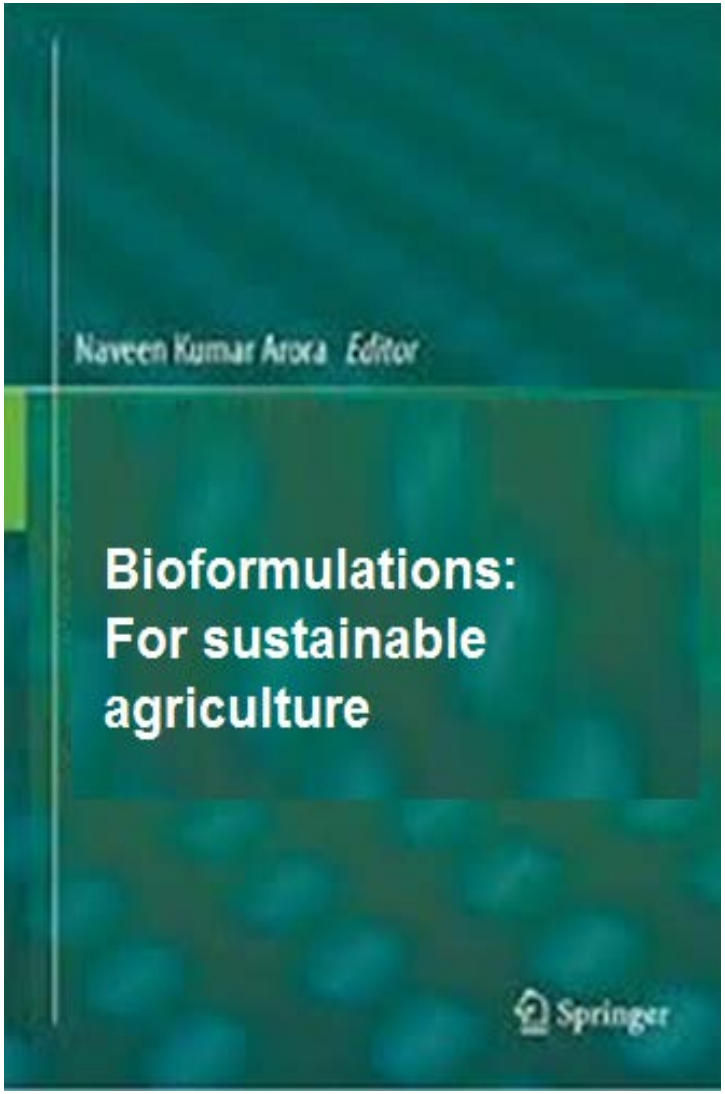
[4]Tewari and Arora 2014, Cell Mol Bio, vol 60

Books Published





Forthcoming



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THANKS