Characterization and cross inoculation studies of *rhizobia* isolated from crop wild relatives of *Vigna*

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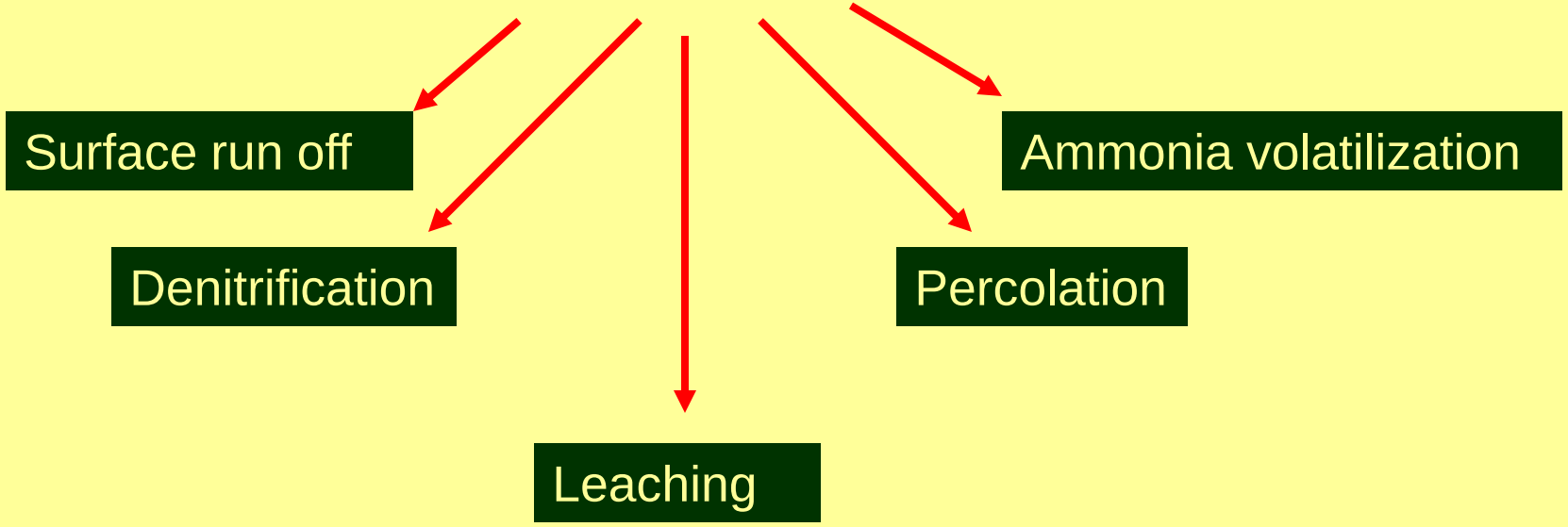
INTRODUCTION

❖Importance of **N**

- N - a limiting nutrient for crop production because it easily lost through



•By adding nitrogen fertilizer (nitrates, ammonia, urea etc.) 25%

soil environment may be enriched in N

•Non biological N fixation (storms bring 10 to 15 kg of nitrogen per ha to the soil every year) - 15%

•Through biological fixation (by free fixing agents , symbiotic fixing agents, cyanobacteria) - 60%

BIOLOGICAL N_2 FIXATION

Major contribution – Symbiotic Systems

Rhizobium – Legume associations

50% - all N fixed on Earth

Rhizobia

- Facultative microsymbionts
- Rod shaped
- 1.2-3.0 μm in length 0.5 - 0.9
- Gram negative
- Non spore forming
- Fix N_2 only when symbiotic
- Aerobic
- Optimal growth conditions : T^0 25^0 - 30^0C ;pH 6 - 7

Important of rhizobia in wild relatives of V*igna*

- The successful exploitation of the *rhizobium*-legume symbiosis requires the presence of appropriate strains in the soil.

- *Rhizobia* from the wild relatives of crop legumes are a natural, that has a tremendous potential to offset the use of expensive N-fertilizers

Important of the rhizobial inoculants

Unlike chemical N-fertilizers, rhizobial Inoculants **:**

- **are cheap**
- **improve soil fertility by recycling of additional nitrogen obtained from the fixation**
- **improve crop productivity**
- **replace the mineral N-fertilizer use for grain legumes**
- **do not promote weed growth- reduce labour cost and/or use of herbicides for weed control**
- **do not promote pollution of soil and water with nitrate.**

CWR Project

A project was aimed to study and explore *rhizobia* from wild relatives of *Vigna* for the crop improvement of *Vigna* group of crops species - (*Vigna radiata* [green gram],*Vigna mungo* [black gram] and *Vigna unguiculata* [cowpea] for crop improvement programmes.

Research Plan

1. Field Survey
2. Nodule Collection
3. Nodule Characterization
4. Isolation and Purification of *rhizobia*
5. Characterization of stock cultures
6. Authentication
7. Pot Experiments
8. Field Experiments

Wild relatives of *Vigna* and their distribution in Sri Lanka

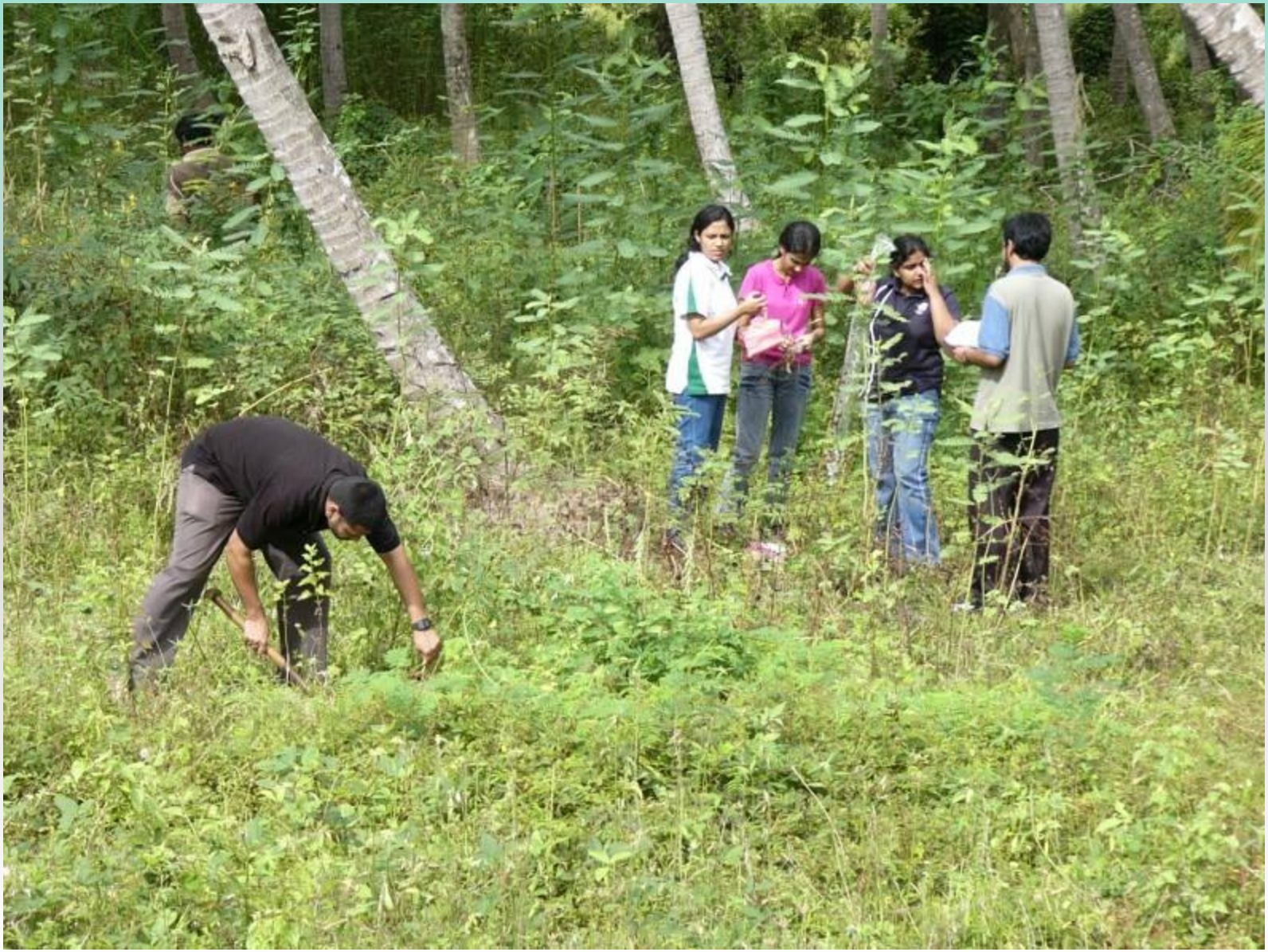
Species	Location
Vigna aridicola	Baticalow,Mhiyangana,Polonnaruwa,Yala
Vigna dalzelliana	Badulla,Ramboda,Walimada
Vigna radiata, var. sublobata	Mahiyangana
Vigna stipulacea	Humbanthota,Puttalama,Yala
Vina trilobata	Ampara,Chalow,Mountlawaniya,Puttalama Yala
Vigna trinervia	Hakgala,Haliela,Pusallawa,Ramboda, Badulla,Udapussallawa
Vigna marina	Hikkaduwa

Tomooka et al., 2003

Germplasm of Rhizobial isolates from wild relatives of Vigna

Host plant	Code for Isolates	Location
Vigna aridicola	Vr1	Polonnaruwa
Vigna dalzelliana	Vd1	24/7Paradeka/Gampola- Nuwaraeliya
Vigna dalzelliana	Vd2	24/7Paradeka/Gampola- Nuwaraeliya
Vigna dalzelliana	Vd3	34/2Helboda/Gampola- Nuwaraeliya
Vigna dalzelliana	Vd4	Kotmale-Talawakelle
Vigna dalzelliana	Vd5	Hunnasgiriya
Vigna marina	Vma1	Hikkaduwa
Vigna minima	Vmi	Kirinda
Vigna radiata var sublobata	Vrs1	Kitulhitiyaya
Vigna radiata var sublobata	Vrs2	Mhiyangana
Vigna stipulacea	Vst1	Tissa-kirinda
Vigna stipulacea	Vst2	Tissa-kirinda
Vigna stipulacea	Vst3	Kirinda
Vigna radiata var sublobata	Vsu1	Nalanda
Vigna trilobata	Vtril1	34/2Helboda/Gampola- nuwaraeliya
Vigna trinervia	Vtrin1	Ramboda
Vigna trinervia	Vtrin2	Mhiyangana
Vigna trinervia	Vtrin3	Mhiyangana
Vigna trinervia	Vtrin4	24/7Paradeka/Gampola- nuwaraeliya
Vigna wild 1	Vsp1	Putlum -krunagala
Vigna wild 2	Vsp2	Laksapihilla
Vigna wild 3	Vsp3	Kotmale-Talawakelle

1.Field Survey



Vigna marina at Hikkaduwa

Vigna dalzelliana at Hunnasgiriya

Vigna stipulacea at Thissamaharama

Vigna trinervia at mahiyangana

V.stipulaceae and*V. (minima)* growing together in Kirinda

V.aridicola

Arachis pintoi

Pueraria sp.

***Sesbania* sp.**

***Phaseolus* sp.**

2.Nodule collection

3.Characterization of nodules

Vigna unguiculata **(Semiglobose)** ***Vigna aridicola***

Vigna mungo

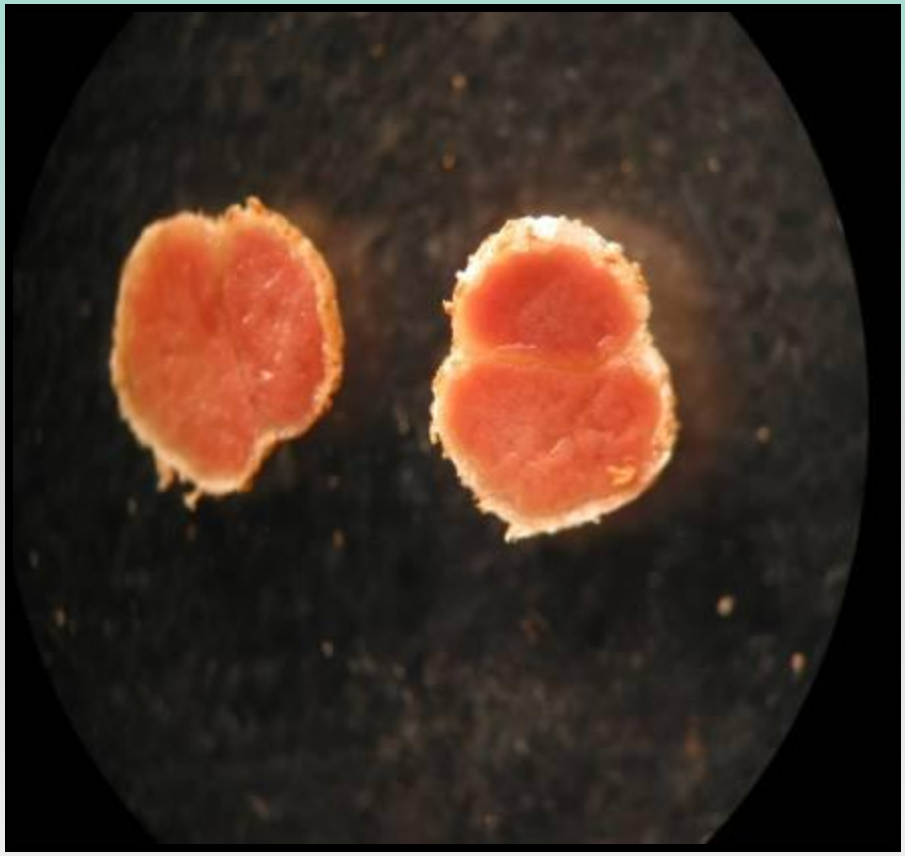
Vigna radiata

Vigna dalzelliana

Vigna* t*rinervia


L 38
(1)

Internal appearance of the nodules



T.S. of a active nodules

4.Isolation and Purification of *rhizobia*

5.Characterization of Stock Culture

Streaking on CRYMA

- All were well grown in to white gummy colonies with in 3 days.

Streaked lines

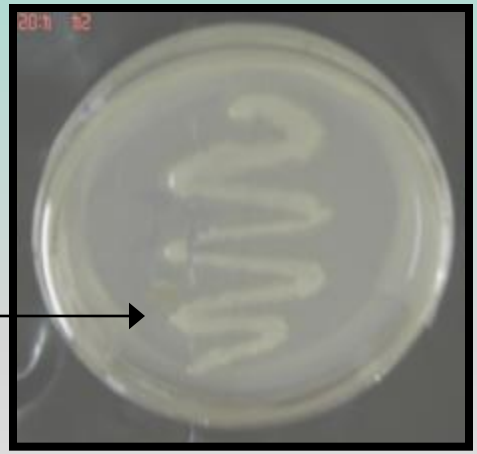
Red coloured CRYMA medium

Ex. A CRYMA plate of V S

Multiplication and Storage

Grown on YMA

White gummy colonies on **YMA**



Stored on Agar slants

($YMA + CaCo_3$)

Cold Storage (long time)

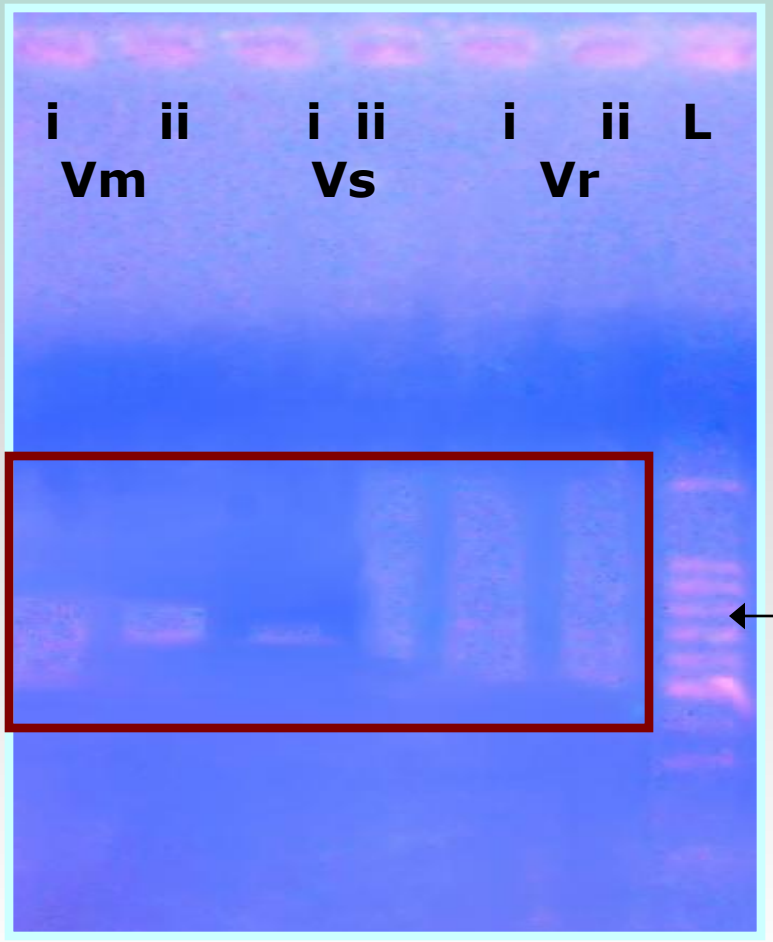
Stock cultures of different isolates

YMB medium, Different strains

Rotary shaker.

Molecular Characterization

Conformation of the Presence of
16 s rDNA IGS Sequence



Wells

Bands Around 700 bp.

100 bp ladder

6. Authentication of the *Rhizobium* isolates

Experimental set-up:

- **Free draining pot method**
- **CRD was used.**

Phase I:Pot experiments

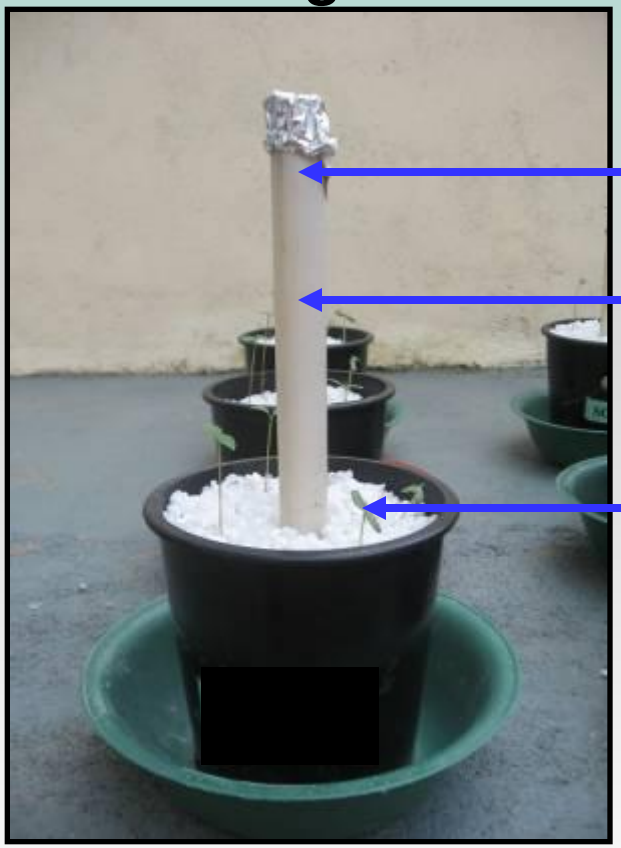
- Pot experiments were conducted to test the infectivity of isolated Rhizobia strains from the wild *Vigna* species.
- It was highly promising that the isolates performed well and produced several healthy nodules

Pot setup just after sowing the seeds

Root inoculation (after 3 days)

Inoculation

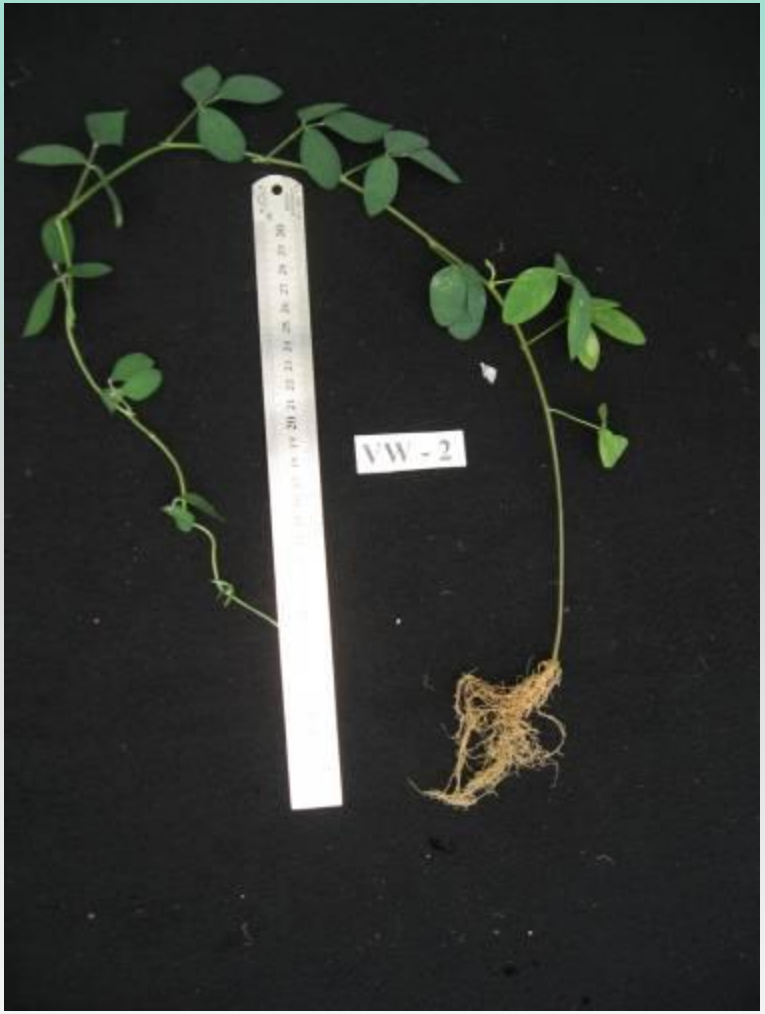
- Colonies were washed with 1% sucrose solution
- Inoculated with 1ml of the inoculum
- Covered with sterilized gravel



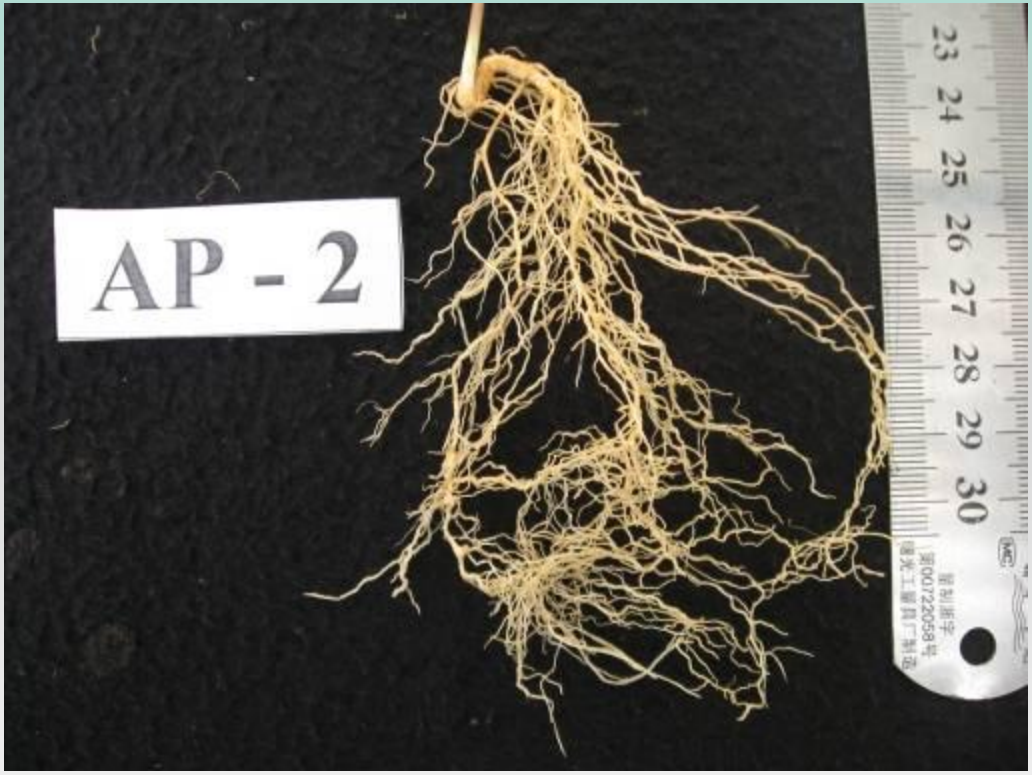
Pot after inoculation

N+ control / Effective / Ineffective / N- control

Effective strain



Ineffective strain



Phase II: Field experiments

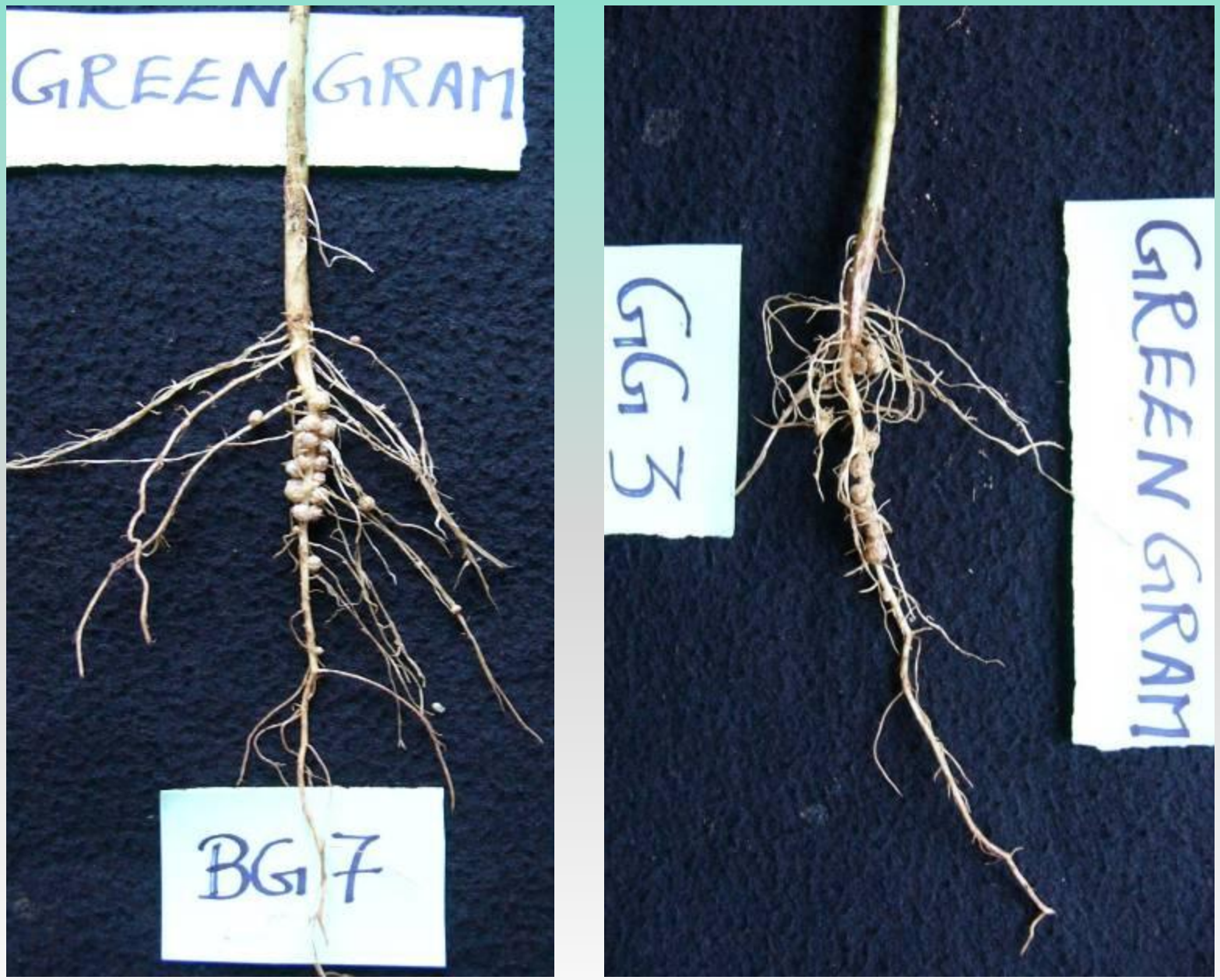
Preparation of field for Cross Inouclation Studies

RCBD plot design for field experiments

Greengram and Blackgram plots inoculated with different rhizobial isolates

Greengram inoculated with isolate GG8 showed luxurious vegetative growth compared to adjoining plots

Fig.10 Blackgram inoculated with isolate GG8 showed luxurious vegetative growth compared to adjoining plots



Greengram plants inoculated with BG7 and GG3 showing large and healthy nodules – vital character for effective N fixation

Blackgram plants inoculated with GG3 and GG8 showing nodules

mature plants filled with healthy pods

Harvesting

Note: *the white ribbons hanged over the filed to threaten birds and protect yield. White fence for protecting plants from wild animals.*

Effective rhizobial isolates obtained ferom CWR

Code number	Host plant	Collected Location
Group1		
CWR1(GG3)	*Vigna radiata* var.*sublobata*	Kakirawa
CWR2(GG8)	*V.radiata* var.*sublobata*	Nalanda
CWR3(GG12)	*V.trilobata*	Puttalm
Group2		
CWR4 (BG3)	*Macroptelium* sp.	Meegalawa
CWR5(BG4)	*Vigna dalzelliana*	Peradeniya
CWR6(BG5)	*V.radiata* var.*sublobata*	Kekirawa
CWR7(BG7)	*V.trilobata*	Puttalm
CWR8(BG8)	*V.trilobata*	Puttalm
CWR9(BG11)	*V. dalzelliana*	Peradeniya

Disscussion

- Rhizobial isolates CWR1, CWR2 and CWR3 (group 1) showed better nodulation on both green gram and black gram plants.

- Although the nodule number was significantly lower with CWR1 at 6^{th} week on both crops, it has significantly increased at 10^{th} week.

  (This result has realized that slow growth rate of this isolate in the colonization after the inoculation and later infection has improved the nodule number.)

- All the isolates (CWR1, CWR2 and CWR3) showed high plant dry matter production than that of the N fertilizer application in both crops but it was significantly higher in black gram (Fig 1).

Plant dry matter (group I)

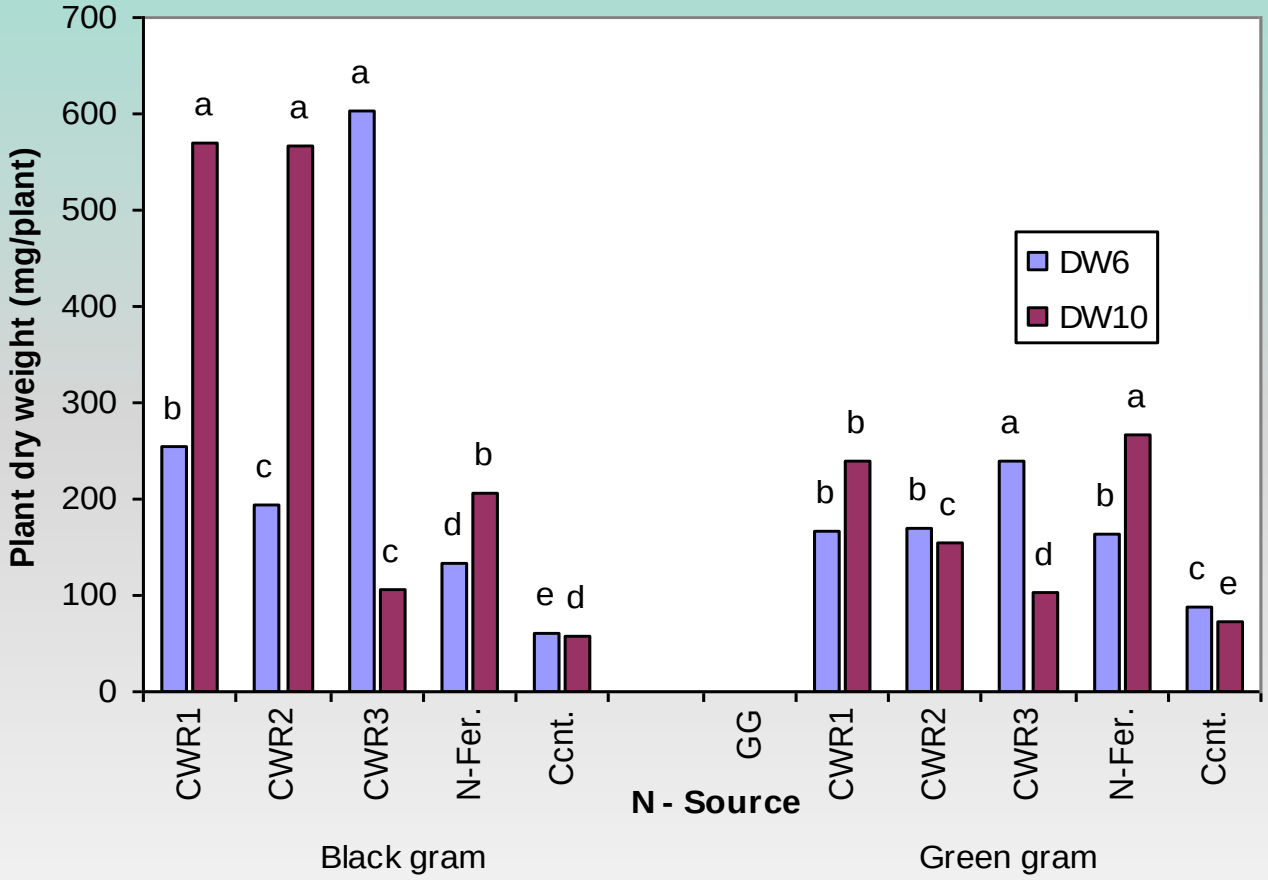


Figure 1. ***Plant dry matter production of Black gram and Green gram with the inoculation of the isolates of group 1, N fertilizer application and the control***

- In group 2, nodule formation was observed with the strains CWR7, CWR8 and CWR9.

- Out of these isolates CWR7 showed significantly highest nodulation and increased the plant dry matter production with both Green gram and Black gram at 6 and 10 weeks after the planting (Fig 2).

- According to the results CWR7 is an effective isolate and has the potential to use as rhizobial inoculants both in Green gram and Black gram.

Plant dry matter (group II)

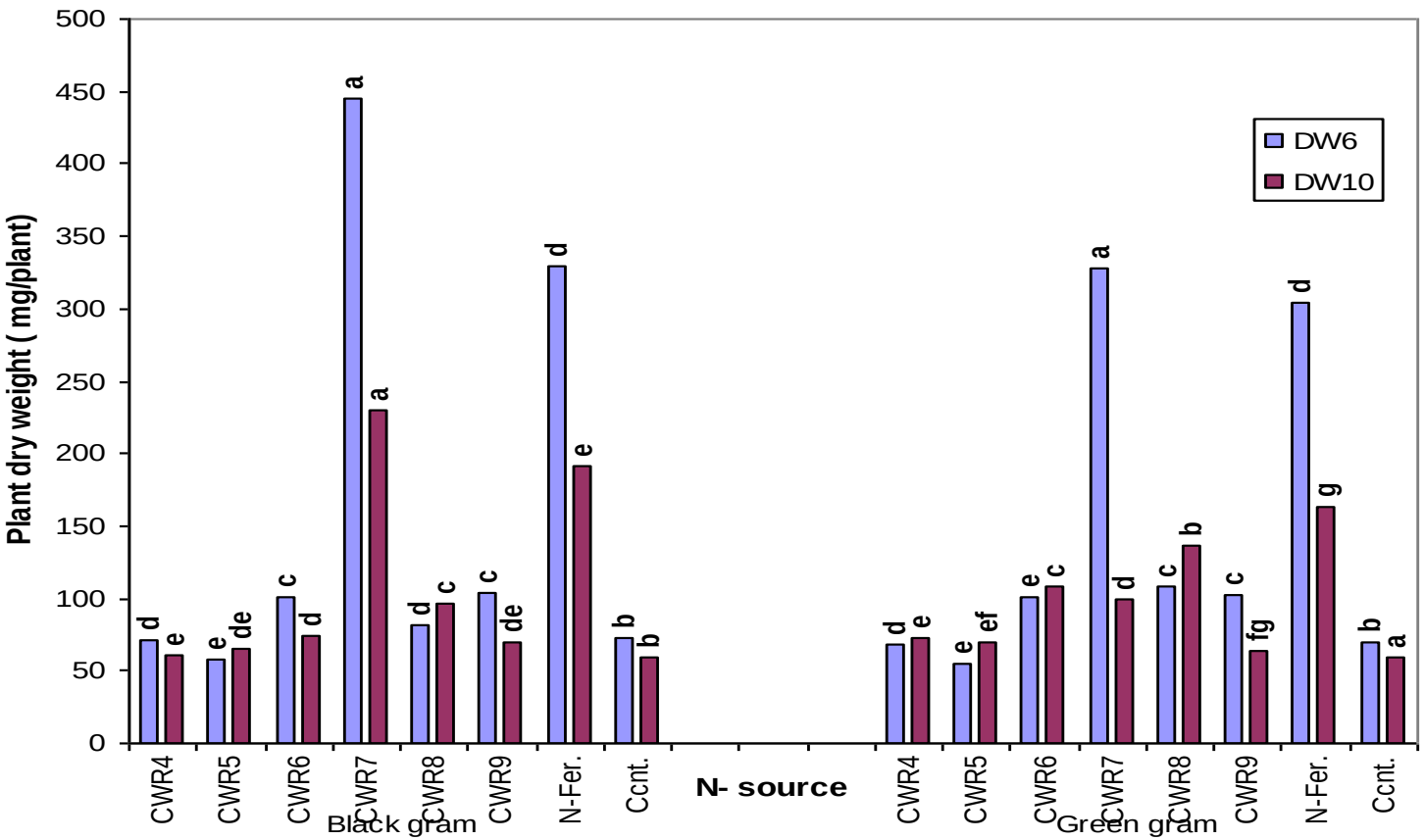
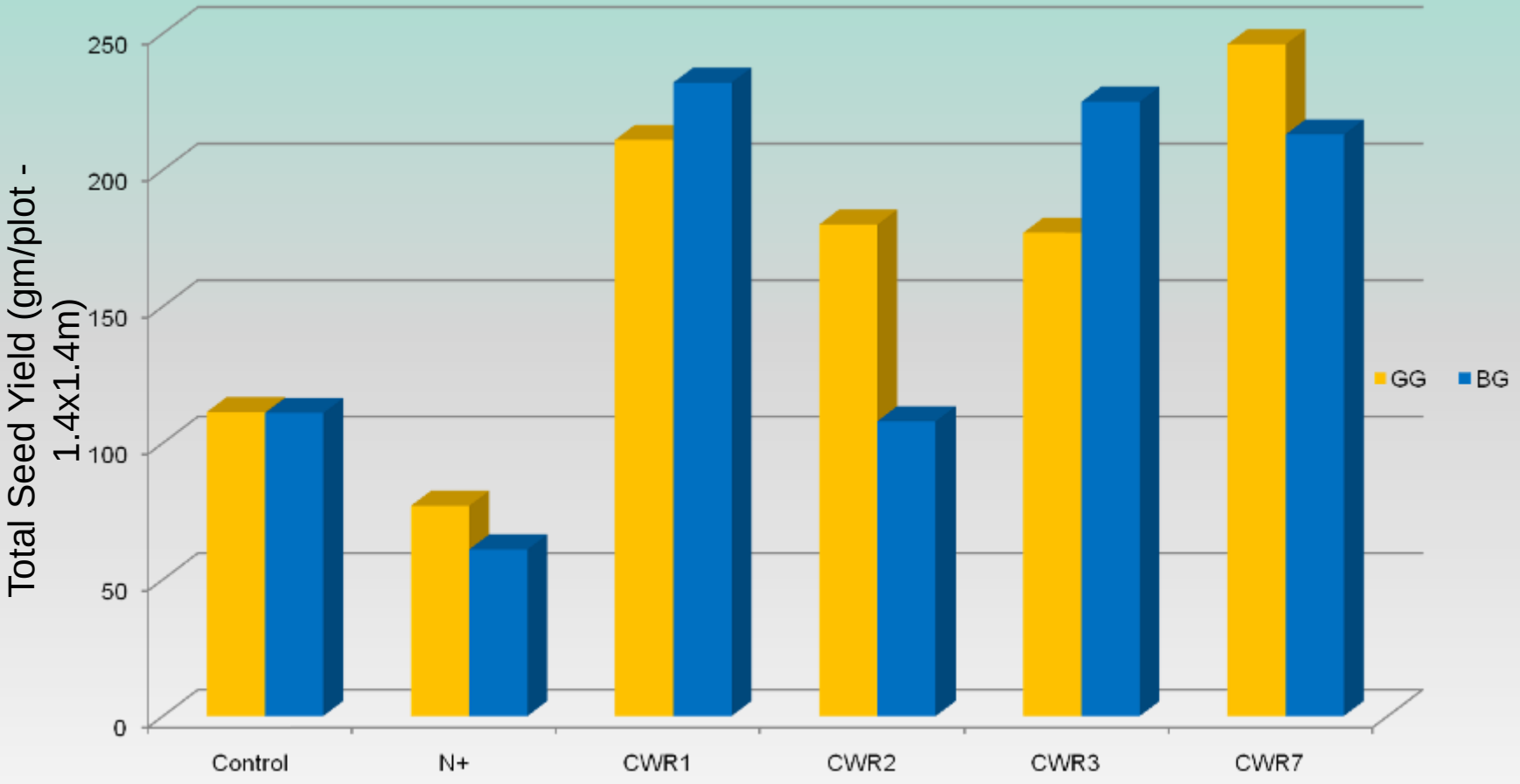


Figure 2. ***Plant dry matter production of Black gram and Green gram with the inoculation of the isolates of group 2, N fertilizer application and the control***

Seed yield comparison in Black and Green gram crops inoculated with CWR rizobial isolates



Conclusion

- The results revealed that the isolates have high infectivity and the effectivity which are important parameters to use as inoculants for crop legumes.

- The results has proven that the *rhizobia* isolated from crop wild relatives of *Vigna* has an ability to form nodule on their cross inoculating group of edible grain legumes such as green gram and black gram.

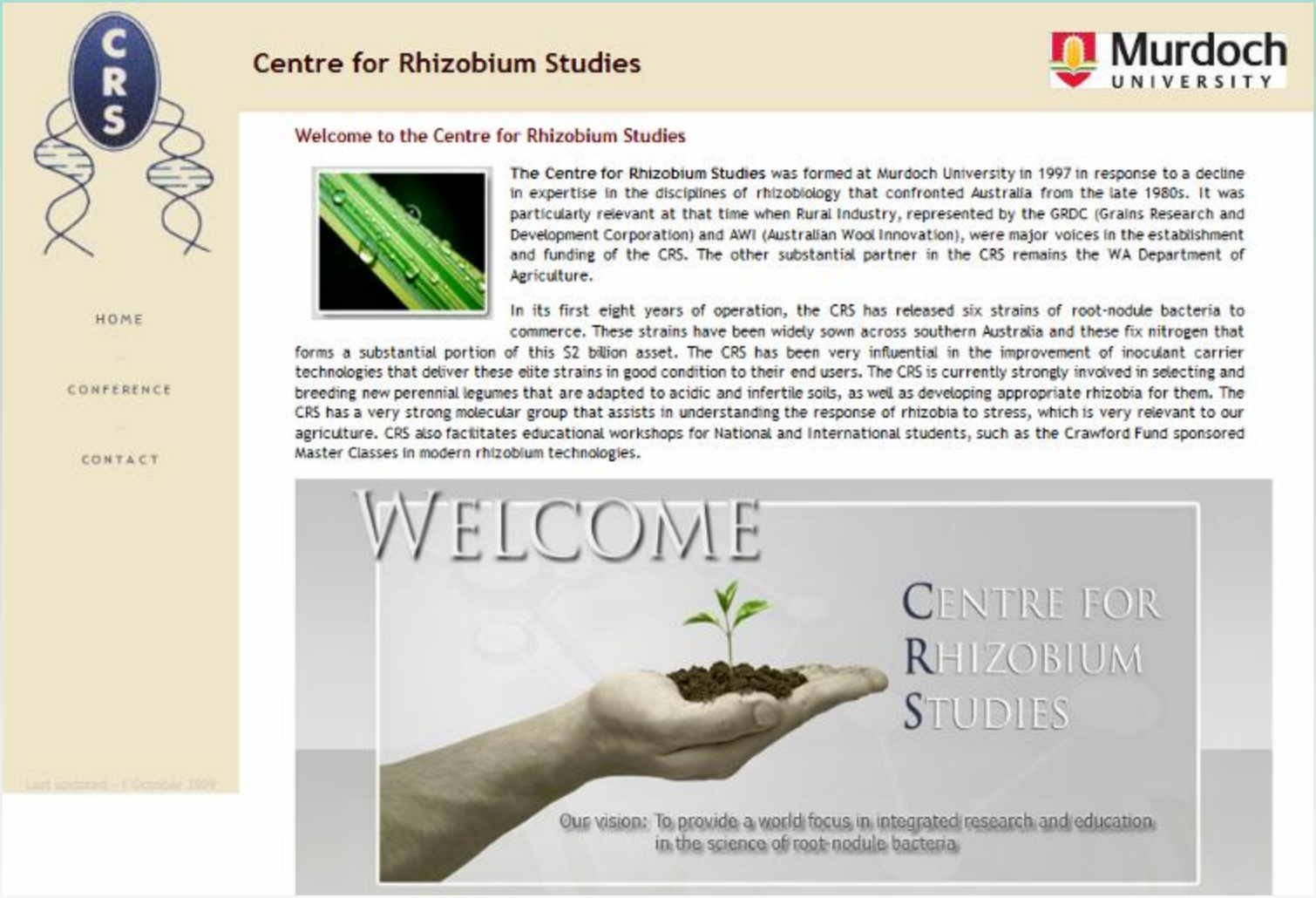
Conclusion (cont.)

- Isolates CWR1, CWR2, CWR3 and CWR7 are well adapted and successfully overcome the competition among native rhizobia in the experimental conditions.

- Finally, these strains have the potential to replace the recommended N-fertilizer by using inoculants produced by these isolates.

- **Future studies: Adaptive trials at Farmers field in different localities.**

Collaboration



Dissemination

Acknowledgement

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Thank You!