Identification of genetic sources of improved responses to nitrogen fixing rhizobacteria and other plant growth promoting microbes (Planned deliverables #2)

Activity 1: Symbiotic nitrogen fixation (SNF) in chickpea- I

Title	**:**	Studies on (G_L x G_R) x E x M for N_2-fixation in chickpea
Objectives	**:**	Determination of the best combination of variety x rhizobia x fertilization for SNF Effectiveness of strains on N_2-fixation under different growing conditions
Activities	**:**	New
Expected outcomes	**:**	Factor(s) limiting SNF in different production environments identified Agronomic practices for enhancing SNF developed
Observations to be taken	**:**	Crop phenology Growth and yield attributes Yields and harvest index Nodulation Efficiency SNF NDVI, SPAD and hyperspectral imaging
Genotypes	**:**	Arifi; Moubarak; FLIP09-213C; FLIP09-314C
Results	**:**	The experiment was conducted at ICARDA-Marchouch, Morocco during winter and spring 2015-16. Application of recommended dose of fertilizer (RDF) in chickpea along with Rhizobium inoculation and seed treatment with ammonium molybdate 1 g/kg of seed, significantly increased number and dry weight of nodule, plant height and pods/plant, chlorophyll content in leaves over control (RDF alone).

Activity 2: Symbiotic nitrogen fixation (SNF) in chickpea-II

Title	**:**	Improving biological nitrogen fixation (BNF) capacity and productivity of kabuli chickpea (*Cicer kabulinum* L.) varieties by molybdenum and PSB applications
Objectives	**:**	Determine the effect of PSB and Molybdenum on growth, yield and quality of Kabuli chickpea varieties Study the effect of PSB and Molybdenum on root nodulation behavior and SNF efficiency in Kabuli chickpea varieties Work out the economics of the systems
Activities	**:**	New
Expected outcomes	**:**	Legume-rhizobium symbiosis for SNF determined Agronomic practices for enhancing SNF developed
Observations to be taken	**:**	Crop phenology Growth and yield attributes Yields and harvest index Plant and soil nutrient content Nodulation

		Efficiency SNF Chlorophyll content Economics
Genotypes	**:**	RVSJKG 102; Phule G 0517; PKV 4
Results	**:**	The trial was conducted at ICARDA-Amlaha, India during winter 2015-16. The seed inoculants with Rhizobium + PSB + Mo was found best among other inoculants in respect to productivity and profitability. Variety PKV 4 produced higher values of growth and yield attributing parameters, seed and biological yields of kabuli chickpea (Table 1 and 2). Treatment combination Rhizobium + PSB+ Mo with Phule G 0517 on seed index proved better combinations for higher production.

Table 1: Yield attributing traits influenced by inoculants and varieties.

Treatments	Pods/ plant (No.)	Seeds/pod (No.)	Seed yield/plan t (g.)	Seed index (g)
Seed inoculants (I)				
I1 : Control	26.7	1.06	17.5	46
I2 : Molybdenum Seed inoculants	29.6	1.07	17.9	48.4
I3 : *Rhizobium* + PSB Seed inoculants	32	1.07	18.4	48.9
I4 : *Rhizobium*+ PSB + Mo seed Inoculants	34.2	1.08	19.9	51.1
SEm±	0.5	0.003	0.50	1.03
CD @ 5%	1.4	0.009	1.5	3.01
Varieties (V)				
V1 : RVSJKG 102	29.8	1.06	17.17	55.4
V2 : Phule G 0517	30.5	1.07	17.88	54.7
V3 : PKV 4	31.5	1.08	20.18	35.8
SEm±	0.4	0.003	0.43	0.9
CD @ 5%	1.2	0.008	1.3	2.6

Table 2: Response of seed inoculant and variety on seed yield kg/ha, straw yield kg/ha and harvest index (%).

Treatments	Seed yield (kg/ha)	Biological yield (kg/ha)	Harvest index (%)
Seed inoculants (I)			
I1 : Control	1253	3490	35.9
I2 : Molybdenum Seed inoculants	1402	4037	35.1
I3 : *Rhizobium* + PSB Seed inoculants	1599	4315	37.2
I4 : *Rhizobium*+ PSB + Mo seed Inoculants	1878	5224	36.1
SEm±	43	152	0.7
CD @ 5%	125	445	NS
Varieties (V)			

		V1 : RVSJKG 102	1435	3895	37.0
		V2 : Phule G 0517	1539	4321	35.9
		V3 : PKV 4	1625	4584	35.4
		SEm±	37	131	0.6
		CD @ 5%	108	385	NS
Thesis	**:**	Master thesis entitled "Improving biological nitrogen fixation capacity and productivity of kabuli chickpea (*Cicer kabulinium* L.) varieties by PSB and molybdenum applications" submitted to Jawaharlal Nehru Agricultural University, Jabalpur, India by Mr. Rahul Badole.			

Activity 3: Effect of herbicides on Rhizobium Nodulation in faba bean

Title	**:**	Evaluation of the effect of herbicides on rhizobium nodulation in faba bean
Objectives	**:**	Evaluate the effect of imazethapyr and metribuzin
Activities	**:**	New
Observations to be taken	**:**	Number of nodules per plants Fresh weigh of nodules per plant Dry weight of nodules per plant
Genotypes	**:**	15 genotypes
Expected Outcomes	**:**	Effect of herbicides on rhizobium nodulation evaluated.
Results		15 faba bean genotypes with different tolerance level for both Metribuzin and Imazethapyr were evaluated at Terbol station for rhizobium nodulation in alpha design with two replication and three treatments (T1: Metribuzin, T2, Imazethapyr, T3: combined Metribuzin and Imazethapyr) and control. Analysis of variance showed significant differences among treatments for number rhizobium nodulation, wet and dry weight of rhizobium nodulation. Also significant differences among genotypes for number of rhizobium nodulation has been detected (Table 1). **a. Number of Nodules per Plant:** Number of nodules per plant varied significantly among evaluated genotypes (G) and among the three treatments (T) and control (C) (Table 1). It varied between 5.56 to 37.75 among genotypes. 11 genotypes were considered to have a less number of nodules per plant between 5.56 and 11.95, 3 genotypes were considered to have an intermediate number of nodules per plant (13.42 and 13.66) and 1 genotype was considered to have a high number of nodules per plant equal to 37.5 (Fig. 1). The accessions IG100096 collected from Morocco showed high nodulation.

The control had the highest number of nodules per plant: 16.66, followed by treatment 1 (Metribuzin): 11.24, then treatment 2 (Imazethapyr): 8.21 and last of all treatment 3 (Metribuzin and Imazethapyr): 6.77. (Table 2)

b. Fresh Nodule Weight per Plant:

Fresh nodule weight per plant varied significantly among the three treatments (T) and control (C). The control had the highest fresh nodule weight per plant: 0.51 g, followed by treatment 1 (Metribuzin): 0.23 g, then treatment 2 (Imazethapyr): 0.21 and last of all treatment 3 (Metribuzin and Imazethapyr): 0.19. (Table 2)

c. Dry Nodule Weight per Plant (DNWP):

Dry nodule weight per plant varied significantly among the three treatments (T) and the control (C). The control had the highest dry nodule weight per plant: 0.15 g, followed by treatment 3 (Metribuzin and Imazethapyr): 0.086 g, then treatment 1 (Metribuzin): 0.081 g, and last of all treatment 2 (Imazethapyr): 0.06. **(Table 2)**

d. Conclusion

The application of Metribuzin and Imazethapyr herbicides as post-emergence treatments in faba bean reduced the root nodulation for most of evaluated genotypes and consequently the nitrogen uptake from the soils.

Table 1: ANOVA table for Dry Nodule Weight per Plant (DNWP), Fresh Nodule Weight per Plant (FNWP) and Number of Nodules per Plant (NNP),

	DNWP	FNWP	NNP
Replication	0.001	0.07	6.53
Replication x Bloc	0.016*	0.09 ***	295.28 ***
Genotype (G)	0.002	0.01	196.22 *
Treatment (T)	0.041 **	0.60 ***	571.47 ***
G x T	0.008	0.03	81.65
Residual	0.008	0.03	78.49
Total	0.01	0.05	135.78

*** Highly significant at $p < 0.001$, ** Significant at $p < 0.01$, * Significant at $p < 0.05$.

Table 2: means of rhizobium in different treatments and in the control.

Treatment	DNWP	FNWP	NNP

Herbicide 1: Metribuzin	0.08	0.24	11.24
Herbicide 2: Imazethapyr	0.06	0.21	8.21
Herbicide 3: Metribuzin and Imazethapyr	0.09	0.20	6.77
Control	0.15	0.51	16.66
LSD at 0.05	0.05	0.09	4.72

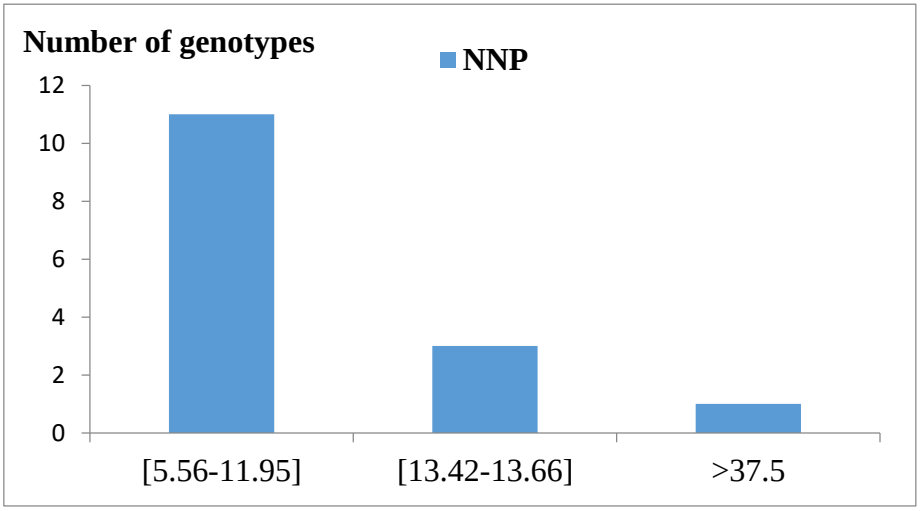
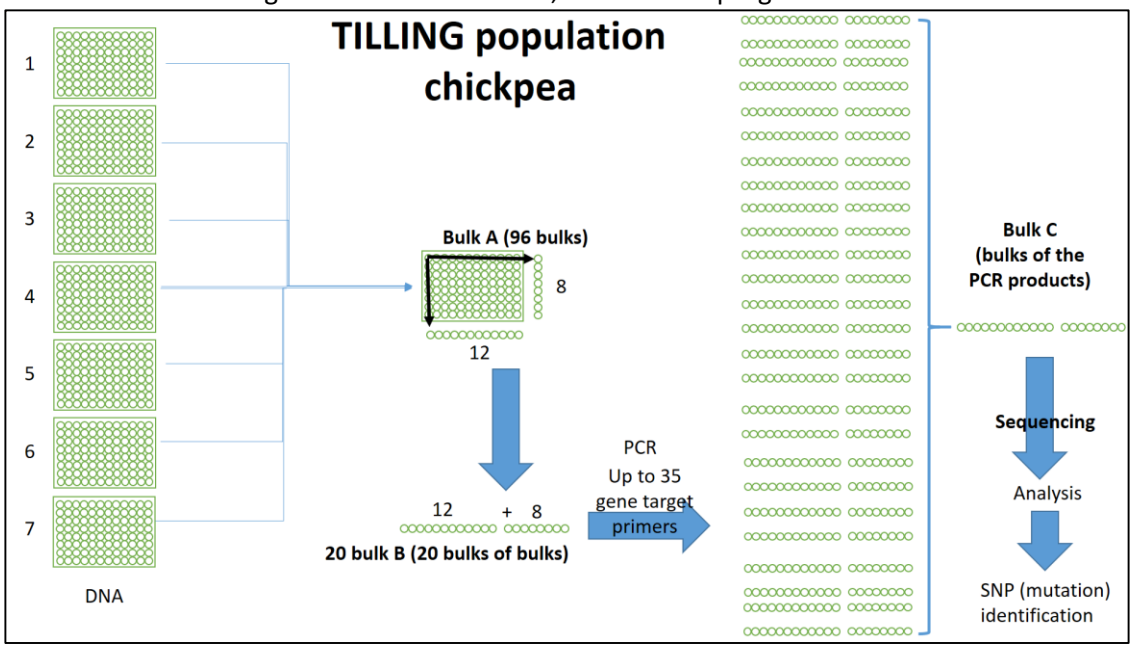


Figure1: Variation of the number of faba bean genotypes according to the number of nodules per plant (NNP).

Activity 4: TILLING mutants reveal hyper-nodulation in chickpea

Title	**:**	Identification of hyper nodulation mutant lines in chickpea using TiLLING population
Objectives		Identify new source of hyper nodulated line in chickpea.
Activity		New
Expected outcomes		New source of genotype (mutant line) for hyper-nodulation in chickpea
Method of evaluation		For genotyping the following steps were performed for 1300 TiLLING lines: 1. Fresh leaf tissue was collected from 1300 mutant lines 2. DNA was extracted. 3. DNA quality and quantity was measured by using NanoDrop, and test agarose gels 4. DNA concentration was fixed and bulked in a systematic structure 5. PCR was conducted to for 43 primer pairs to cover 8 genes reported as genes related to hyper-nodulation in legumes. 6. Samples were sent then to Australia for genotyping. Genotyping was conducted by the following steps: 1. Libraries were prepared and run on Miseq 2. Data was trimmed for adaptors and primer sequences

		3. Denovo assembly was generated from control sample. 4. Trimmed reads from all other samples were reference aligned to the denovo reference generated from control sample
Genotype		1300 TiLLING population lines
Results		Two mutations have been identified in 2 different bulks, but only one mutant line (548) have been detected with high nodulation. However, this is still in progress to confirm this mutation. 

Activity 5: Drought/nodulation interaction in chickpea

Title	**:**	**Chickpea nodulation response to drought in different locations.**
Objectives	**:**	Identify the best genotypes performing the nodulations under drought stress
Activities	**:**	New
Expected outcomes	**:**	New genotypes to be identified producing nodules under drought stress under natural inoculation.
Observations to be taken	**:**	Size, wet and dry weight of nodules
Genotypes	**:**	200 chickpea subset (drought) selected by using FIGS
Results:		Five genotypes (IG114795, IG70270, IG70278, IG70293, IG70764) showed better nodulation than the average under drought stress and non-stress environments