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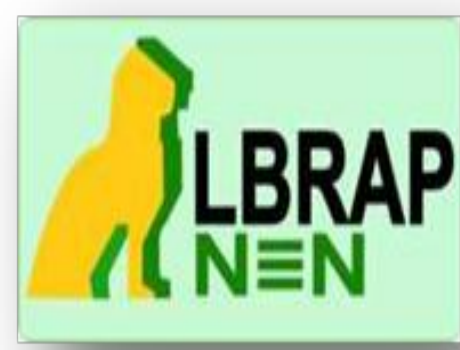
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# Rhizosphere symbiots valorisation: Common bean-rhizobia symbiosis adaptation to P deficiency in Ain Temouchent agro-ecosystem in Algeria

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## I. Introduction

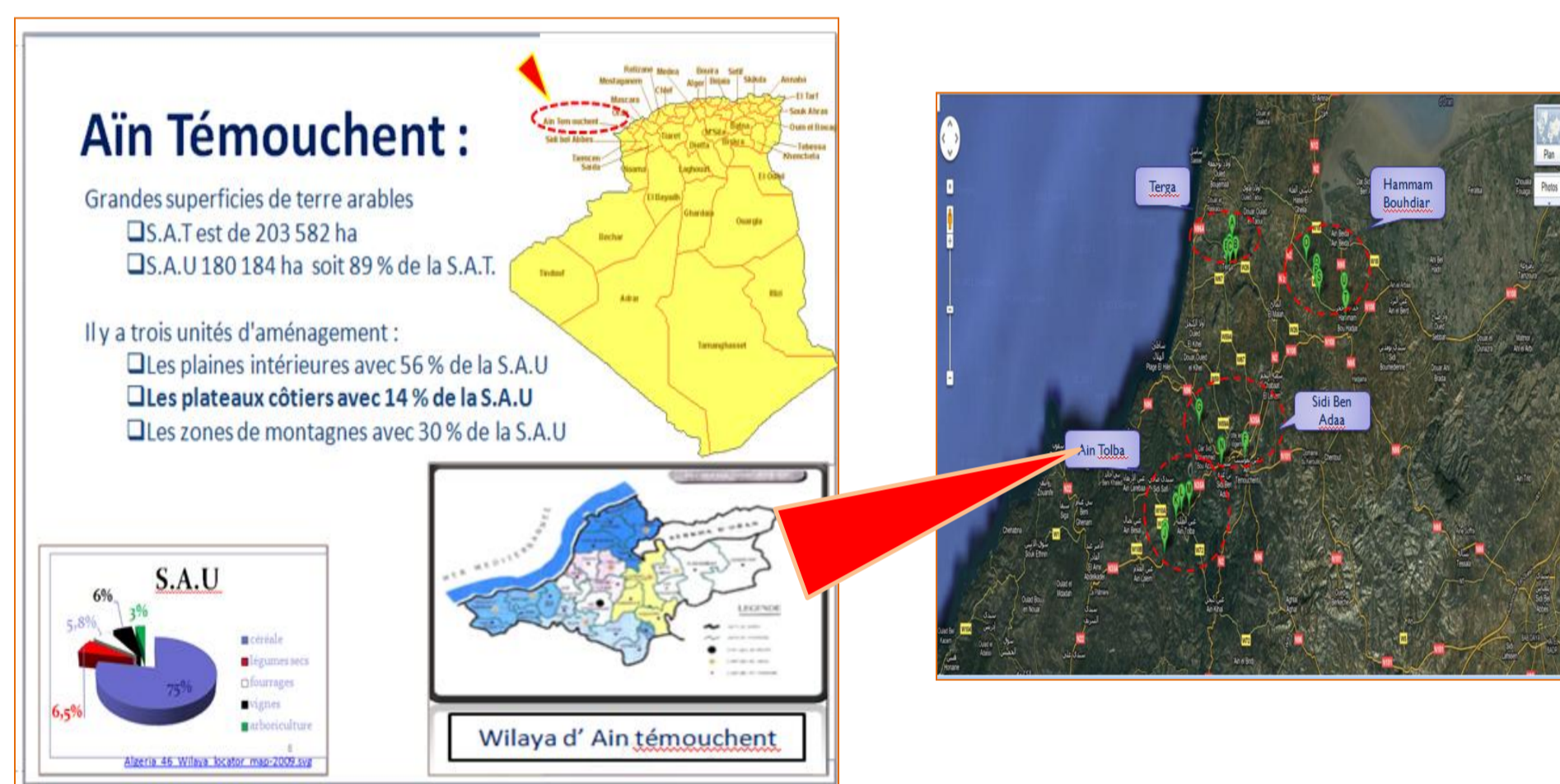
Leguminous have a high environmental, dietary and socio-economic importance especially for the African countries. Despite of their interest, during these last years, their culture is decreasing caused by biotic and abiotic stresses: Temperature variations, impairment of Mediterranean soils in minerales especially phosphorus. The ability of a symbiotic association with rhizobia allows the biological nitrogen fixation resulting can be exploited to improve plant growth and fertility soil. The inoculation by rhizobia plays an important role in improving and increasing the potential of fixing the atmospheric nitrogen through increasing number and weight of nodules. In this context that is our study whose objective is to select an efficient rhizobia and bean genotypes to improve growth and production of this very important beans and adapt it to address constraints in particular soil phosphorus deficiency.

The biological nitrogen fixation in symbiosis established with bean-rhizobia and nodule formation provides annually to the plant and soil an amount of nitrogen equivalent to chemically synthesized in the fertilizer industry (Oliveira *et al*, 1998). Inside Common beans are often considered as a poor nitrogen fixer (ISOI and Yoshida, 1991). A study conducted by CIAT has also shown that 60% of cultivated soils beans are deficient in P (vadez, 1996).Witch are our challenges in this work : Improve symbiotic potential of *Phaseolus vulgaris* in adverse conditions and Increase its adaptation to infertile soils with Reasoned Inoculation that is study of natives rhizobia from selected agroecosystem to preservate of biodiversity and agroecosystem functions.

## II. Material & Methodologies

Six recombinant inbred lines, namely RILs: (CIAT) and one common bean variety widely cultivated in Algeria. Lines 115, 104 and 75 have been characterized as P-efficient whereas 147, 83 and 29 have been categorized as P-inefficient based on plant growth and seed yield in relation to the availability of P. This RILs was sowing both *in vitro* trapping and *in natura* multilocus test for 20 plots from Ain Temouchent agroecosystem chose in northwest of Algeria. After 45 days after transplanting the nodules are collected, according to macroscopically aspect 40 strains was selected to PCR-RFLP analysis amplified 16S rDNA genes. Total DNA was extracted as Laguerre (1992) described. Aliquots of PCR products were digested with restriction endonucleases. The following enzymes were used: *Msp* I and *Nde* II. All strains were tested in glass house at hydroponic cultural condition. The preparation begins before sowing, by sterilization of the seeds (Tajini *et al*, 2009 & Bargaz *et al*, 2010). After, seeds were cultivated in bottles 1<sup>-1</sup> warped with aluminum foil to maintain the rooting darkness condition, and contain the same nutriment solution changed weekly, (vadez, 1996 & Adelson *et al*, 2008).

### 1 Agroecosystem selected



the wilaya of Ain temouchent by his large areas of arable is considered as a reference region for the culture of f legumes despite decreasing its performance in the last year because the farmers unknown sustainable agriculture strategy and don't use the biofertilizer, to another hand the climate instability have a major impact.

In this Agroecosytem , 20 plots disperse in Four regions how has a same microclimat are selected, . The 8 Rils sowing ,iat the flowering stage the nodules are recovered .

### 2 Strains collection

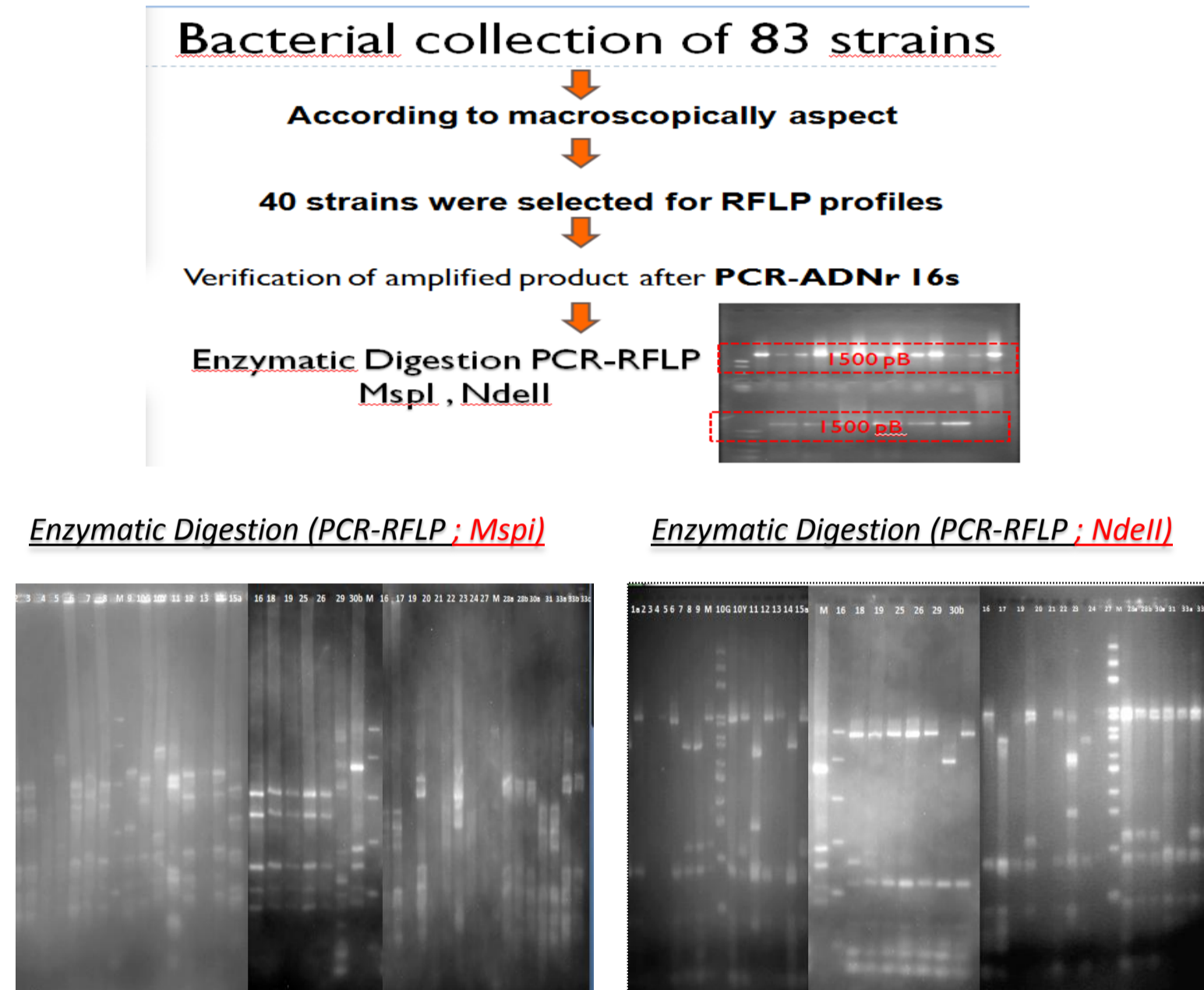
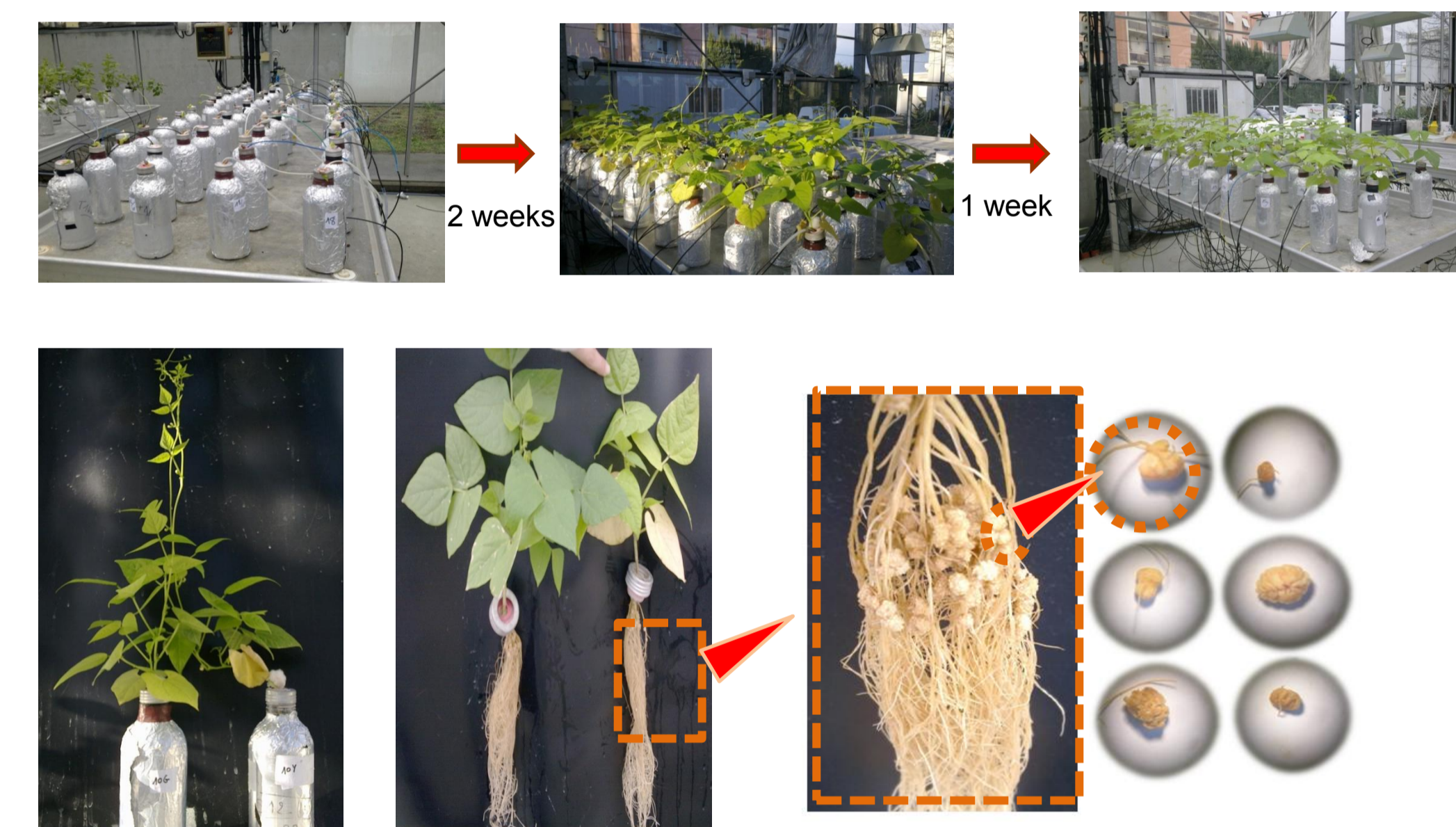


Fig. 2. Profil obtained after a PCR-RFLP by *Msp*I enzym restriction.

Fig. 3. Profil obtained after a PCR-RFLP by *Nde*II enzym restriction.

### 3 Test Nod



The inoculum was prepared *in vitro* in liquid YEM medium for 72 hours with stirring to obtain cellular density of 10<sup>8</sup>. The inoculation was as follows proceeded: At sowing ,seeds are emerged in the inoculum for 15 min and 1 ml was also added to the nutritive solution. After 5 days nutritive solution is inoculated with 5 ml.

## V. Result & Discussion

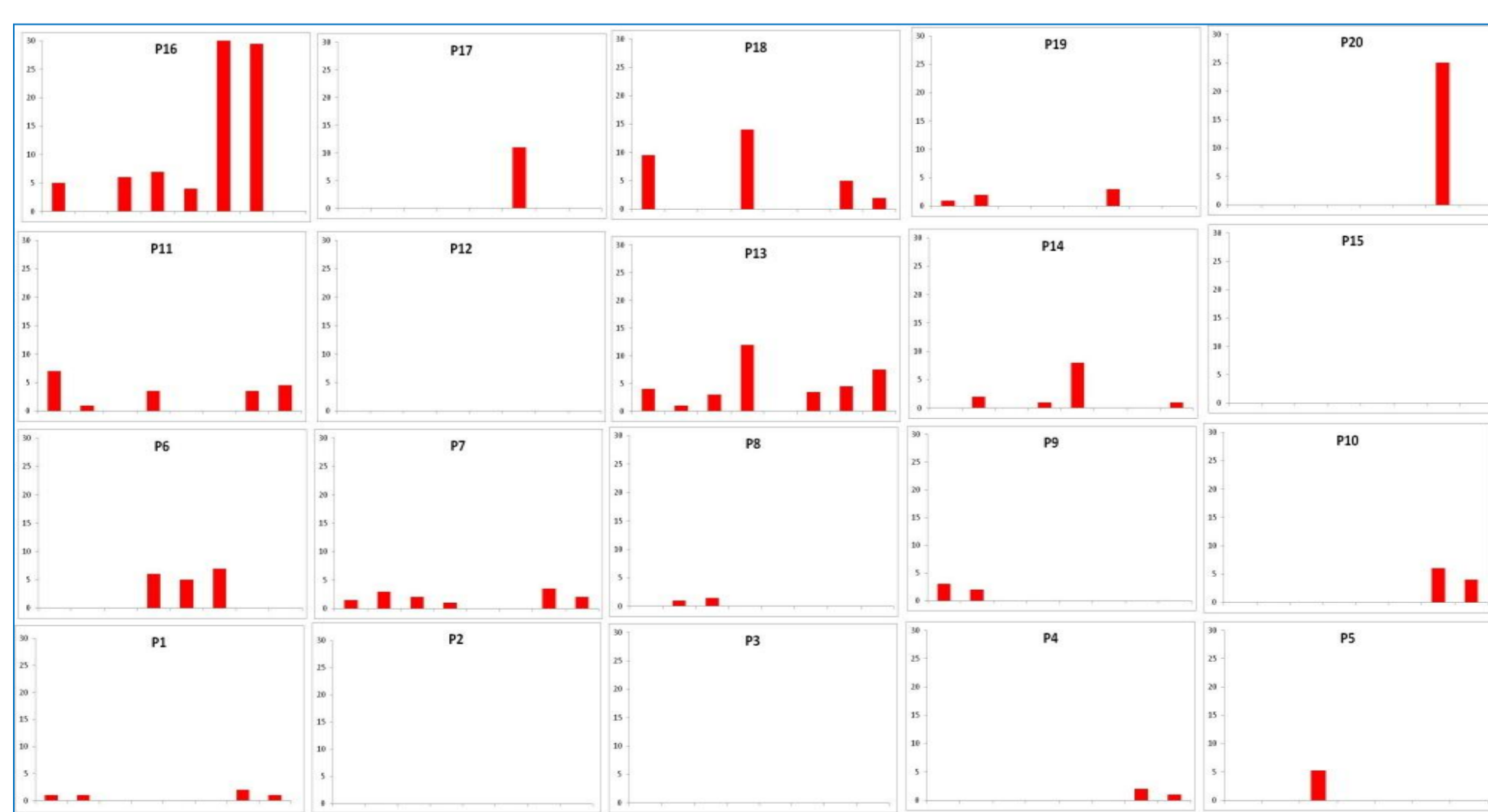
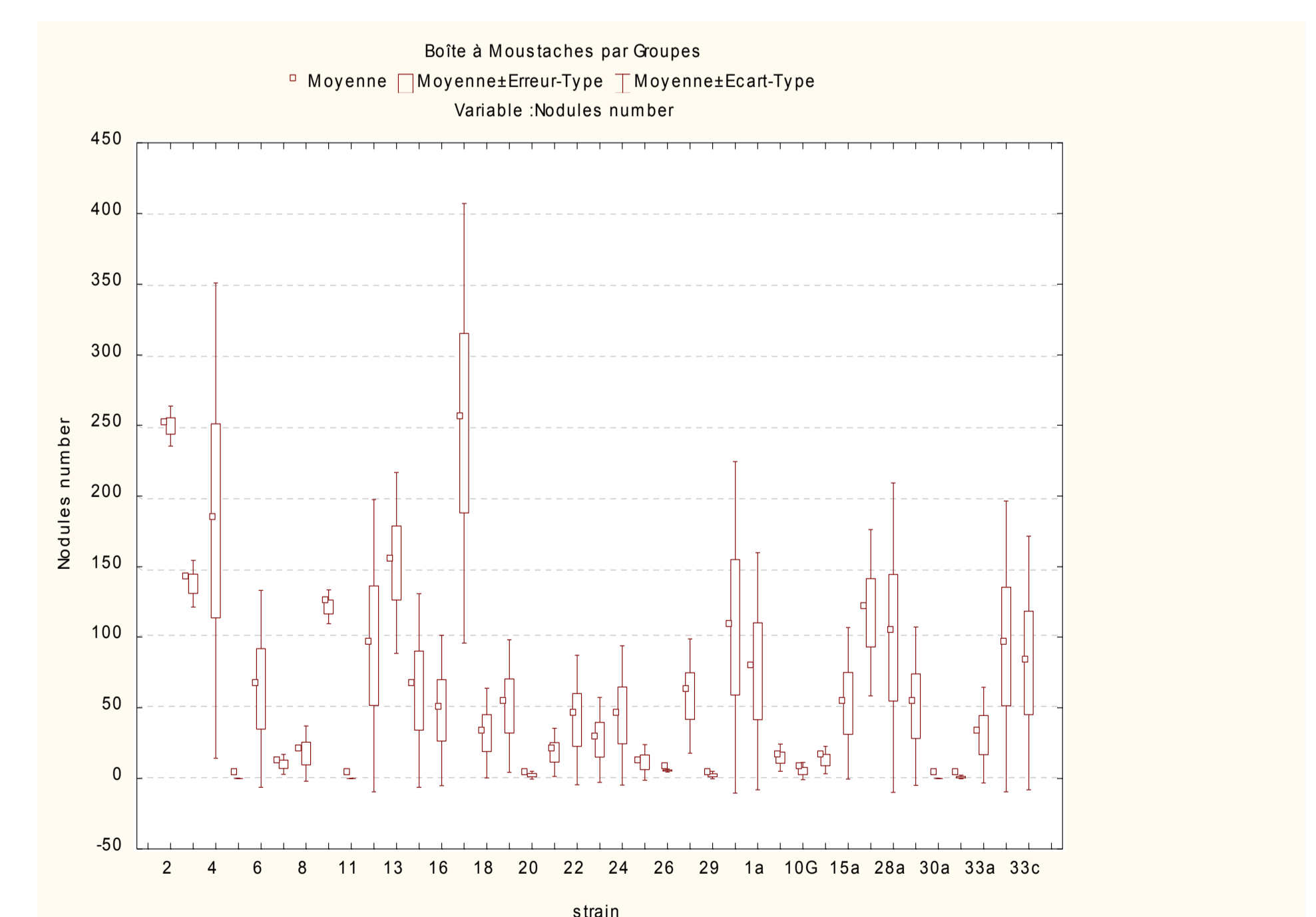


Fig. 3. Data are means of five replicates of nodules number after 60 days after transplanting *in natura* for 20 plots from AinTemouchent agroecosystem

Strains	plots	Rils	Control RIL	Affiliation
1a	P2	104	115	R.leguminosarum
1b	P2	104	115	///
2	P6	115	115	R.etli
3	P3	29	115	R.gallicum
4	P3	83	115	///
5	P3	loc	115	Agrobacterium
6	P4	83	115	R.gallicum
7	P6	104	115	R.leguminosarum
8	P2	104	115	R.leguminosarum
9	P6	115	115	R.loti/ciceri
10G	P3	loc	115	///
10Y	P3	loc	115	Agrobacterium
11	P7	loc	115	///
12	P4	106	115	R.gallicum
13	P8	83	115	R.gallicum
14	P2	34	115	R.etli
15a	P20	loc	115	R.loti/ciceri
15b	P20	loc	115	///
16	P18	loc	115	R.gallicum
17	P12	115	115	R.etli
18	P2	34	115	R.gallicum
19	P8	83	115	R.gallicum
20	P3	loc	115	Agrobacterium
21	P18	loc	115	///
22	P2	115	115	///
23	P2	104	115	///
24	P12	115	115	R.etli
25	P13	115	115	R.gallicum
26	P3	83	115	R.gallicum
27	P2	147	115	///
28a	P2	115	115	Agrobacterium
28b	P2	115	115	///
29	P3	83	115	R.etli
30a	P6	29	115	Agrobacterium
30b	P6	29	115	///
31	P9	147	115	R.gallicum
32	P16	34	115	///
33a	P12	115	115	R.gallicum
33b	P12	115	115	///
33c	P12	115	115	Agrobacterium

Fig. 4. Interaction Rils-Strains affiliated : Test Nod The green box demonstrate the formation of the nodule

Small collection of isolates revealed an interesting diversity. For 40 strains studied five were identified as *Rhizobium etli*, 3 *R. leguminosarum*, 11 *R.gallicum*, 1 *R.loti*, 1 *R. ciceri* and 6 *Agrobacterium* and 10 strains remaining will be sequenced to be identified. For this strain studied, 29 can establish a nodule and 17 and 2 strains were more efficiency that was identified as *R.etli*.



In this small collection studied 80% of the isolates were able to induce nodulation  
The strain 17 and 4 that come from the same region are a highly infective ability

- Nodulation are limited *in natura* conditions
- The common-bean is very sensitive to agro climatic system
- The microsymbiot of studied agroecosytem initiate nodulation with the common-bean host.
- The genotypic variability affect nodulation

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