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Soil fertility and rhizobial inoculation perenity in a system maize/bean intercropping

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Abstract

In the aim to increase knowledge on the beneficial effect of rhizobial inoculation in an intercropping system cereals/legumes, a trial on *Phaseolus vulgaris* intercropped with *Zea mays* was conducted. Beans have been inoculated with three strains: S1, affiliated to CIAT 899 and two strains affiliated to *R. tropici* and *R. leguminosarum*. The effect of intercropping maize with beans was expressed by a significant improvement in biometric parameters and yield as well as the soil organic matter and total nitrogen. However, the response of beans to inoculation was different from one strain to another, especially in their respective perenity verified by RAPD-PCR. However, in overall, the effect was statistically significant, except the S1-S2-S3 mixinocula, which has brought no significant difference compared to the uninoculated control.These results expresses the interest of rhizobial inoculation in intercropping systems.

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Introduction

Today, to meet the growing demand for crop production while facing the decrease in viable agricultural areas, farmers and producers are turning to intercropping that encourage the simultaneous production of different cultures and to limit and control agricultural land that were under fallow (Thobatsi, 2009). Increasingly, intercropping are involving food legumes. Indeed, due to their ability to fix atmospheric nitrogen, they represent alternative, non-polluting and inexpensive organic soil fertilization (Zhang *et al.,* 2010; Matusso *et al.*, 2012). Therefore, in either crops rotation or association, legumes can enrich the nitrogen status of the soil. Thus, improving soil fertility, reducing weeds, improving grains and fodder quality, legume crops can increase crop productivity and increase the gross incomes by complementing and/or supplementing the fertilizer costs (Coulibaly *et al.,* 2012; Kheroar and Patra, 2014) to increase the place of legume use in farms, this study focused on the impact of the combination of a cereal, *Zea mays*, with a legume *Phaseolus vulgar*is inoculated with biological nitrogen-fixing strains, on bean and maize yield.

Materials and methods

Biological materials

The vegetal varieties used for bean was El Djadida and for maize "Crazics" obtained by the National Institute of Agricultural Researches, H'madna-Algeria. The studied strains were selected from the Laboratory of Biotechnology of Rhizobia and Plant Breeding (LBRAP)at the University of Oran 1 Ahmed Ben Bella and were referred as S2and S3. These strains were affiliated to *Rhizobium tropici* and *Rhizobium leguminosarum* respectively on the basis of their restriction pattern of the 16S gene with restriction enzymes MBOI and MspI (Laguerre *et al.,* 1994). S2 strain, isolated from the region of AinTemouchent was selected for its high NaCl tolerance on TY, reaching 800mm NaCl. S3 strain, isolated from the region of Sidi Bel Abbès, was selected for its symbiotic efficiency.

The reference strain CIAT 899, obtained by INRA-Montpellier, France and characterized as *Rhizbium etli*, was also tested, and referred as S1for comparison.

Experimental site

The trial was conducted in 2015 at the Technical Institute of Vegetable and Industrial Crops (ITCMI) located at Staouali region, 20 km northwest of Algiers. Annual rainfall is of around 700 mm and the average annual temperature is 17.9 °C (National Office of Meteorology). The physicochemical characteristics of station soil were kindly provided by ITCMI. The soil contained about 4% of each of clay and silt, and 92% sand; pH 8.85; soil organic matter (OM) 1.42%; total nitrogen (N) 0.065% ; available phosphorus (P) 24,2ppm; electrical conductivity of 16.83 mS /m..

Experimental device

The experimental trial, in completely randomized blocks, was divided into four blocks corresponding to four repetitions; each block was divided into six plots. Each plot was used for one of the six cropping systems: Bean-Corn inoculated with the strain S1, Bean-Corn inoculated with the strain S2, Bean-Corn inoculated with the strain S3, Bean-Corn inoculated with the mix of the three strains, Bean-Corn without inoculation and finally pure corn.Experimentation covered an area of 880 m^2, each plot wasof4 m × 4 m. Beans and corn were planted in alternate rows with a total for each plot of two lines for bean and two lines for corn. As for the plot with the maize alone, corn lines were alternated with unsown rows. The seeds were planted just under 30 cm distance to drip from one another with an average density of 13 plants /line.The technique of coating was followed for bean seed inoculation as described by Burton (1984).

Biometric analysis

Plant and nodules fresh weight

At flowering stage (37 DAS for beans and 53DAS for corn), three plants per line were harvested, with a total of 24 plants per treatment, as well as nodules, they were then weighed to estimate fresh weight.

Yield parameter

For beans, 50 DAS, when plants were mature, the pods were sampled. As for the analysis of fresh and dry weight, three plants per line were dug up so24 plants per treatment. The pods were collected in plastic bags labeled as Block N° and type of treatment and then they were weighed.For corn, 65 DAS, three plants per line, with a total of 12 plants per treatment were selected for harvesting. Thus, the weight of corncobs was recorded for each treatment.

Evolution of soil organic matter content (OM%) and total soil nitrogen (N%)

Fifty grams of soil, of each treatment and each repetition, were sampled at 20 cm depth at three times interval. At T0 just before planting, at T1 and T2 for 37 DAS and 65 DAS respectively. The used technique for OM% was the method of Anne. For N% the analysis was performed by the Kjeldahl technique.

Sustainability of inoculated strains

DNA extraction

The 20 heavier nodules, for each treatment, were selected for strain isolation. A pure colony for each strain was grown on TY agar 1.5% of the inclined Gibson tube. After incubation for 48 h at 28°C, 8 ml of sterile miliQ water was added to the tube and then vortexed to obtain a homogeneous bacterial suspension. The volume to be sampled for the following extraction, depended on the optical density (OD) read at 620 nm of the bacterial suspension obtained which must be equal to 1.After centrifugation for 3min at 13.000rpm, cell debris in the supernatant were removed. The resulting pellet was resuspended in Tris-HCl (10 mM, ph 8.3) and proteinase K (10mg/ml). The suspension was incubated overnight at 55 ° C, then increased to 100 ° C for 10 min to stop the proteinase digestion reaction. The tubes were finally stored at -20 ° C.

PCR-RAPD

A total of 100 isolates were analyzed. The amplification was performed in 200 µl tubes containing 25µl of final reaction volume.

The reaction mix comprises 2,5µl DNA, 10pmol primer, 10 mM dNTPs, 2 mM MgCl2, Taq buffer to final 1x, 0,5U of Go Taq (Promega) and 14,15µl ultrapure water. The primer used was OPI-06 (Naz *et al.,* 2009). The PCR program followed was: 94 ° C for 4 minutes; 94 ° C for 1 min, 34 ° C for 1mn30, 72 ° C for 5 min x 40; 72 ° C for 10 minutes.

Statistical analyses

The effects of cultivation type (monoculture and association) and bacterial inoculation on plant growth, nodulation, yield and soil OM% and N% content were tested by the analysis of the variance (ANOVA) of a single factor, as well as the linear regressions were studied with the XLSTAT software 2014. The averages were compared with multiple comparison Tukey test at 0.05 probability.Comparison of different profiles RAPD-PCR and construction of UPGMA dendrograms were performed with the 1D-Cliqs Phoretix1 2015 software.

Results

Biometric analysis

Bean

Plant and nodule fresh weight

Compared to non-inoculated control, inoculation with the strains S1, S2 and S3 have made highly significant improvements in the dry and fresh weight of the host plant (Table 1), S2 strain enhanced by a factor of 2 the fresh weight. Followed by S3 and S1 strains, which increased, respectively, by 113.94% and 65% plant fresh weight. However, with the co-inoculation, no significant effect in comparison to non-inoculated plants was noted.

Regarding the nodule weight, the same results were observed as for the biomasses of the host plant. The inoculated seedlings with S2 and S3 strains have a nodule's weight significantly greater than non-inoculated control with an increase of 1.4gr and 0.85gr respectively. With the S1 strain, a significant improvement of 100% was achieved. At the opposite, co-inoculation, where nodular weight, although was higher than the non-inoculated control of 50%, it was not significant.

Yield parameters

Inoculation improved weight of pods (Table 1). Thus, the plants inoculated with the strains S2 and S3 have a pod weight significantly higher than the non-inoculated control of 80%. Plants inoculated with the strain S1 exhibit a significant improvement of 36.67%. As for co-inoculated plants, no significant differences were noted.

Table 1. One-Way Anova of biometric parameters of non-inoculated maize and bean (MH), inoculated (S1, S2, S3, S1+S2+S3) and cultivated maize alone (M). Values represented the mean of 4 replicates ± SE (Standard Erreur). The letters indicate significant difference between the means at P <0.05 in Tukey test HSD.

Samples	Bean			Maize	
	Total fresh weight (g/plant)	Nodule fresh weight (g/plant)	Pods weight (T/Ha)	Total fresh weight (g/plant)	Podsweight (T/Ha)
MH	28,98± 6,91 d	0,6±0,04 d	0,32±0,03 c	155,08±10,17 bc	0,3±0,03 b
S1	47,88±5,99 bc	1,2±0,04 b	0,44±0,02bc	214,5±11,79 ab	0,36±0,01 ab
S2	87,29±10,21 a	2±0,05 a	0,65±0,08 a	267,25±14,43 a	0,42±0,04 a
S3	62±3,01 b	1,45±0,04 b	0,57±0,02 a	214,25±31,05 ab	0,39±0,06 a
S123	44,13±8,12cd	0,9±0,02 cd	0,41±0,04bc	196,25±52,48 b	0,35±0,01 ab
M	-	-	-	95,5±11,00 c	0,3±0,05 b
F Test					
Bloc	0,6662	< 0,0001	0,0018	< 0,0001	0,0024
Sample	< 0,0001	< 0,0001	< 0,0001	< 0,0001	< 0,0001
Bloc*Sample	0,9886	0,9501	0,7428	0,9886	0,9391

Maize

Total fresh weight

In this part, the plots of corn grown in association with beans, inoculated or not, were compared to maize intercropped with empty lines and which will be referenced as fallow control. Nevertheless, the corn associated with beans had a highly significant positive effect on all biometric parameters of maize (Table 1). For Fresh weight, all the plots of maize intercropped with inoculated beans, maize had a significant higher fresh weight than the control, except for the co-inoculated bean where, as in the non-inoculated bean, no significant effect was observed.

Yield parameters

For corn cobs weight, we obtained a significant increase in maize associated with beans inoculated with strains S2 and S3 with 40.94% and 23.98% respectively. The rest of the treatments made no significant differences.

Evolution of soil organic matter and total nitrogen

Organic matter

For corn and as for beans and fallow, relative to the stage before sowing, cultures hada weak organic matter level at the two sampling stages (Fig.1). For maize, all modalities of crops had an organic matter content reduced at both 37DAS and65DAS. The most important reduction was recorded in maize associated with non-inoculated bean of68.42% at37JAS and of 33.1% at65JAS. The lowest value has been observed with S3strain inoculation with 51.42% at37JAS and S1 strain inoculation with 19.01% at 65JAS. For bean, the same trend was observed with a significant reduction from the initial rate at37DAS and 65DAS. The lowest rate was noted at 37DAS among the bean inoculated with the strain S2 with 73.24%. At 65DAS in the bean inoculated with the strain S1 with 21.12%.

The smallest decrease was found in the non-inoculated bean with 43.66% and11.27% at37DAS and65DAS.However, and for all samples without exception, collected rhizospheric soils had an increased OM% rate in 65JAS than in 37DAS.

In common bean, this increasing was more pronounced while inoculation was done with the strain S2 with a difference of 0.88% and was the lowest while there was non-inoculation with a difference of 0.64%. In maize, these results were 0.74% and 0.58% during inoculation with the strain S1 and S3 respectively.

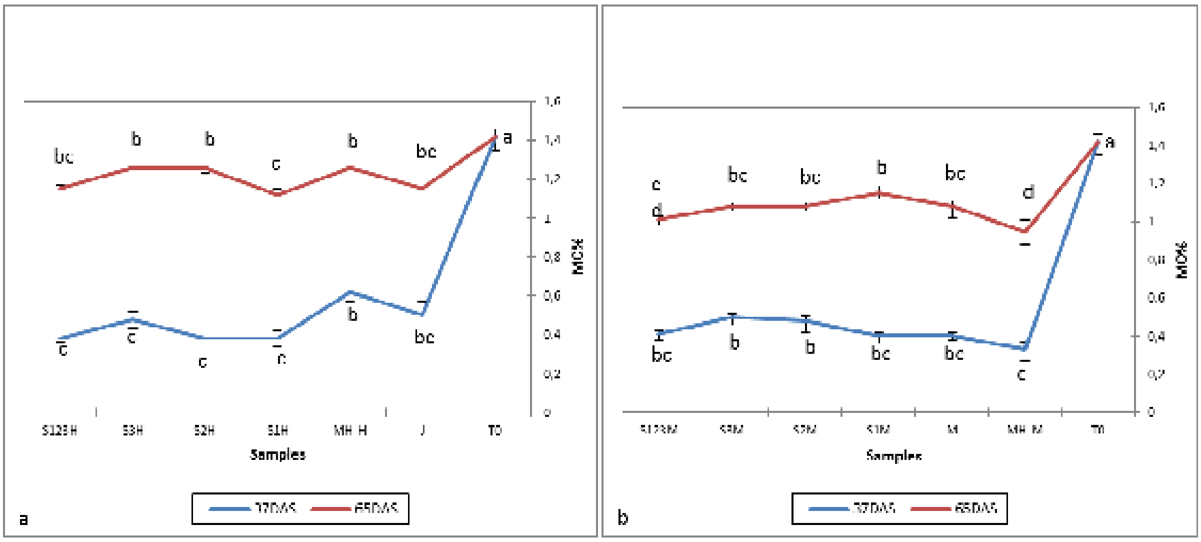

Fig. 1. Evolution of soil organic matter of bean rhizosphere (a) and maize rhizosphere (b). The data were the average of 4 replicates. Only averages with different letters were significantly different at Tuckey HSD test at P <0.05.

Total nitrogen

As the organic matter, at 37DAS, the intercropping had been accompanied by a significant reduction of the rhizospheric total nitrogen content. However, at 65DAS it was noticed a significant increase in nitrogen rate in comparison to the nitrogen level before sowing (Fig.2)In Corn, at 37DAS, sole the association with uninoculated bean and maize alone have a significant reduction of 62% and 52% respectively. At 65DAS, we observed an increase in the total nitrogen content of the rhizosphere.It was highly significant while corn was intercropped with bean inoculated with strains S1, S3 with 60% and 54% improvement respectively.

It was significant while associated with beans inoculated with the strain S2 with 40%.In bean, at37DAS only beans rhizospheric soil inoculated with strains S1, S2 and S123 showed a significant decrease of 50%.

At 65DAS, highly significant increases were noticed in the bean inoculated with the strain S3 (80%) and S2 (62%) respectively.

Competitively of strains.

Fig.3 represents the RAPD-PCR profiles on a sample of 100 nodules obtained from the roots of bean both inoculated and not inoculated. Thus, the rate of nodular occupation (Fig. 4) was determined by frequency of RAPD-PCR profiles of the introduced strains with respect to the native strains.No RAPD profile of the three introduced strains was observed in nodules of non-inoculated bean.

In the case of inoculation, the rate of nodular occupation differed from one strain to another. Indeed, it was more important with the S2 and S3 strains with 35% and 30% of harvested nodules; followed by the S1 strain which represents 20% of the nodules obtained.

However, we observed in case of co-inoculation, the nodular occupancy of the three introduced strains decreased relative to their inoculation apart. This reduction was 5% for the strain S1, 15% for strain S2and 20% for strain S3.

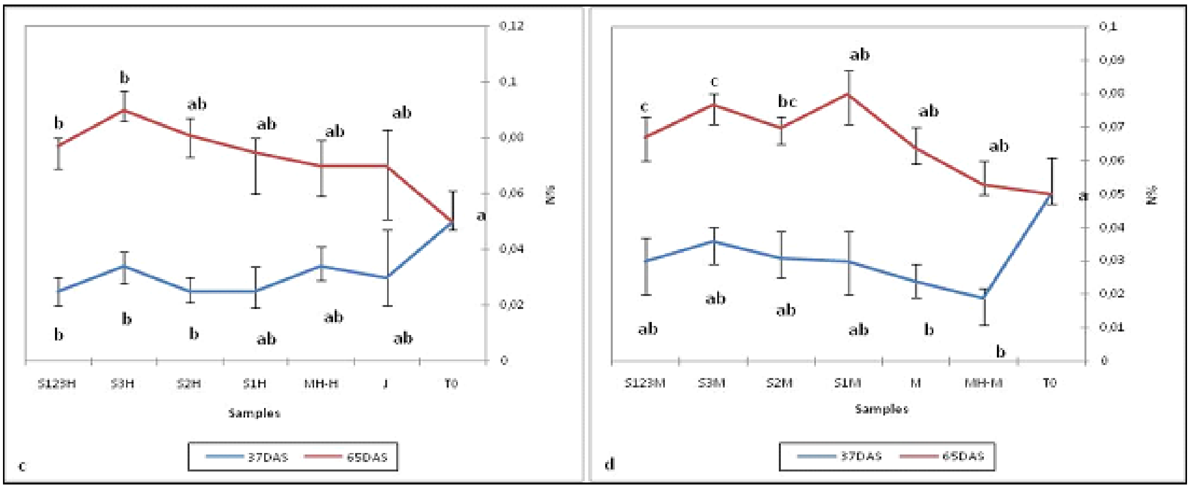

Fig. 2. Evolution of total nitrogen of the soil rhizosphere of bean (a) and maize (b). The data were the average of 4 replicates. Only averages with different letters were significantly different with Tuckey HSD test at $P < 0.05$.

Discussion

This study highlighted the various symbiotic interactions that occurred between maize (*Zea mays* L.) and common bean (*Phaseolus vulgaris* L.) inoculated with atmospheric nitrogen-fixing strains.

Effect of intercropping

Tosti and Guiducci (2010) reported that the association wheat-faba bean had doubled the nitrogen content of the cereal grain. Prins and de Wit (2006) noted an increase in the protein content of 2% in wheat when grown in intercropping with faba bean and *Pisum sativum*, which was very important especially for livestock feed.

According to Mariotti *et al.* (2015), intercropping barley with bean or faba beans reduced the rate of NO^{-3} leached and thus nitrogen losses through leaching and fertilizer inputs, thus complying with several objectives of sustainable development (Kheroar and Patra, 2014; Yao *et al.,* 2015).

This beneficial effect may be attributed to an improvement of the mineral nutrition due to changes in the composition of the microbial community by the inoculation (Sun *et al.,* 2009) and also by their complementarity in nitrogen resources use (Tosti and Guiducci, 2010), since the legume take its nitrogen from the symbiosis, leaving the soil nitrogen for the maize. Furthermore, root exudates of legumes induced acidification of the rhizosphere facilitating the dissolution of calcium phosphates and phosphorus, thus increasing the availability of as similable phosphorus and its absorption by the subsequent culture, particularly in the alkaline pH soils (Sun *et al.,* 2009; Akédrin *et al.,* 2010).

Effect of inoculation

The obtained results in our study were consistent with Howeison *et al.* (2011) and Gerding *et al.* (2014) who observed different responses, in the field, of respectively *Lotus ornithopodioides* and *L. diffusa* to inoculation.

This is explained by the host legumes root exudates that were specific to certain species of rhizobia and that the inoculation depended also on the inoculated strains (Yates *et al.,* 2005) or the host legume cultivar (Chiansu *et al.,* 2011). However, Trabelsi *et al.* (2011) and Gerding *et al.* (2014) attributed this to the genomic variations between strains.

Moreover, this latter and Berenji *et al.* (2015) found positive correlations between the rate of nodular occupation and aerial dry biomass.

This showed that the legume inoculation response in the field was a much more complex phenomenon and was not always positive (Chiansu *et al.,* 2011).

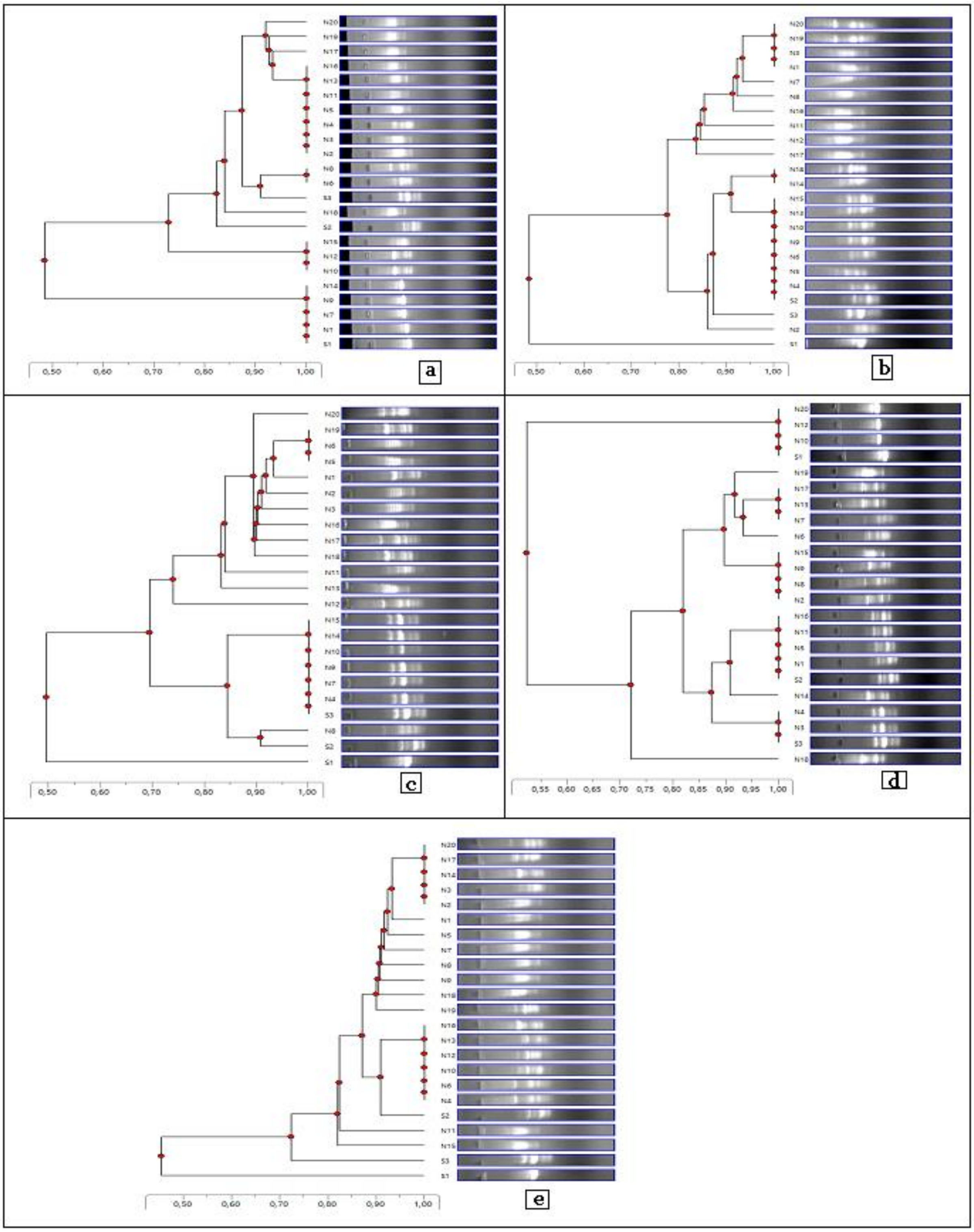

Fig. 3. UPGMA Dendrogram of RAPD-PCR patterns of bean nodules inoculated with the strain S1 (a), the strain S2 (b), the S3 strain (c) mixed inoculation S123 (d) and non-inoculated (e).

Soil organic matter and total nitrogen

The soil organic matter plays an important role in maintaining soil fertility by contributing to several of its properties including the cation exchange capacity, water holding capacity, and the physical structure of the soil (Kamara *et al.,* 2012).

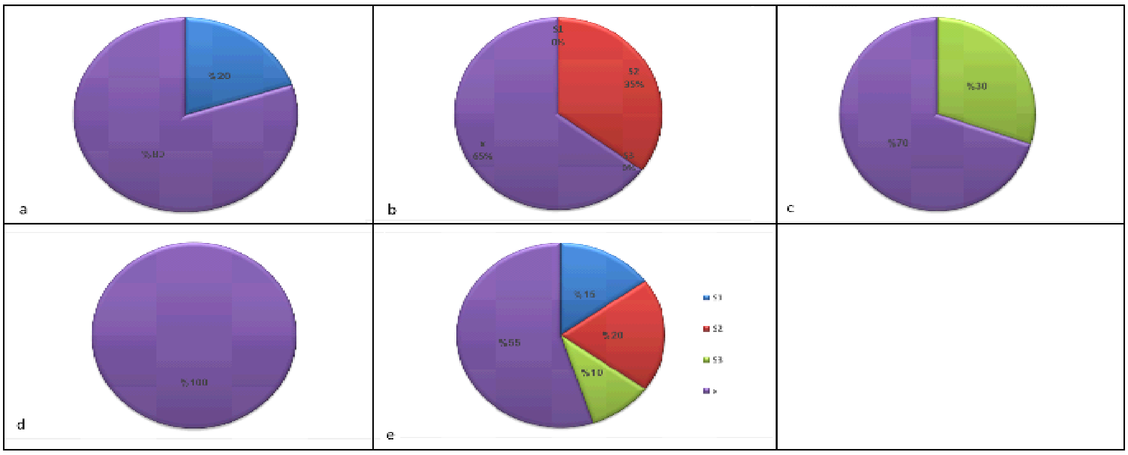

Fig. 4. Proportional quantities of different inoculated strains present in *Phaseolus vulgar*is nodules inoculated with the strain S1 (a), the S2 strain (b), the strain S3 (c) non-inoculated (d) and with the mixed inoculum S123 (e).

The effect of inoculation on the total soil nitrogen content was in the same way as the organic matter with significant increases compared to the initial status To.

This had also been reported in *Sesbania* and *Leucaena* grown together with annual grasses, which even allowed a transfer of nitrogen from legumes to grass between 20 and 70 kg.N/Ha (Abbas *et al.,* 2001). Rawat *et al.* (2013) noted even a 30kg economy of nitrogen fertilizer/ha with wheat in rotation with inoculated soybeans. On the contrary to Wang *et al* .(2014) who noted no significant difference when maize was in combination with faba bean.The observed effect was explained by the fact that during the growth of grain legumes, considerable amounts of nitrogen were released from the roots in the soil. In addition, residues of these crops had a higher nitrogen content in comparison to other cultures and nitrogen was released into the soil while decomposition.

Competitiveness of strains

The analysis of the nodular occupancy was a very interesting technique to determine the competitiveness and efficiency of the introduced strains (Mnasri *et al.,* 2007).

In this study, we observed disparities in nodular occupancy for the single variety El Jadida of *P. vulgar*is when it was tested with different strains. In addition, we noted positive correlations between dry plant biomass and nodular dry mass.

The disparity in the rate of nodular occupancy can be explained by: i. the competitiveness of the introduced strains with the native ones; ii. The introduced strains' effectiveness; iii. host specificity (Blanco *et al.,* 2010; Nangul *et al.,* 2013); iv. Soil and the environmental conditions which characterized the fields (Thies *et al.,* 1992). *Phaseolus vulgaris* is a promiscuous legume (Valverde *et al.,* 2006), it is likely that these differences were due to the competitiveness and/or efficiency of the introduced strains rather than the host specificity. Thus, the results of PCR-RAPD of nodules obtained and the efficiency of Nitrogen Fixation is consistent with this hypothesis.

Conclusion

This study highlighted the beneficial legume/cereal association as well as rhizobial inoculation on yield of either the two crops and even on soil fertility characteristics. The rhizobial inoculation improved significantly the fresh weight of bean, nodules maize and yield parameters of the two crops.

Also, the inoculation affected significantly the evolution of rhizospheric organic matter and total nitrogen where they exhibited the best improvement at 65DAS. However, coinoculation showed nonsignificant if effect on either biometric nor rhizospheric parameters. This may be due to a competitively between the strains. These results are good indicator on how to manage rhizobial inoculation for a better crop productivity.

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