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Senegalia senegal response to inoculation with rhizobial strains vary in relation to seed provenance and soil type

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Abstract

Aims The focus of the study was to determine the symbiotic and growth response of three *Senegalia senegal* (Syn. *Acacia senegal*, gum arabic tree) provenances, namely Dahra (Senegal), Tera (Niger) and Makueni (Kenya) to inoculation with selected *S. senegal*-nodulating rhizobia in soils from Dahra and Goudiry regions of Senegal, representing typical soil and enviromental conditions for establishing gum arabic production plantations.

Methods A greenhouse experiment was performed to evaluate the effect of 11 rhizobial strains on nodulation and growth of three *S. senegal* provenances in two field soils, differing in nutrient status and indigenous rhizobia. After 4 months, plants were harvested for determination of nodulation, shoot and root dryweight.

Results Nodulation and growth of *S. senegal* varied in relation to rhizobial strain, provenance, soil type, and their interactions. Generally, nodulation was higher in Dahra than Goudiry soils, while Makueni provenance was the most compatible host. Inoculation had a significant effect on all parameters measured in Dahra field soil. By contrast, inoculation had a significant effect on height (shoot length), and shoot, root and total dry matter but not on nodulation. In the two field soils, seed provenance effect was significant for all parameters measured. The interaction between inoculation and provenance showed a significant effect on all parameters measured except nodule number in Dahra field soil while in Goudiry, the interaction had a significant effect on seedling height and shoot, root, and total dry matter but this effect was not significant with nodulation parameters.

*Conclusion*s *S. senegal* is variable in its response to inoculation, it is therefore advantageous to select and match effective rhizobia-provenance symbionts for each site.

Introduction

In the arid and semi-arid lands of Africa, low and erratic rainfall, high temperatures and poor soil water and nutrient availability limit agricultural productivity (Mertz et al. 2012). Thus, multipurpose trees such as *Senegalia* (*Acacia)* species that provide a means to maximise agricultural potential and stabilise yields under stressful, unpredictable growing conditions are important for reforestation and reclamation of marginal lands, for fuel wood, timber, shelterbelts and soil improvement (Midgley and Bond 2001; Raddad and Luukkanen 2007). Previous phylogenetic studies indicated that *Acacia* Miller *s.l.* is polyphyletic. Recently, Kyalangalilwa et al. (2013) segregated genera for *Acacia s.l.* and proposed new combinations for the African species in *Senegalia* and *Vachellia.* The *Senegalia* clade is represented in Africa, Central and South America, and Asia with more than 60 species. *S. senegal* (L.) Britton & P. Wilson [Syn. *Acacia senegal* (L.) Willd.] is a complex group formed by closely related species widely distributed through the arid and semi-arid lands of sub-Saharan Africa (Odee et al. 2015). This tree is adapted to survive under harsh environmental conditions such as low and erratic rainfall, intense solar radiation, and high wind velocity (Cossalter 1991).

S. senegal is a N_2-fixing shrub or tree with considerable economic and ecological importance, producing a natural gum (gum arabic) widely used in the food and beverage industry, pharmaceuticals, other technical applications and provisioning of severalecosystem services in the drylands of tropical Africa (Ballal et al. 2005; Gaafar et al. 2006; Gray et al. 2013; Odee et al. 2011; Omondi et al. 2010) and a well-established traditional agroforestry tree component (Raddad et al. 2005). However, in Senegal the number of *Senegalia* (*Acacia)* species is believed to have reduced over the past years. The species remains under pressure as a result of its overexploitation by human population, shortage of rainfall in the Sahel, overgrazing. In addition, it is due to inappropriate agricultural practices, leading to the degradation and/or lack of regeneration of *S. senegal* parklands. Therefore, there is a need to conserve and sustainably manage the species if they are to meet the increased demand for fuelwood, fodder, soil improvement through N_2 fixation, protection of the environment and to cater for gum production, which is an important source of cash (Fagg and Allison 2004). The N_2-fixing capacity of a legume tree is often used to explain its ability to grow better on and restore the fertility of N-depleted soil (Dommergues 1995).

S. senegal is a promiscuous species that could be nodulated with various rhizobial taxa and strains (Bakhoum et al. 2014; de Lajudie et al. 1998; Fall et al. 2008; Nick et al. 1999; Njiti and Galiana 1996; Odee et

al. 1995; Odee et al. 1997; Sarr et al. 2005). Nevertheless, previous studies have shown that *S. senegal* is mainly nodulated in Senegal by rhizobial strains phylogeneticaly close to *Mesorhizobium plurifarium* (Bakhoum et al. 2014; Fall et al. 2008; Sarr et al. 2005a). Recent studies showed that inoculation with *Mesorhizobium* strains significantly improved nodulation and *S. senegal* plant growth under water-limited conditions (Fall et al. 2011), and enhanced plant nutrient content and rhizospheric soil fertility of *S. senegal* plants (Bakhoum et al. 2012). While in another study, inoculation improved plant nodule number but not shoot N content (Ndoye et al. 2012). Like several other African acacias, *S. senegal* has the potential to fix N_2 under a range of soil and environmental conditions if nodulated by effective rhizobia (Gray et al. 2013; Ndoye et al. 1995; Raddad et al. 2005). *S. senegal* is morphologically variable. It has four distinct varieties, namely vars. *senegal*, *kerensis*, *leiorhachis* and *rostrata* (Fagg and Allison 2004), of which three (*senegal*, *leiorhachis* and *kerensis*) are found in East Africa and one (*senegal*) in West Africa. Rangewide genetic studies of the species also show differentiations among varities and provenances across its native range, with clear genotypic distinction between west African and east and southern Africa (Chevallier and Borgel 1998; Odee et al. 2012; Odee et al. 2015). Therefore, there is an important need to select appropriate plant phenotypes/genotypes and rhizobia that are the most compatible to each other. The essential requirement to realize this objective is to increase understanding of the effect of abiotic factors such as soil characteristics and climatic conditions on nodulation and growth of different plant provenances.

As part of an international consortium aimed at improving growth and sustainable production of gum arabic, we evaluated the symbiotic and growth response of *S. senegal* provenances (Dahra, Senegal; Tera, Niger and Makueni, Kenya) inoculated with 11 *Mesorhizobium* (rhizobial) strains and grown in two Senegalese (Dahra, arid and Goudiry, semiarid) soils under greenhouse conditions.

Material and methods

Sampling and analyses of soils

Composite soil samples were collected from Dahra (15°21’ N**,** 15°29’ W) and Goudiry (14°11’ N**,** 12°43’ W), in the northern and the southern part of Senegal, respectively. The climate is influenced by a strong north-south dominated precipitation gradient, resulting in about 400-500 mm and 800-1200 mm per year at Dahra (arid) and Goudiry (semiarid), respectively (Bakhoum et al. 2012). Soil samples were collected in April 2008 from the top 0 - 25-cm-deep of rhizosphere soil of *S. senegal* trees grown in plantations at Dahra and Goudiry. The soils were passed through a coarse sieve (2 mm mesh) to remove stones and large pieces of organic matter, and stored at

4°C. The physical and chemical soil properties were analyzed at LAMA (*Laboratoire des Moyens Analytiques*, IRD, Dakar, Senegal). The total amount of carbon and nitrogen was determined by the combustion system ThermoFinnigan Flash EA 1112 (ThermoFinnigan, France). The colorimetric determination of total and available phosphorus was performed according to the method of Dabin (1965). Soil pH was determined in 2 M KCl suspensions at a solid liquid ratio of 1:2.5. Soil physical characteristics were determined according to the method of Gee and Bauder (1986), and exchangeable cations followed the method of Thomas (1982).

The most probable number (MPN, Brockwell 1980) method was used to estimate the number of *S. senegal*-nodulating rhizobia (per g^{-1} soil) indigenous to Dahra and Goudiry field soils. The seeds were then transferred in aseptic conditions into Gibson tubes (four replicates per soil) containing a sterile Jensen nitrogen-free medium (Vincent 1970). *S. senegal* seeds of the Dahra provenance were inoculated with soil samples and grown in a controlled environment (Easy-lighting, 200 W 8U 8500LM 6400K° blue – 2700K° red, Cis products, Paris, France) for three months with a photoperiod of 16 hours (under daylight) and eight hours (night), temperature of 30 ± 1 °C (night), relative humidity of 70 ± 5% and a photosynthetically active radiation (PAR) of 120 µmol m^{2-1} s^{-1}.

Rhizobial strains used

Table 1 shows the 11 rhizobial strains strains used in this study. They were all isolated from *S. senegal* in Senegal and selected on the basis of their symbiotic infectivity and effectiveness (Bakhoum et al. 2012; Fall et al. 2008; Sarr et al. 2005b). *S. senegal* nodulating rhizobial strains used in this study have identical *nod*A, *nod*C, and *nif*H gene sequences, and are closely related to *Mesorhizobium plurifarium* (Bakhoum et al. 2015; Fall et al. 2008).

Plant test

The three *S. senegal* provenances tested originated from Makueni County, Kenya (2° 9' S, 37° 46' E); Tera, Niger (14° 0' N, 0° 45' E), and Dahra, Senegal (15° 21’ N**,** 15° 29’ W). Germination of the seeds was done as described previously (Fall et al. 2008). Pre-germinated seedlings were transplanted into 12 cm x 8 cm (height x diameter) plastic bags filled with 800 mL of field soil from Dahra or Goudiry. The eleven strains were grown in glass flasks containing liquid yeast extract mannitol (YEM) medium (Vincent 1970) at 28°C for 2 days on an orbital shaker. Seedlings were inoculated during transplanting with 5 ml of the rhizobial culture in YEM liquid containing approximately 10^9 cells ml^{-1}. Non-inoculated treatments received 5 ml of autoclaved YEM medium.

Experimental design

The experimental design was a randomized complete block at Bel Air Station, Senegal. Each block was divided into seven plots; two plots represented soil origins (Dahra and Goudiry); three plots represented seed provenances (Dahra, Senegal; Tera, Niger and Makueni, Kenya); two plots represented the inoculation treatment (inoculated separately with eleven rhizobial strains and non-inoculated control). Each plot had twelve replicates. All plants were grown in a greenhouse (daylight approximately 10 h, average daily temperature 25°C day, 20°C night) and watered regularly with tapwater. After 4 months of growth, seedling height measurements were taken, then plants were uprooted, their root systems gently washed with tap water and the nodules counted. The oven dry weight (80°C for 72 hours) of the shoots, roots, and nodules were recorded.

Statistical analysis

Data on seedling height, nodule number, and shoot, root and nodule dry matter were statistically analyzed using one - and two-way ANOVA with XLSAT software version 2010. Student-Newman-Keuls range test ($P<0.05$) was performed to indicate the level of differences between the means. The means of soil physical and cemical characteristics of the two soil sources were compared using unpaired *t*-test.The hierarchical classification associated with correlation matrix were done with R software (64 3.1.0) to show the clustering charateristics based on the correlation between nodulation (nodule number, nodule dry matter), shoot and root characteristics (root, shoot and total dry matter, and shoot length) parameters measured in each soil type. A principal component analysis (PCA) was carried out in each soil type to determine the correlation between inoculation treatment, plant provenance and soil parameters using XLSAT software version 2010.

151 **Results**

152 **Soil characteristics**

153 Soils from the arid Dahra and semi-arid Goudiry regions of Senegal used in this study were both sandy (Table
154 2). However, soil from the semi-arid Goudiry had a higher percentage of clay and silt than the soil from Dahra.
155 Soils from Dahra can be characterised as poorly developed soils formed on sandy parent material of dunes or
156 fluvial deposits (with less than 3 % clay). These soils are reddish and have previously been classified as
157 Arenosols (Batjes 2001). The soils from Goudiry are classified as high in ferric lixisols, with clay-enriched lower
158 horizon (FAO 1995, 2003). Soil pH was slightly acidic in both sites and did not vary significantly. Total C, N, P,
159 contents, percentages of Ca, K were significantly higher in Goudiry than in Dahra field soil ($P < 0.05$). By
160 contrast, the difference of available P, percentage of Mg and Na were not significant between Dahra and
161 Goudiry field soils.

162 The number of rhizobia able to nodulate *S. senegal* (MPN) was also higher in Goudiry (4.02 $x10^4$ cells g^{-1})
163 compared to Dahra (34 cells g^{-1}) field soil (Table 2).

164 **Effect of rhizobial inoculation on nodulation**

165 Uninoculated plants were nodulated except plants of *S. senegal* provenance grown in Goudiry field soil, and
166 plants of Tera (Niger) provenace in Dahra field soil, thus reaffirming the presence of compatible indigenous
167 rhizobia (Table 3). Interestingly, *S. senegal* provenance from Makueni (Kenya) showed better nodulation than
168 the West African provenances, Dahra (Senegal) and Tera (Niger), especially in Dahra field soil. Generally, for
169 each provenance, the nodule number and nodule dry matter was higher in Dahra field soil than in Goudiry field
170 soil. Thus, Dahra field soil was more responsive to rhizobial inoculation. Compared to uninoculated plants,
171 significantly high ($P<0.05$) nodule dry matter were obtained by strains CiradF300 and ORS 3610 on Dahra
172 (Senegal) provenance in Dahra and Goudiry field soils, respectively; strains ORS 3604 and ORS 3416 on Tera
173 (Niger) provenance in Dahra field soil, and strain ORS 3607 on Makueni (Kenya) provenance in Dahra field soil.
174 The highest mean nodule number and dry weight were recorded in Dahra field soil on Makueni (Kenya)
175 provenance plants inoculated with strains ORS 3600 (8.25 nodules $plant^{-1}$) and ORS 3607 (51.3 mg $plant^{-1}$),
176 respectively (Table 3).

177 **Effect of rhizobial inoculation on plant shoot and root dry weight**

Makueni (Kenya) and Dahra (Senegal) provenances showed contrasting shoot and root dry matter accumulation in Dahra and Goudiry soils (Fig. 1A & B). Makueni provenance had better shoot than root growth, while Dahra provenance had better root than shoot growth in both soils. However, shoot and root growth of Tera (Niger) provenance did not show any differences between the two field soils.

In Dahra soil, the best inoculation response was recorded with the strains ORS 3607 which showed the best nodule dry weight (Table 3). Strain CiradF 300 significantly (P<0.05) improved the root dry weight of Makueni provenance by 47% compared to uninoculated plants. In Goudiry soil, inoculation with rhizobial strains ORS 3416, ORS 3607, and ORS 3593 signicantly (P<0.05) increased shoot dry weight of provenance Makueni (Kenya). These strains showed hagh nodule dry weight (Table 3). All rhizobial strains significantly increased root dry weight in Makueni (Kenya) provenance. In Dahra soil, rhizobial strains ORS 3574, ORS 3593, ORS 3604, ORS 3607, CIRAD F300 and ORS 3616 significantly (P<0.05) increased shoot dry weight of Dahra (Senegal) provenance plants compared to uninoculated plants (Fig. 1A). All of them showed high nodule dry weight (Table 3). Nevertheless, no significant effect of inoculation was observed on root dry weight. In Goudiry soil (Fig. 1B), all rhizobial inoculation treatments significantly increased the shoot and root dry weight of Dahra provenance (Senegal) plants, except the strain ORS 3628. In Dahra soil, inoculation with rhizobial strains ORS 3573, ORS 3574, ORS 3588, ORS 3604, ORS 3610, ORS 3628 and ORS 3588, ORS 3604 to Tera (Niger) provenance, significantly improved shoot and root dry weight, respectively, in comparison with uninoculated plants (Fig. 1A & B). In Goudiry soil, inoculation with the strains ORS 3604 and ORS 3610 increased significantly (P<0.05) the shoot dry weigth of plants by 40% and 32%, respectively. In contrast, strains ORS 3604 and ORS 3610 showed low nodule dry weight (Table 3). Only the root dry weight of plants inoculated with the strains ORS 3604 was significantly increased by 82% compared to uninoculated plants.

Interactions and correlations between factors tested

The two-way interaction of plant provenances and strain were significant (P < 0.05) for most parameters, except nodule number in Dahra soil, and both nodule and nodule number in Goudiry soil (Table 4). For Dahra soil, ANOVA test with two factors showed that inoculation had a significant effect on height (shoot length), nodulation, root, shoot and total dry matter of seedlings (Table 4). Provenance also had a significant effect on all parameters measured. The interaction between inoculation and provenance showed a significant effect on all parameters measured except nodule number. Regarding Goudiry soil, inoculation had a significant effect on the seedling height, and shoot, root and total dry matter. However, inoculation had no effect on nodulation. Seed

provenance effect was significant for all parameters measured. The interaction between inoculation and plant provenance had a significant effect on the height and shoot, root, and total dry matter but this effect was not significant with nodulation parameters.

Hierarchical classification and correlation between inoculation and plant growth parameters

Hierarchical classification associated with correlation matrix are represented in Fig 2. A and B. In our study, we used this method to identify the impact of field soil on the hierarchichal clustering and the correlation of plant parameters measured. Results of Dahra soil showed two clusters in relation to the correlation of parameters: the first comprises correlation between root dry matter (RDM) and total dry matter (TDM), and the second formed by nodule dry matter (NDM), nodule number (NN), shoot dry matter (SDM) and seedlings height (shoot length) which were correlated. Three clusters were revealed in Goudiry (Fig. 2 B): correlation between total dry matter (TDM), shoot dry matter (SDM) and seedlings height (shoot length); among nodule number (NN) and nodule dry matter (NDM); and root dry matter (RDM). In Dahra field soil, the improvement of TDM was linked to RDM; however, in Goudiry field soil, it was correlated to SDM and height (shoot length). There is an influence of the soil type on plant growth parameters.

PCA distribution of inoculation and provenance treatments, and plant growth parameters

To reveal the similarities and differences between samples and to assess the relationships between the observed variables, principal component analysis was performed. We used this method to identify which rhizobial strain inocultated to a *S. senegal* provenance is able to improve plant parameters measured in relation to the soil type. PCA showed that variables were condensed into two principal components that together were extracted and accounted for 90% and 82% variance for Dahra (Fig. 3) and Goudiry (Fig. 4) soils, respectively, suggesting that rhizobial inoculation and provenance treatments had positive effect on nodulation and plants growth parameters measured. Inoculation effects changed significantly depending on soil type and *S. senegal* provenance. However, the provenance impact was most pronounced in Dahra than in Goudiry soil.

In Dahra soil, three major clusters were clearly separated: Cluster A represented by nodule number (NN), nodule and shoot dry weight (NDM and SDM) and the height (shoot length) values correlated with the Makueni (Kenya) provenance inoculated with ORS 3573, ORS 3574, ORS 3588, ORS 3593, ORS 3600, ORS 3604, ORS 3607, ORS 3610, CiradF 300 and ORS 3416 in the positive values of F1. Cluster B consisted inoculated plants from Dahra (Senegal) provenance with ORS 3573, ORS 3588, ORS 3600, ORS 3610, ORS 3628, ORS 3416 and

inoculated plants from Tera (Niger) provenance with ORS 3574, ORS 3588, ORS 3593, ORS 3600, ORS 3607, ORS 3610, ORS 3628, ORS 3416 wich were linked to root dry matter (RDM) in the negative values of F1. The plants of provenance Dahra (Senegal) inoculated with ORS 3574, ORS 3593, ORS 3604, ORS 3607, CiradF 300 and plants of provenance Tera (Niger) inoculated with ORS 3573, ORS 3604 constitued the Cluster C, which is correlated to total dry matter (TDM) in the positive values of F2.

In Goudiry soil two clearly distinct clusters can be identified: The cluster A is represented by an association of TDM, SDM, NDM, NN and height with treatments of provenance Makueni (Kenya) inoculated with ORS 3573, ORS 3588, ORS 3593, ORS 3607, ORS 3610, CiradF 300, ORS 3416 and treatment of provenance Tera (Niger) inoculated with ORS 3604 in the positive values of F1 axis. The cluster B was formed with treatments of provenance Dahra (Senegal) inoculated with ORS 3573, ORS 3588, ORS 3593, ORS 3604, CiradF 300, treatments of provenance Tera (Niger) inoculated with ORS 3607, ORS 3610, CiradF 300 and treatment of provenance Makueni (Kenya) inoculated with ORS 3604, associated with RDM in the positive values of F2 axis.

Discussion

Our results demonstrate provenance variation in symbiotic association with selected *Mesorhizobium* strains as influenced by soil characteristics (nutrient status and indigenous rhizobia). Several authors have shown similar results, for example, in the common bean, *Phaseolus vulgaris* (Cardoso et al. 2009) and several woody legumes (Elbanna et al. 2009; Mnasri et al. 2007; Odee et al. 1995; Sanginga et al. 1991). In our study, Makueni provenance had the best nodulation response when inoculated seedlings were grown in arid Dahra soils that had low nutrients status and number of indigenous *S. senegal*-nodulating rhizobia (Table2). These results also suggest that Makueni provenance has a higher N demand compared to the West African provenances. This is also corroborated by a previous study that reported higher shoot N contents of the Makueni provenance (Kenyan) than the West African demostrating differences in their N requirements (Bakhoum et al. 2012). Thus, high nodulation capacity may indicate higher N-demand in the Makueni than Dahra and Tera provenances.

Nodulation tended to be higher in Dahra soils poor in nutrients compared to fertile Goudiry soils. This was probably due to the differences in level of available N in the soils, which was higher in Goudiry than Dahra (Table 2). These results showed that nodulation was inversely related to soil N. This could be attributed to that the act that nodule number and N_2 fixation are regulated in response to the N status of the plant as described by

263 Ruffel et al. (2008). Dart (1974) showed that N compounds like nitrates may affect nodulation regardless of
264 plant age, size or prior to inoculation status. Plants use the available nitrogen in soil and form nodules to
265 complement the quantity of nitrogen required, thus the observed nodulation may also reflect differences in the
266 relative N-limiting status between the soils. However, indigenous rhizobia could also be responsible for the
267 observed diffierence in nodulation of *S. senegal* plants grown in Dahra and Goudiry soils. Singleton and Tavares
268 (1986) and Turk et al. (1993) indicated that the response of rhizobial inoculation mostly occurs when the
269 indigenous population densities are <50 rhizobia g^{-1} of soil; Dahra soil rhizobial MPN (Table 2) was within this
270 threshold. On the other hand, MPN of rhizobia was high in Goudiry soil (4.02×10^4 g^{-1} of soil), a large number
271 which could also outcompete the inoculant strain. In studies solely dependent on indigenous rhizobia, it has been
272 shown that low rhizobia counts in the soil reduce nodule numbers and biomass while high rhizobial counts in
273 soil enhance nodulation, for example cowpea (Kimiti and Odee 2010), *Acacia saligna* (Benbrahim et al. 1998)
274 and *Cajanas cajan* (Mapfumo et al. 2000). Therefore, the inoculation response in Goudiry soil cannot only be
275 explained by available N in soil, but also the number of competitive indigenous rhizobia in soil. Notwithstanding
276 the difference in MPN estimates of indigenous rhizobia capable of nodulating *S. senegal* in Dahra and Goudiry
277 soils, inoculation in most cases improved nodulation. In addition, indigenous rhizobial strains originally isolated
278 from Goudiry generally performed better than exogenous strains in Goudiry soil despite high indigenous
279 populations, indicating the importance of selection and re-inoculation with an effective indigenous strain as
280 previously demonstrated in *Sesbania sesban* by Makatiani and Odee (2007). Besides natural adaptation, the re-
281 inoculated strain is also expected to reduce competion for nodulation from other compatible indigenous soils.

282 Another important finding of this work is that nodulation (nodules number and nodule dry matter) were
283 correlated with shoot dry matter and seedlings height in Dahra and Goudiry soils indicating effectiveness of the
284 symbioses and contribution of N_2 fixation to the growth of *S. senegal* seedlings. Nodule dry weight and numbers
285 were negatively correlated with root dry matter in Dahra soil and not in Goudiry soil. These results suggested
286 that the control and biomass partitioning for nodule development in *S. senegal* is driven by the soil available N,
287 but other factors such as host provenance and rhizobial strain may also be equally important. Other workers (e.g.
288 Laguerre et al. 2007; Rodiño et al. 2011) have reported variability of nodulation, root and shoot characteristics in
289 relation to rhizobial strain and plant genotype.

290 Our results showed that inoculation with rhizobial strains significantly improved growth of *S. senegal* seedlings.
291 These results are in agreement with several previous studies in nursery conditions which reported enhanced

growth of *S. senegal* species due to inoculation with effective microsymbionts (Badji et al. 1988; Räsänen et al. 2001). Nevertheless, there was high variability in plant development among the provenances studied. It is important to note that effectiveness of rhizobial strain on improving plant growth parameters varied according to provenance and soil source. This is also reflected in the variable interaction effects of inoculation × plant provenance on the various growth and nodulation parameters between Dahra and Goudiry soils (Table 4). Corollary to this result, PCA showed that in Goudiry soil, the rhizobial strain ORS 3604 inoculated to Tera (Niger) provenance seedlings had improved growth parameters except root dry matter. In contrast, the strain was only correlated with the total dry matter in Dahra soil. This reaffirms the importance of soil type, hence plant available N on the nodule development and functioning. In our study, Dahra provenance generally performed better in produced more roots biomass than other provenances, especially in Dahra soil, indicating its adaptation grow in poor soils, by growing an extensive root system in order to get nutrient from wider soil area. Therefore, these results implied that nodulation and thus effectiveness of symbiosis is regulated by plant provenance, rhizobial strain and soil origin.

Conclusions

The nodulation and growth of *S. senegal* seedlings was variable and dependent on complex interactions of rhizobial strain inoculation, plant provenance and soil type. This study has shown that it would be advantageous to select effective combinations of rhizobia × provenances in relation to soil and environmental conditions where they are to be planted.

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Table 1 List of Senegalese *Mesorhizobium* strains originally isolated from rhizosphere soils of *S. senegal* and used in this study

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Rhizobial strain	Genbank accession number (16S rRNA)	Site in Senegal	Climatic zone	Reference
ORS 3573	JQ039728	Dahra	Arid	Bakhoum *et al*. (2014)
ORS 3574	JQ039729	Dahra	Arid	Bakhoum *et al*. (2014)
ORS 3588	JQ039735	Goudiry	Semiarid	Bakhoum *et al*. (2014)
ORS 3593	JQ039736	Goudiry	Semiarid	Bakhoum *et al*. (2014)
ORS 3600	JQ039741	Goudiry	Semiarid	Bakhoum *et al*. (2014)
ORS 3604	JQ039739	Goudiry	Semiarid	Bakhoum *et al*. (2014)
ORS 3607	JQ039737	Goudiry	Semiarid	Bakhoum *et al*. (2014)
ORS 3610	JQ039732	Goudiry	Semiarid	Bakhoum *et al*. (2014)
ORS 3628	JQ039740	Goudiry	Semiarid	Bakhoum *et al*. (2014)
ORS 3416	EU584256	Kamb	Arid	Fall *et al*. (2008)
CiradF 300	Unknown	Kebemer	Semiarid	Sarr *et al*. (2005)

Table 2 Physical and chemical characteristics of Senegalese Dahra (arid) and Goudiry (semiarid) soils used. For each parameter analyzed, means followed by the same letter on each row are not significantly different according to Newman-Keuls test at 5% level. Means ± SE (n = 3)

Soil characteristics	Dahra soil	Goudiry soil
% Clay	3.57±0.50[a]	6.87±0.267[b]
% Silt	10.30±1.14[a]	19.10±1.51[b]
% Sand	85.30±1.00[b]	73.87±1.56[a]
% Total C	0.52±0.06[a]	0.77±0.04[b]
% Total N	0.05±0.01[a]	0.07±0.01[b]
Available P (mg kg^{-1})	8.01±1.05[a]	8.29±0.01[a]
Total P (mg kg^{-1})	49.00±7.55[a]	79.33±4.70[b]
% Ca (meq)	0.92±0.11[a]	1.33±0.07[b]
% Mg (meq)	0.42±0.03[a]	0.42±0.07[a]
% K (meq)	0.20±0.01[a]	0.28±0.02[b]
% Na (meq)	0.11±0.05[a]	0.15±0.02[a]
pH H_2O	5.97	5.96
MPN*	34	$4.02x10^4$

* Most probable number (MPN) estimates of rhizobia (g $soil^{-1}$) able to nodulate *S. senegal* Dahra provenance

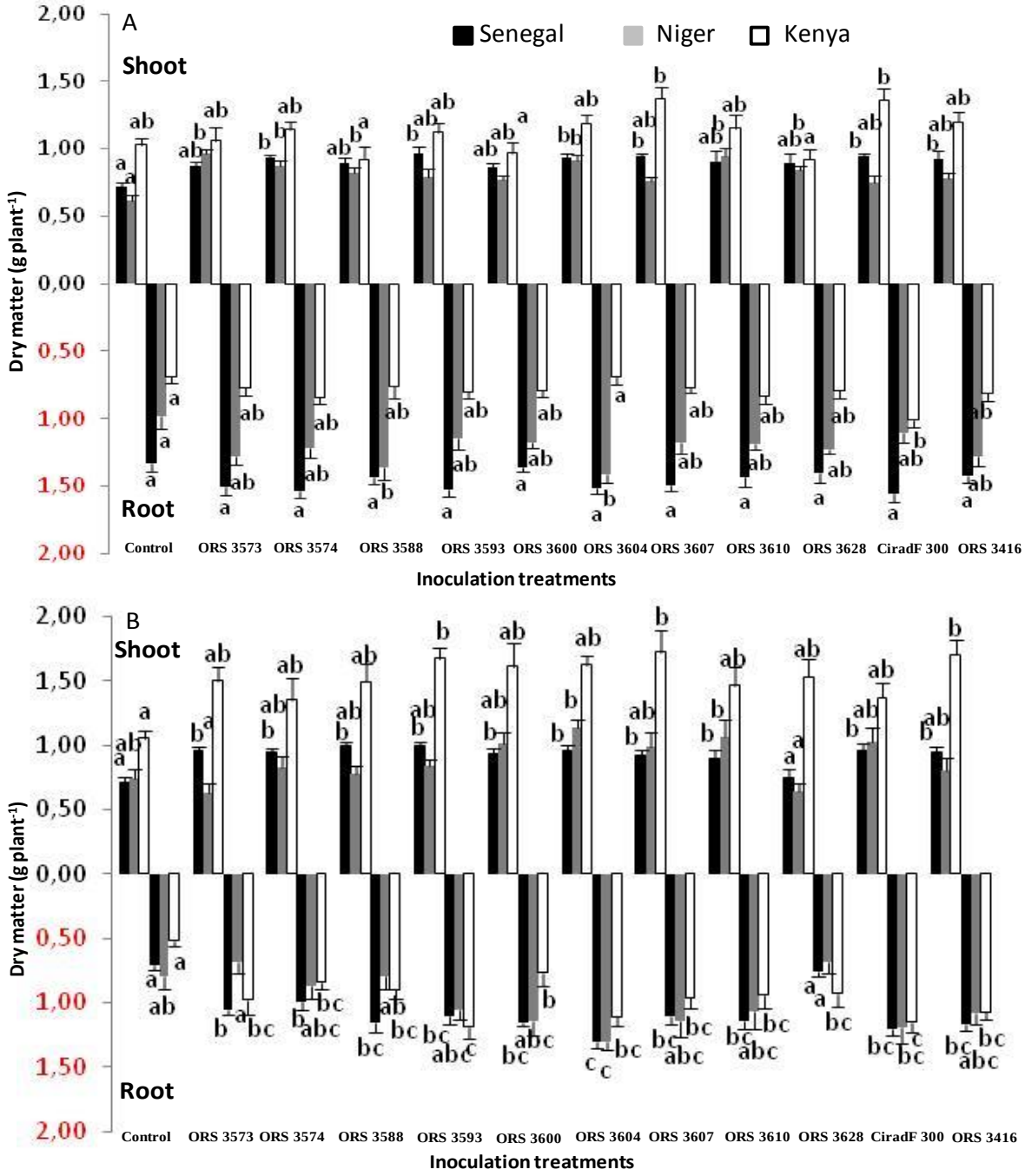
Table 3 Nodulation (mean nodule number, nodule dry matter plant^{-1}) of *S. senegal* seedlings of three provenances (Dahra, Senegal; Tera, Niger and Makueni, Kenya) grown in two different non-sterilised soils (Dahra and Goudiry, Senegal) after four months in greenhouse conditions at Bel Air Station, Senegal. For each soil type, means of values (n = 10) with the same letter are not significantly different according to Student-Newman-Keuls range test (P<0.05). Nod number: nodule number plant^{-1}; NDM: nodule dry matter plant^{-1}.

	Dahra provenance				Tera provenance				Makueni provenance			
	Dahra soil		Goudiry soil		Dahra soil		Goudiry soil		Dahra soil		Goudiry soil	
Treatments	Nod number	NDM (mg)	Nod number	NDM (mg)	Nod number	NDM (mg)	Nod number	NDM (mg)	Nod number	NDM (mg)	Nod number	NDM (mg)
Control	0.17^{a}	1.55^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0.17^{a}	2.06^{a}	1.08^{a}	12.85^{a}	1.08^{a}	3.48^{a}
ORS 3573	1.42^{a}	3.26^{ab}	0.33^{a}	0.12^{a}	2.58^{bc}	3.36^{ab}	0^{a}	0^{a}	5.33^{abc}	18.36^{a}	1.36^{a}	6.76^{a}
ORS 3574	1.58^{a}	3.75^{ab}	1^{a}	0.53^{a}	0.91^{abc}	4.46^{ab}	0^{a}	0^{a}	4.25^{abc}	18.6^{a}	0^{a}	0^{a}
ORS 3588	0.42^{a}	0.65^{a}	0.5^{a}	2.53^{ab}	2.92^{c}	8.49^{b}	0^{a}	0^{a}	5.7^{abc}	25.04^{a}	2.45^{a}	8.54^{a}
ORS 3593	1.83^{a}	5.25^{ab}	0.42^{a}	0.08^{a}	0.58^{ab}	3.4^{ab}	0.1^{a}	0.54^{a}	5.08^{abc}	22.05^{a}	1.27^{a}	8.67^{a}
ORS 3600	1.08^{a}	3.18^{ab}	0.83^{a}	1.87^{ab}	0.08^{a}	0.08^{a}	0.75^{ab}	3.97^{a}	8.25^{c}	33.5^{ab}	0.78^{a}	0.48^{a}
ORS 3604	0.58^{a}	3.39^{ab}	0.42^{a}	1.62^{ab}	1.75^{abc}	8.34^{b}	1.08^{b}	3.56^{a}	2.73^{ab}	13.34^{a}	0.08^{a}	0.31^{a}
ORS 3607	1.67^{a}	6.81^{ab}	1^{a}	1.47^{ab}	0.42^{ab}	0.55^{a}	0.09^{a}	1.81^{a}	7.5^{bc}	51.65^{b}	0.45^{a}	5.76^{a}
ORS 3610	1.5^{a}	3.9a^{b}	0.64^{a}	4.82^{b}	0.67^{abc}	1.85^{ab}	0.17^{a}	0.04^{a}	5.09^{abc}	28.77^{a}	1.91^{a}	8.78^{a}
ORS 3628	0.83^{a}	1.24^{a}	0.5^{a}	0.35^{a}	0^{a}	0^{a}	0^{a}	0^{a}	3.6^{abc}	24.67^{a}	0^{a}	0^{a}
CiradF300	1.08^{a}	8.7^{b}	0.67^{a}	1.22^{ab}	2.42^{bc}	7.2^{ab}	0^{a}	0^{a}	5.55^{abc}	16.5^{a}	1.18^{a}	4.07^{a}
ORS 3416	0.17^{a}	0.7^{a}	0.25^{a}	0.29^{a}	1.33^{abc}	8.74^{b}	0.1^{a}	0.31^{a}	4.14^{abc}	26.37^{a}	1.45^{a}	7.65^{a}

Table 4 Significance level obtained from two-way ANOVA testing the effects of inoculation and provenance level on different parameters measured on field soils, Dahra (arid) and Goudiry (semi-arid) inoculated with *Mesorhizobium* strains on three *S. senegal* provenances cultivated during four months at greenhouse conditions.

Soils	Factors tested	Nod numb	NDM	SDM	RDM	TDM	Height (shoot length)
Dahra	Inoculation	*	*	***	**	***	***
	Provenance	***	***	***	***	***	***
	Inoc*Prov	NS	***	***	**	**	**
Goudiry	Inoculation	NS	NS	***	***	***	***
	Provenance	**	**	***	***	***	***
	Inoc*Prov	NS	NS	*	**	**	***

Significant values are indicated: *P<0.05; **P<0.01; ***P<0.001; NS, not significant to student-Newman-Keuls test. Inoc: inoculation treatments; Prov: provenances; Nod number: nodules number; NDM: nodules dry matter; SDM: shoot dry matter; RDM: root dry matter; TDM: total dry matter



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500 **Fig. 1** Shoot and root dry matter yield of Dahra, Senegal; Tera, Niger and Makueni, Kenya *S. senegal*
501 provenances grown in Dahra arid (A) and Goudiry semi-arid (B) Senegalese non-sterilised soils inoculated with
502 selected rhizobial strains. For shoot and root dry matter taken separately, bars with the same letters are not
503 significantly different according to Student-Newman-Keuls range test (P<0.05) for each *S. senegal* provenance.
504 Error bars are standard errors of the mean (n=10).

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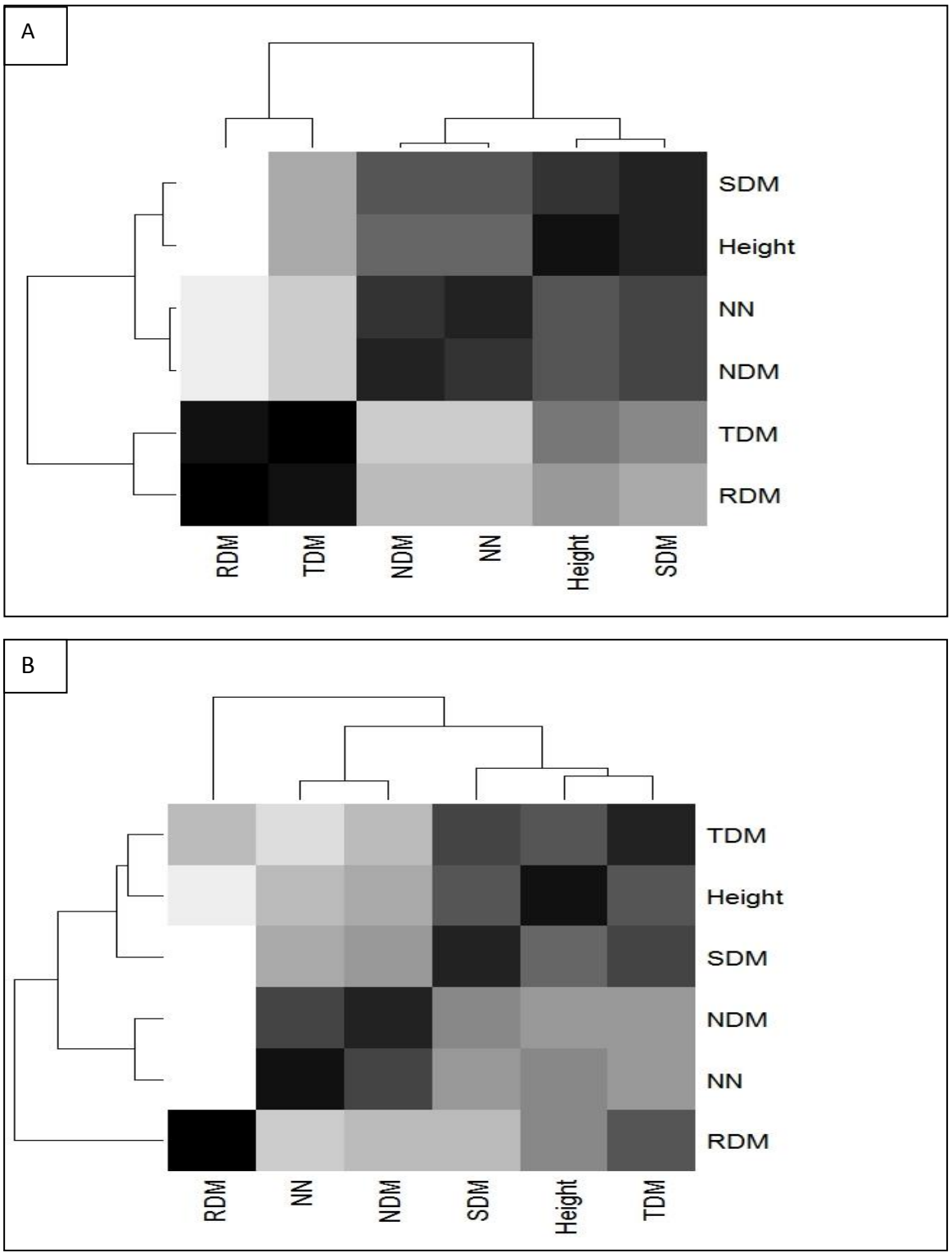
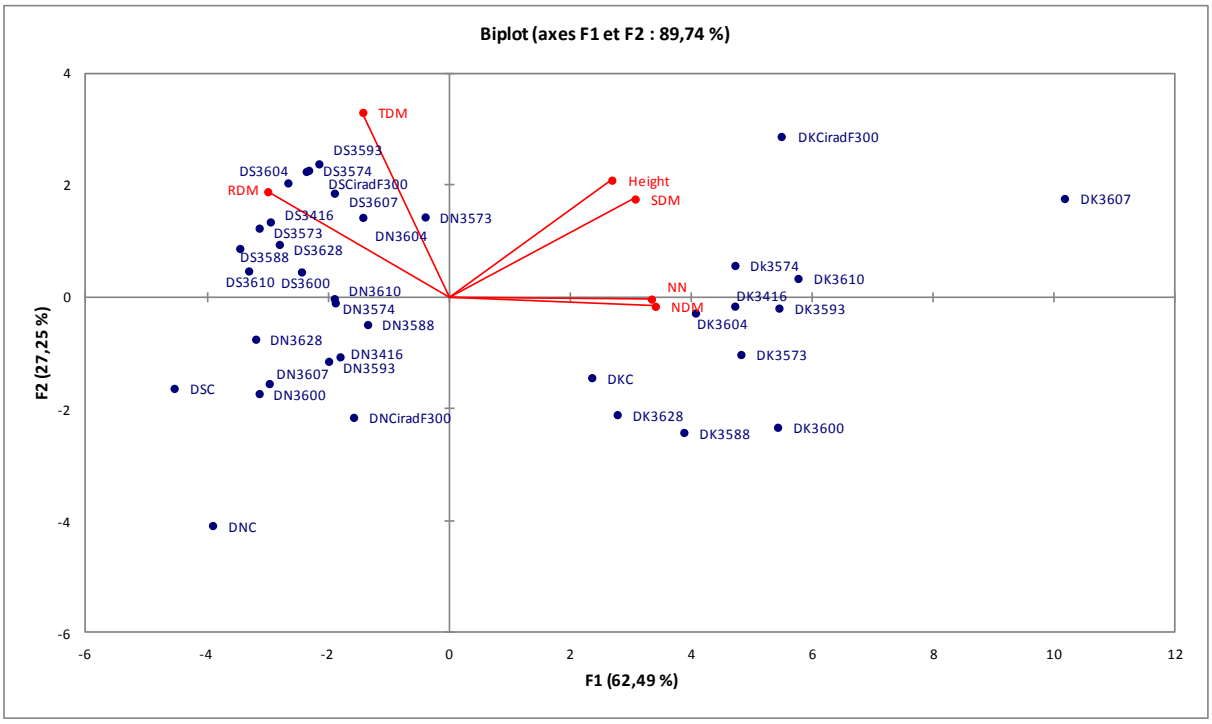


Fig. 2 Hierarchical classification associated with correlation matrix of nodulation (NN, NDW), shoot and root characteristics (RDM, SDM, TDM and Height (shoot length)) of three *S. senegal* provenances inoculated with selected rhizobial strains in Dahra (A) and Goudiry (B) Senegalese soils. The colour gradations from black to light shades correspond with high to low correlation between the parameters. The letters are defined as follows: NN, nodule number per plant, NDW, nodule dry weight per plant, RDM, root dry weight per plant, SDM, shoot dry weight per plant, TDM, total dry weight plant per plant



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532 **Fig. 3** Principal component analysis representing the relationships between nodulation (NN, NDW), shoot and
533 root characteristics (RDM, SDM, TDM and Height (shoot length)) of three *S. senegal* provenances inoculated
534 with selected rhizobial strains in Dahra soil. The % variance explained by each component is given in
535 parenthesis. The letters are defined as follows DS, Dahra soil associated to Dahra (Senegal) provenance; DN, Dahra soil associated to
536 Tera (Niger) provenance; DK, Dahra soil associated to Makueni (Kenya) provenance, and C, control.

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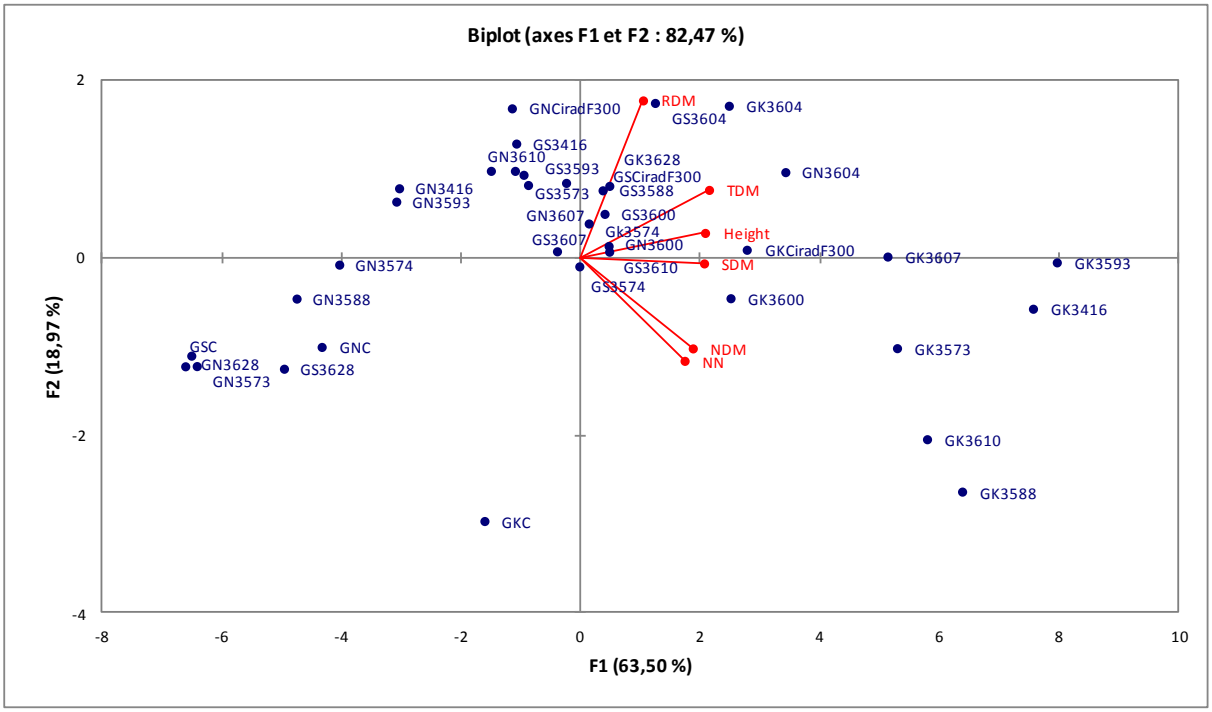

Fig. 4 Principal component analysis representing the relationships between nodulation (NN, NDW), shoot and root characteristics (RDM, SDM, TDM and Height (shoot length)) of three *S. senegal* provenances inoculated with selected rhizobial strains in Goudiry soil. The % variance explained by each component is given in parenthesis. The letters are defined as follows: GS, Goudiry soil associated to Dahra (Senegal) provenance; GN, Goudiry soil associated to Tera (Niger) provenance; GK, Goudiry soil associated to Makueni (Kenya) provenance and C, control.