



Genotypic and symbiotic diversity of *Rhizobium* populations associated with cultivated lentil and pea in sub-humid and semi-arid regions of Eastern Algeria

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This paper is dedicated to the memory of
Gisèle Laguerre.

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ABSTRACT

The genetic structure of rhizobia nodulating pea and lentil in Algeria, Northern Africa was determined. A total of 237 isolates were obtained from root nodules collected on lentil (*Lens culinaris*), proteaginous and forage pea (*Pisum sativum*) growing in two eco-climatic zones, sub-humid and semi-arid, in Eastern Algeria. They were characterised by PCR-restriction fragment length polymorphism (RFLP) of the 16S–23S rRNA intergenic region (IGS), and the *nodD-F* symbiotic region. The combination of these haplotypes allowed the isolates to be clustered into 26 distinct genotypes, and all isolates were classified as *Rhizobium leguminosarum*. Symbiotic marker variation (*nodD-F*) was low but with the predominance of one *nod* haplotype (g), which had been recovered previously at a high frequency in Europe. Sequence analysis of the IGS further confirmed its high variability in the studied strains. An AMOVA analysis showed highly significant differentiation in the IGS haplotype distribution between populations from both eco-climatic zones. This differentiation was reflected by differences in dominant genotype frequencies. Conversely, no host plant effect was detected. The *nodD* gene sequence-based phylogeny suggested that symbiotic gene diversity in pea and lentil nodulating rhizobial populations in Algeria was low compared to that reported elsewhere in the world.

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Introduction

In Algeria, food and forage legume productions are, along with cereals, the major challenges for agriculture. In a poor soil context, legume plants are interesting since they have the capacity to enter into nitrogen fixing symbiosis with rhizobial soil bacteria. They develop nodules on their roots, where bacteria reduce atmospheric nitrogen to ammonia that is then available for plant nitrogen nutrition. This symbiosis may have environmental and agronomic impacts due to the reduced needs for nitrogen chemical fertilizers. Legumes are frequently cultivated in rotation or in association with cereals in order to improve their nitrogen nutrition, crop yields and soil fertility. Among food legumes, lentil (*Lens culinaris*) cultivation has been promoted by the Algerian Ministry of Agriculture since 2007–2008 (<http://www.minagri.dz>). Lentil

is one of the most ancient legume crops cultivated in occidental Asia, Egypt and South Europe, and was probably spread from Western Asia [10]. Many ancient and long cultivated lentil varieties in Algeria have been lost and, nowadays, cultivated lentil are either local – heterogenous mixtures – or of European origin. Several varieties have been introduced, and several new ones have been selected and bred for their adaptation to the various agro-climatic conditions found in this country [17]. Diversification of forage cultures is limited due to only a few research efforts carried out on variety creation and introduction. Proteaginous pea (*Pisum sativum*) was introduced recently to Algeria, but available areas devoted to its culture remain limited, despite its strategic importance. Forage pea (*P. sativum* subsp. *arvense*) is well adapted and productive in the whole northern part of Algeria. Although *Pisum* is a common genus in the Algerian flora, all cultivated peas are introduced varieties. Areas cultivated with forage pea increased a lot during the 1980s but decreased afterwards, despite the plant breeding program for productive varieties conducted locally by the ITGC (Technical Institute for Large Scale Farming). The ITGC

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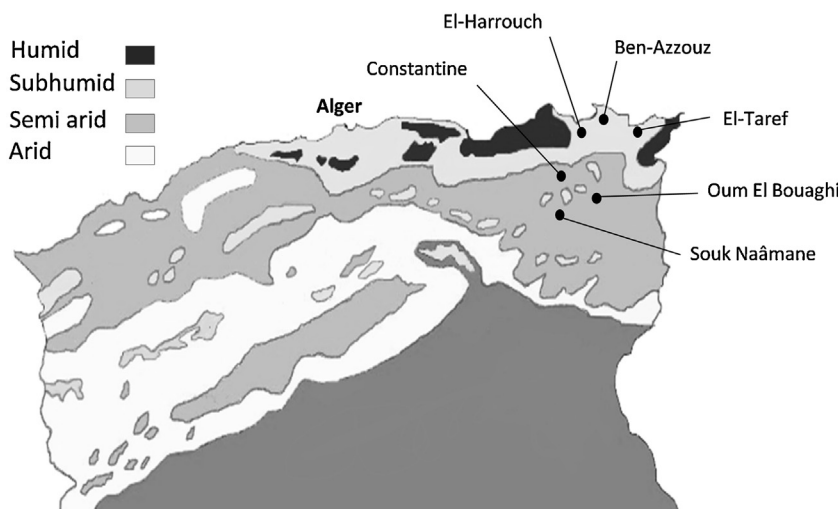


Fig. 1. Bioclimatic zones of Algeria with the sampling sites indicated.

demonstrated that forage pea associations with several crops (pea-oat, -wheat, -barley) were superior to vetch associations due to a more balanced legume/cereal growth [17].

Specificity is one of the main characteristics of the legume-rhizobium symbiotic associations. Indeed a rhizobial strain usually infects only a limited number of host plant species, which is a feature defined as its host range. The symbiovar concept ([41], following the classical pathovar concept for plant pathogenic bacteria) was defined in order to distinguish rhizobia by their symbiotic capabilities (i.e. host range), which is supported by symbiotic gene information irrespective of their species affiliation. For instance, the three symbiovars *phaseoli*, *trifolii* and *viciae* were created within the *Rhizobium leguminosarum* species for strains nodulating the tribes *Phaseolus*, *Trifolium* and *Viciae* (including the genera *Pisum*, *Vicia*, *Lathyrus* and *Lens*), respectively [41]. Symbiovar *viciae* strains were later reported in *Rhizobium fabae* [49] and *Rhizobium pisi* [37]. In *R. leguminosarum* bv. *viciae* (Rlv), the host range is controlled by nodulation (*nod*) genes carried by a symbiotic plasmid (pSym) [23]. Several studies have shown genetic heterogeneity of the *nodD* gene among Rlv strains, and symbiotic specificity towards host plants [13,24,25,28,33,34,48,55].

A large amount of literature has reported genetic diversity among natural rhizobial populations nodulating *Pisum*, *Vicia* and *Lathyrus*, especially *R. leguminosarum* [4,12,13,24,32,34,35,50,56]. *R. fabae* [49] and *R. pisi* [37] were described based on ribosomal RNA, *atpD* and *recA* phylogenies. *R. fabae*, isolated from *Vicia faba* in China, also nodulates pea [49] and its *nodC* gene is similar to that of symbiovar *viciae* [41]. *R. pisi* was created to reclassify a *R. leguminosarum* strain nodulating *P. sativum*, *Trifolium repens* and *Phaseolus vulgaris* [37]. Strains isolated from *Pisum* in Peru are phylogenetically close to *R. leguminosarum* and to *Rhizobium etli*, a species nodulating beans in Mexico [43]. *R. etli* strains were also recovered from pea nodules in China [52].

Several molecular studies on Rlv nodulating lentil were reported [20,26]. Recently Rashid et al. [38] published a phylogenetic study based on 16S rRNA, *recA*, *atpD* and *glnII* genes from *Rhizobium* strains nodulating lentil cultivated in several regions in Bangladesh, showing that the majority of them may represent three separate lineages in the *R. etli* – *R. leguminosarum* clade. Rashid et al. [39] showed that *R. leguminosarum* was the original symbiont of lentil, but that it was associated with new lineages in Bangladesh where it was introduced.

The agronomic and ecological impacts of rhizobia both rely on their symbiotic properties (nodulation, efficiency, specificity and host range) and their adaptation to environmental constraints

affecting nitrogen fixation, such as high temperatures, water stress, salinity or soil acidity [1,53]. The plant genotype itself influences rhizobial population structure in pea nodules [13]. *R. leguminosarum* is sensitive to environmental perturbations, such as agronomic practices and heavy metal soil contamination [12,22,35]. Water deficit, rain irregularity and soil salinity also represent major limitations for plant growth and agronomic production in the Mediterranean zone, particularly in Algeria [29], and rhizobial adaptation to drought stress is variable [5,8,40]. In chickpea, lack of water influences rhizobium diversity, as well as reducing nodule number and aerial biomass [8]. Isolates tolerant to salt stress are often also tolerant to water stress [47]. Large phenotypic and genotypic diversities are often recovered among rhizobial isolates sampled in arid and semi-arid zones (i.e. in Morocco [15], Algeria [9], and Tunisia [30,54]).

Until 2008, legumes of the *Viciae* tribe, including pea and lentil, were considered to nodulate only with Rlv but, since then, *R. pisi* and *R. fabae* have been isolated from pea and faba bean. Most of the studies on Rlv diversity and ecology were conducted in temperate climate zones in Europe. To our knowledge, no study on rhizobia nodulating lentil and pea in Algeria has been reported in the literature. Therefore, in this study, natural *Rhizobium* populations nodulating pea and lentil varieties adapted to environmental constraints were studied in Algeria. The structure and genetic diversity of natural rhizobial populations were examined at several sites representing two contrasting eco-climatic conditions – sub-humid and semi-arid – in Eastern Algeria. Rhizobial isolates were characterised by PCR/RFLP of the 16S–23S ribosomal intergenic region (16S–23S IGS) and the *nodD-F* symbiotic region, as well as by sequence analyses of 16S–23S IGS, 16S ribosomal RNA and *nodD* genes.

Materials and methods

Experimental sites, soil composition and cultivars

The study was carried out on three legume plants, proteaginous pea (*P. sativum* L. subsp. *sativum* var. *sativum* cv. *messire*), forage pea (*P. sativum* L. subsp. *sativum* var. *arvense* cv. *Sefrou*, see Smýkal et al. [44] for new classification of *Pisum*) and lentil (*L. culinaris* L. cv. *P.B. Dahra*). The two pea cultivars cv. *messire* and cv. *Sefrou* were introduced varieties, selected in France and Morocco, respectively. *L. culinaris* L. cv. *P.B. Dahra* was a local cultivar.

The plants were grown in six experimental fields in Eastern Algeria, representing sub-humid to semi-arid climatic zones (Fig. 1). Four well-separated soil samples were collected at the time

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