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Original article

Phenotypic characteristics and genetic diversity of rhizobia nodulating soybean in Egyptian soils

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A B S T R A C T

Twenty rhizobial strains isolated from the root nodules of soybean (*Glycine max* L.) were collected from nine governorates representing different agro-climatic and soil conditions in Egypt. The strains were characterized using a polyphasic approach, including nodulation pattern, phenotypic characterization, 16S rDNA sequencing, *nifH* and *nodA* symbiotic genes sequencing, and rep-PCR fingerprinting. Symbiotic properties assay revealed that all local rhizobial strains showed a wide spectrum of prolific nodulation and a marked increase in plant growth parameters compared to the un-inoculated control. Complete sequencing of 16S rRNA demonstrated that, native soybean nodulating rhizobia are phylogenetically related to *Bradyrhizobium*, *Ensifer* and *Rhizobium* (syn. *Agrobacterium*) genera. Study of tolerance ability to environmental stresses revealed that local strains survived in a wide pH ranges (pH 5–11) and a few of them tolerated high acidic conditions (pH 4). *Agrobacterium* strains were identified as the highest salt-tolerant and were survived under 6% NaCl, however *Ensifer* strains were the uppermost heat-tolerant and can grow at 42 °C. *Agrobacterium* strains have been shown to harbor *nifH* and *nodA* genes similar to those in other fast growing soybean symbionts and were largely distinct from symbiotic genes of slow growing bradyrhizobia. The symbiotic effectiveness stability of *Agrobacterium* strains to nodulate soybean roots was confirmed using plant nodulation assay.

1. Introduction

Soybean (*Glycine max* L. Merrill) is called a miracle golden bean because of its nutritive value, especially as a substitute or complement of protein. Soybean production was 223 million metric tons (MMT) in 2007 [13], accounted for a half of all the leguminous crops [32]. Within the past 60 years, soybean emerged as the dominant oilseed in world trade. In 2005, the USDA [47] estimated world soybean production at 218 MMT, representing about 56% of total global oilseed production.

Soybean like other legumes forms symbiotic relationship with soil bacteria commonly referred to rhizobia [5]. Biological Nitrogen Fixation (BNF) is a fascinating biological phenomenon, which involves some legumes, whether grown as pulses for food, as pasture, in agro-forestry or in natural ecosystems [19]. The process involves the reduction of atmospheric dinitrogen to ammonia (NH_3) [16]. Worldwide 44–66 MMT of N_2 are fixed every year by agriculturally important legumes [1], with another 3–5 MMT fixed by legumes in natural ecosystems [42]. Symbiotic nitrogen fixation by legumes plays an important role in sustaining crop productivity and maintaining soil fertility of the semi-arid lands, like most Egyptian land [56]. Nevertheless, it is sensitive to various environmental stresses such as soil salinity, acidity, alkalinity, temperature which leads to unsuccessful legume nodulation. Hence, identification of bacterial strains and host cultivars that are tolerant to these stresses [3,53] will give rise to a more sustainable agriculture and it would open the way for alternate, lower cost solutions to these problems.

Soybean-nodulating rhizobia are genetically diverse and are classified into different genera and species. The slow growing bradyrhizobia, that effectively nodulate soybeans are *Bradyrhizobium japonicum* [21], *Bradyrhizobium elkanii* [23], *Bradyrhizobium liaoningense* [52] and *Bradyrhizobium yuanmingense* [4]. Other symbionts of soybean are fast growing and classified as *Ensifer* (*Sinorhizobium*) *fredii* [39] and *Ensifer xinjiangense* [34]. In addition

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to the “traditional” rhizobial genera, strains of the genus *Agrobacterium* (now, rather controversially, included in the genus *Rhizobium* [14,55]) have been frequently isolated from root nodules of various legumes including *Phaseolus vulgaris* [30], *Sesbania* spp. [7] and *Vicia faba* [46]. However, little information has been published regarding to nodulation of soybean by *Agrobacterium* [6]. These strains have been identified as *Agrobacterium* based on biochemical studies, numerical taxonomy and 16S rRNA-restriction fragment length polymorphism (RFLP) [27].

Till now there is no report addressed the phylogeny of rhizobial population associated with soybean in Egypt. Therefore, this original research is aiming at exploration the biodiversity of rhizobial population that nodulate soybean under different Egyptian agroclimatic conditions using ribosomal gene (16S rRNA) and symbiotic genes (*nifH* and *nodA*) sequencing and rep-PCR fingerprinting (REP, ERIC and BOX).

2. Materials and methods

2.1. Germplasm collection

Soybean root nodules have been collected from nine governorates that grow soybean in Egypt (Table 1 and Fig. 1). The emerging nodules which showed nitrogen fixation activity by the presence of leghaemoglobin were collected from healthy plants 50 days after sowing. The collected nodules were carefully excised from the roots with scalpel, washed by water and stored on silica gel containing vials till isolation process.

2.2. Bacterial isolates and reference strains

Root nodules were surface sterilized by washing for 30 s with 95% ethanol, immersed in 3% sodium hypochlorite and finally were washed six times by sterile double distilled water. Following sterilization, nodules were crushed aseptically in 1 ml sterile double distilled water, the nodules extract were streaked on surface of Yeast Extract Mannitol Agar (YEM) plates supplemented with 0.025 g L^{-1} of Congo red and finally were incubated at 28 °C for 3–6 days [45]. The reference strains used in this study were obtained from The Biological Nitrogen Fixation Unit, Agricultural Research Center (ARC), Giza, Egypt and Genetic Engineering and Biotechnology Research Institute (GEBRI), City for Scientific Research and Technology Applications, Alex., Egypt.

Fig. 1. GIS map of soybean nodulating rhizobia collection sites.

2.3. Plant nodulation test

All the 20 rhizobial isolates and two reference strains were tested for their ability to nodulate soybean plants under sandy soil condition in pot experiment. The soil was analyzed according to Page et al. [33]. The main physical and chemical properties of

Table 1
List of rhizobial local isolates used in this study and their geographical origin.

Isolate	Site/governorate	Longitude	Latitude	Soil texture
NGB-SR 1	Nubaria Area/Behaira	29 51 50.634	30 54 48.22	Sandy loam (calcareous)
NGB-SR 2				
NGB-SR 3				
NGB-SR 4	Sakha village/Kafr ElSheikh	30 56 49.652	31 5 38.543	Clay (fertile)
NGB-SR 5				
NGB-SR 6				
NGB-SR 7				
NGB-SR 8				
NGB-SR 9	Agricultural Research Center/Giza	31 12 49.272	29 58 55.046	Clay loam (fertile)
NGB-SR 10	Al Batanoon, Shbeen Al Koom/Menoufia	30 59 14.539	30 37 44.906	Clay loam (fertile)
NGB-SR 11	Tersa, Tookh/Qalyoubia,	31 11 41.472	30 18 25.516	Clay loam (fertile)
NGB-SR 12	Sedse village/Beni Suef	31 05 25.775	29 04 54.349	Clay (fertile)
NGB-SR 13	Behidah, Menia/Menia	31 13 2.0520	30 26 42.942	Clay (fertile)
NGB-SR 14	Samaloot/Menia	30 42 0.4500	28 17 85.896	Clay (fertile)
NGB-SR 15	Abo Qorqas/Menia	30 49 56.280	27 55 34.590	Heavy clay (highly fertile)
NGB-SR 16	Baroot, Beni Suef/Beni Suef	31 01 17.781	29 04 15.800	Clay (fertile)
NGB-SR 17	Fourikah, Bebba/Beni Suef	30 57 53.053	28 56 8.1410	Clay (highly fertile)
NGB-SR 18	Gemizah village/Gharbia	31 7 50.8430	30 44 53.297	Clay (fertile)
NGB-SR 19	Al Khargah, New Valley	30 33 38.726	25 26 6.565	Sandy (low fertile)
NGB-SR 20				