



# Structural and regulatory characteristics of *Bradyrhizobium japonicum* and *Bradyrhizobium elkanii* isolates recovered from soils cultured with soybean in a zero tillage management.

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## ABSTRACT

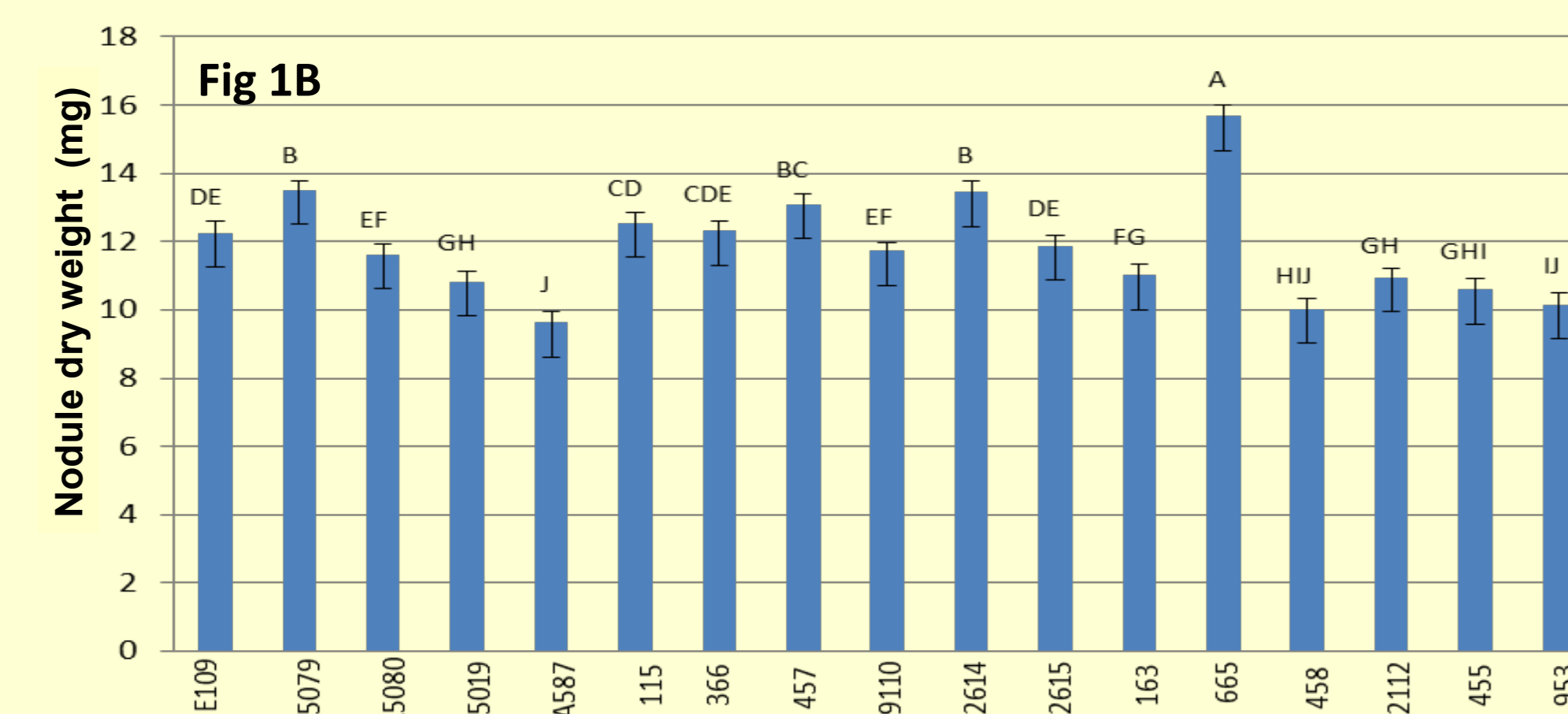
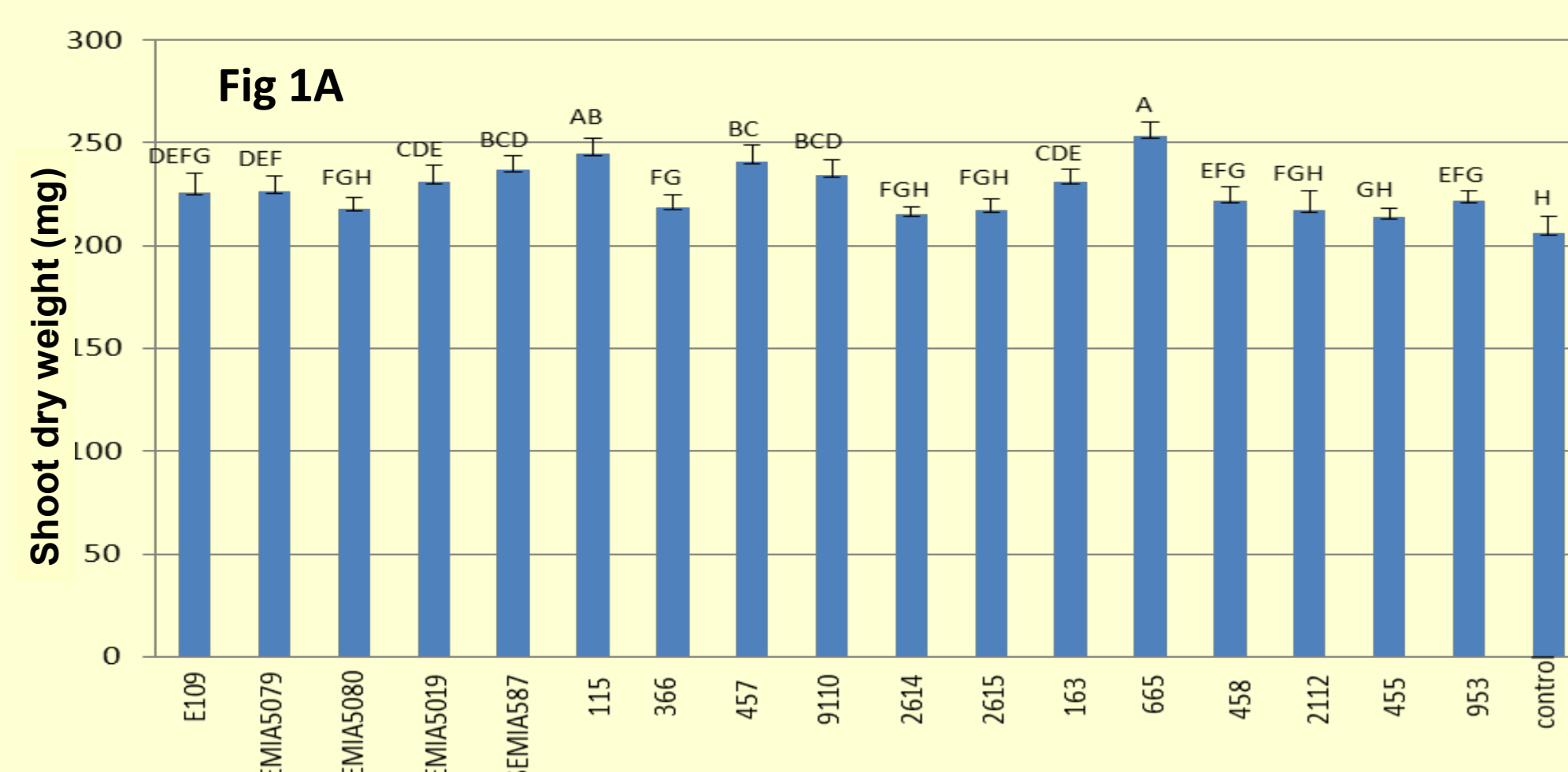
Rhizobia nodulating soybeans were isolated from soils cultivated under zero tillage management for approximately 10 years. The isolates (250) were found to be siblings of strains of *B. japonicum* and *B. elkanii* used in commercial inoculants. A subset of 12 of them were selected based upon either their high or low nitrogen fixing activity, compared to control strains E109, Semia 5080, Semia 5079, Semia 587 and Semia 5019, which are the strains used in commercial inoculants in Argentina, Brazil and Uruguay. The similarity of the isolates was assessed by means of BOX, REP-PCR and Rsa sequences. Our hypothesis is that differences in the strains ability to fix nitrogen might be due to structural alterations that occurred in the two genome fragments containing most of the nitrogen fixing genes (Kaneko *et al.*, 2002) or to differential expression of nitrogen fixation genes. We are currently analyzing the structural features of the DNA fragments containing the nitrogen fixation genes. Our preliminary assays consisted in the amplification of a 1169 bp fragment containing *nifD* that was restricted with *EcoR V*. We found three different restriction patterns. Also we amplified a 3.5 kb fragment containing *Rsa*, *RSB* and *nifD* that is sequences where mutations might occur at different rates. We are currently setting up amplification reactions aimed at amplifying in four different reactions the nitrogen fixation genes in order to evaluate if the strains lack similarity at this regions.

## INTRODUCTION

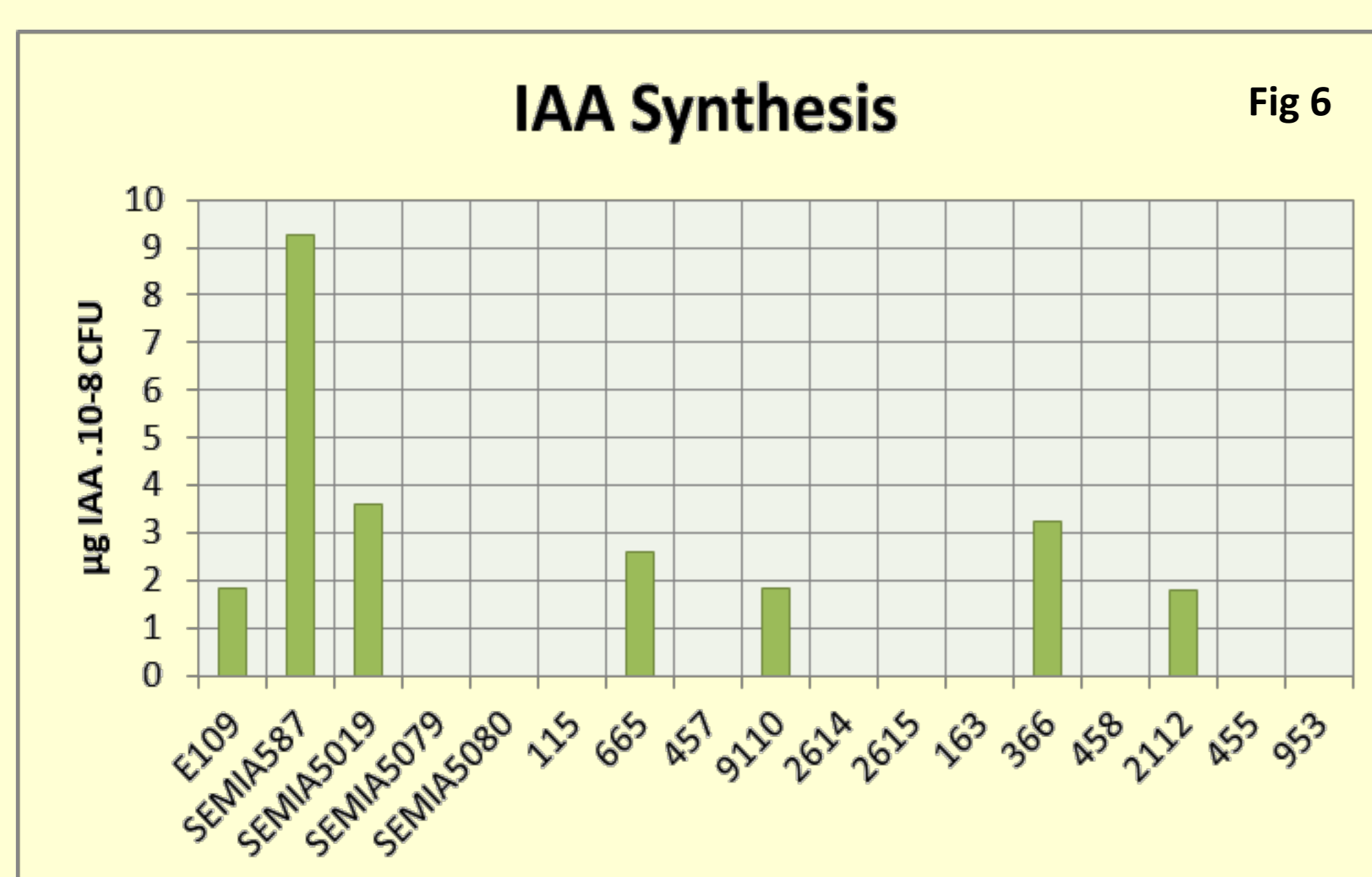
Rhizobia nodulating soybean were isolated from soils cultivated with soybean under conventional and zero tillage. Soil dilutions were used to inoculate an improved soybean cultivar to estimate by the most probable number the population of rhizobia of these soils. We isolated 200 strains that were analyzed based on several physiological characteristics and BOX and ERIC fingerprinting. The soils contained only slow growing strains that were either *B. japonicum* or *B. elkanii*, derived from strains introduced by means of commercial inoculants. The isolates differed in their ability to nodulate and fix nitrogen in association with soybean. Therefore the aim of this research is to identify among the 200 isolates strains that differ in their ability to fix nitrogen, which might be the result of structural differences along the DNA coding for nitrogen fixation genes or due to differential expression of nitrogen fixation genes. Therefore we are analyzing the structural features of these strains among the two most important genome fragments containing nitrogen fixing genes

## MATERIALS AND METHODS

A composed soil sample of fields managed under conventional and zero tillage were taken from 9 different fields. A soil dilution series from each sample was used to inoculate soybean cv to estimate the Most Probable Number of rhizobia. To assess diversity strains were isolated from nodulated soybeans of the most and less diluted soil suspension. The isolated strains were reisolated from inoculated soybeans grown under control conditions in the greenhouse. They were evaluated for their ability to fix nitrogen by measuring nodule and plant biomass. The isolates were fingerprinted by means of BOX, REP and repetitive sequence PCR (de Bruijn, 1992; Minamisawa *et al.*, 1998). As controls we included the strains used to formulate commercial inoculants in Brazil, Uruguay and Argentina. In addition to this the isolates were also characterized by means of the RFLP of the 16S-23S ITS, which was amplified as described by (Kwon S. W. *et al.*, 2005) and then was restricted with *Hind III*. Also, we amplified the *nifD* made a preliminary analysis by *EcoR V* restriction analysis.

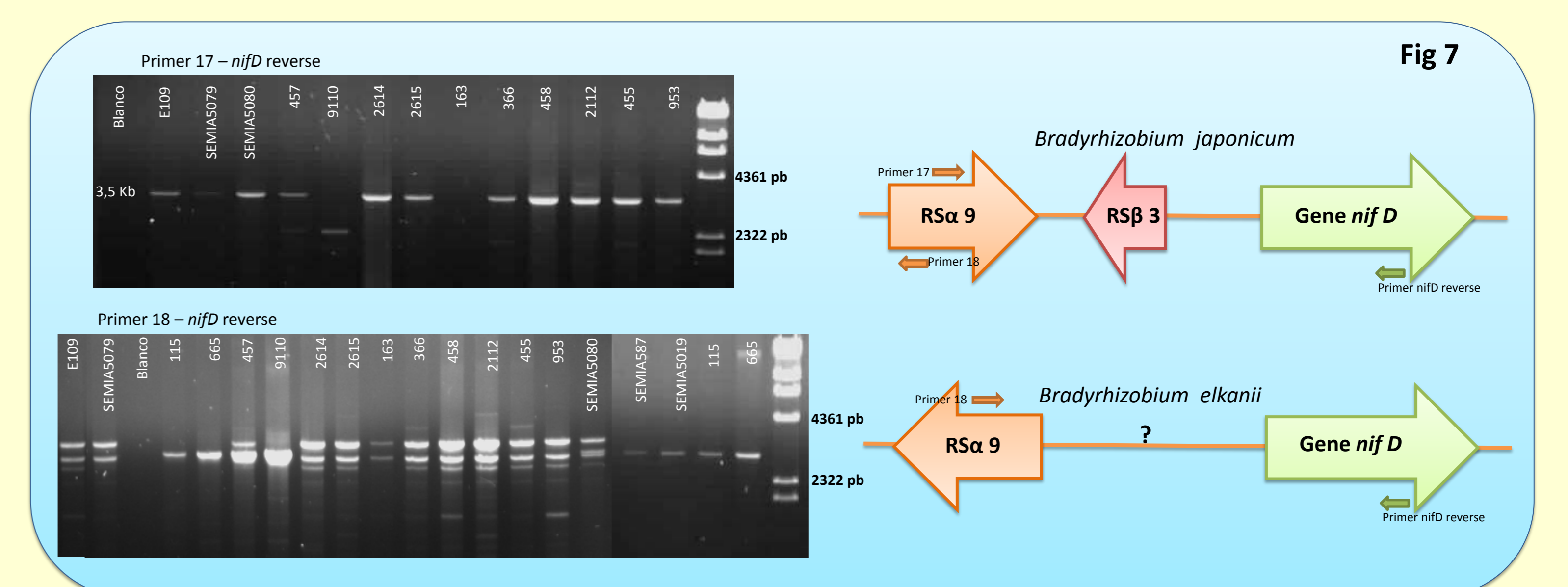
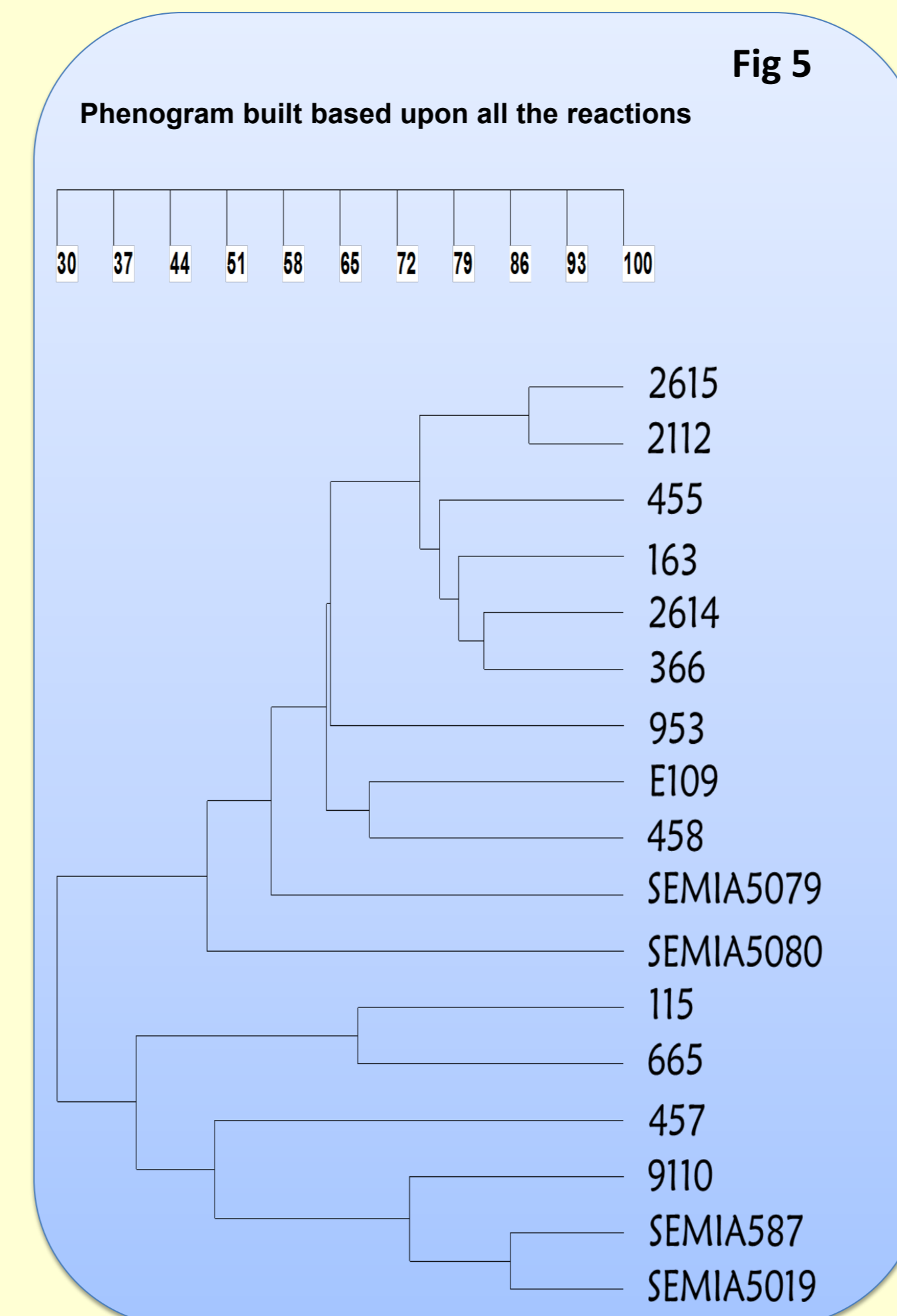
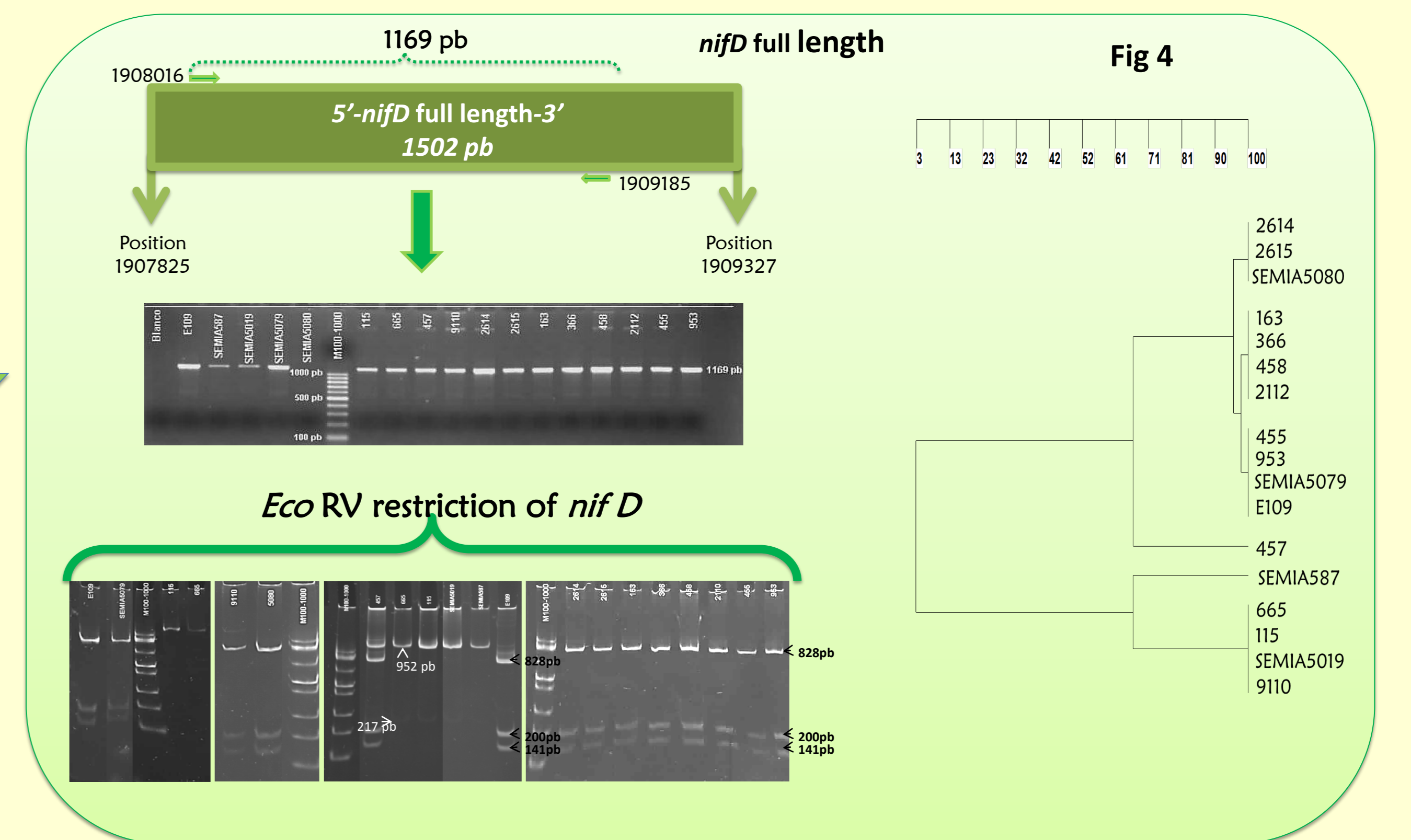
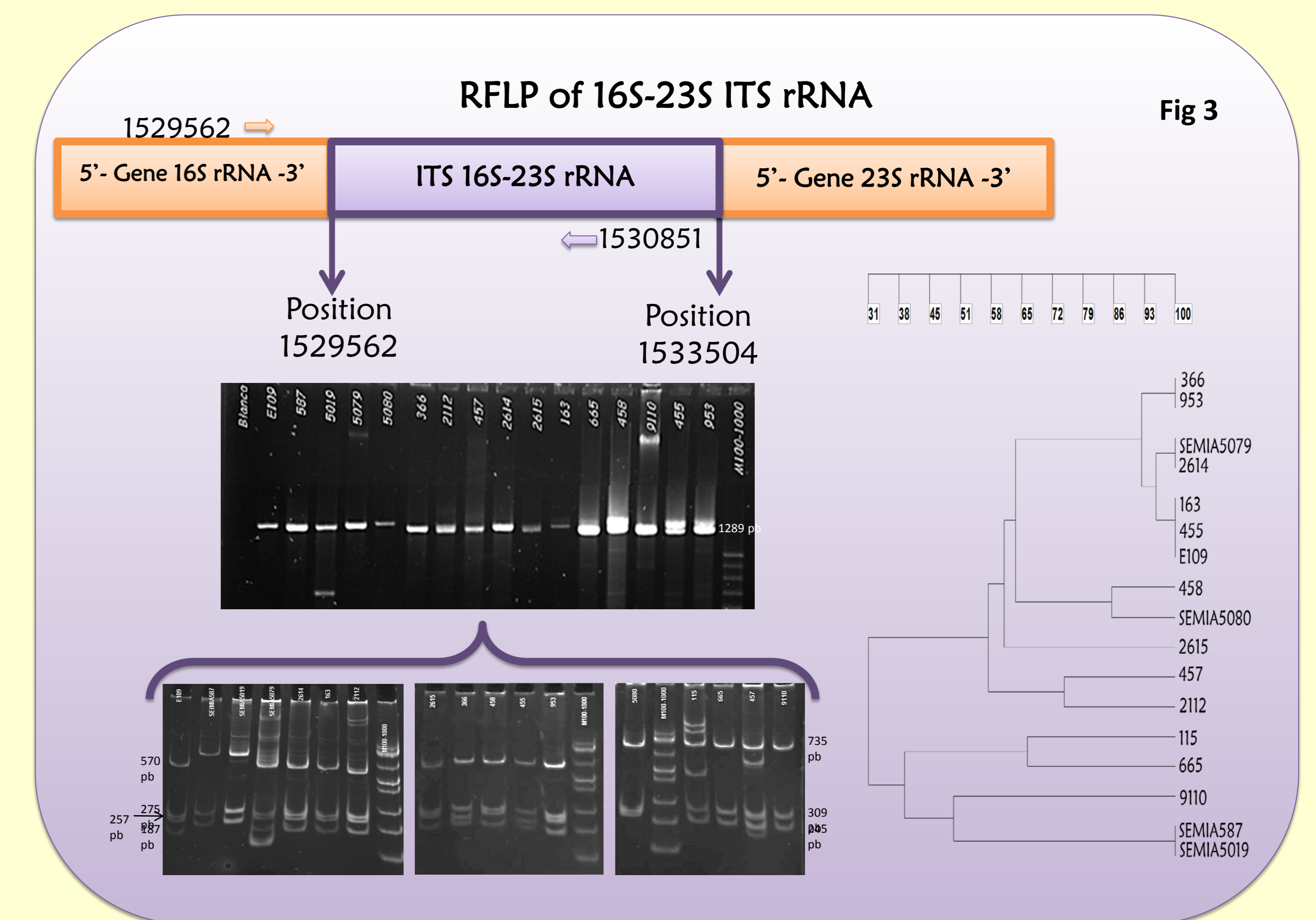
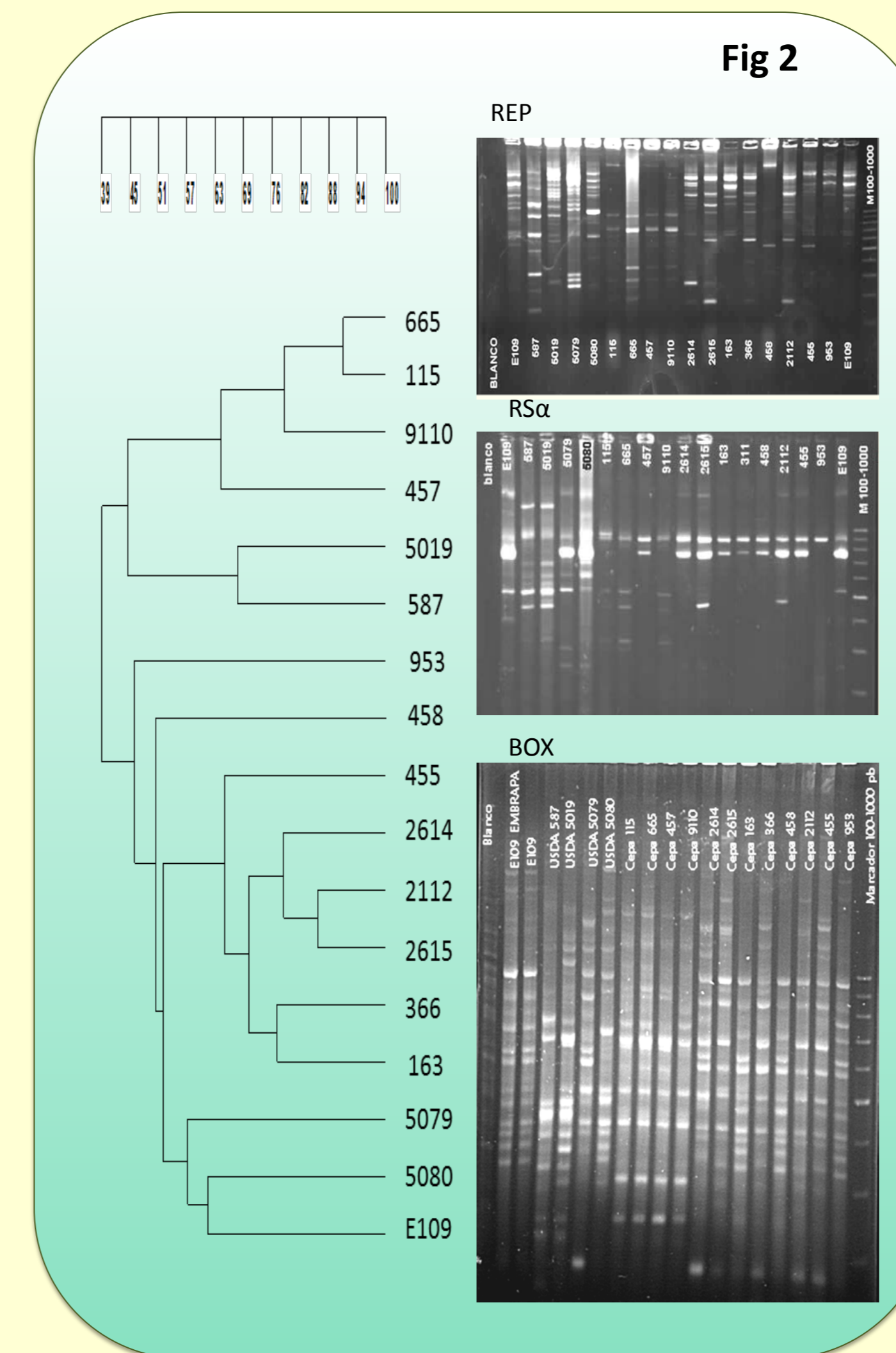


Soybean cultivar La Tijereta Syngenta inoculated with control strains and bradyrhizobia isolated from the soils. The plants were cultivated in modified Leonard Jars filled with vermiculite and watered with a N free nutrient solution. Plants were harvested 21 days after inoculation. Similar letters indicate no significant difference (Tukey at 5%). The bars indicate the standard deviation for each treatment. 1: E109; 2: SEMIA5079; 3: SEMIA5080; 4: SEMIA5019; 5: SEMIA587; 6: 115; 7: 665; 8: 457; 9: 9110; 10: 2614; 11: 2615; 12: 163; 13: 366; 14: 458; 15: 2112; 16: 455; 17: 953; 18: control.



## REFERENCE

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Kwon SW, Park JY, Kim JS, Kang JW, Cho YH, Lim CK, Parker MA, Lee GB. 2005. Phylogenetic analysis of the genera *Bradyrhizobium*, *Mesorhizobium*, *Rhizobium* and *Sinorhizobium* on the basis of 16S rRNA gene and Internally Transcribed Spacer region sequences. Int. J. Syst. Evol. Microbiol. 55, 263-270.  
Minamisawa K., T. Isawa, Y. Nakatsuka and N. Ichikawa. 1998. New *Bradyrhizobium japonicum* strains that possess high copy numbers of the repetitive sequence Rsa. Applied and Environmental Microbiology. 65, 1845-1851.



## RESULTS

The isolates as shown in Fig. 1A and 1B differ in their ability to nodulate and fix nitrogen.

Based on the BOX, REP and Repetitive Sequence fingerprints we found that among all the isolates included in this analysis four were clustered with *B. elkanii* and 8 with *B. japonicum* (Fig. 2). These results were confirmed by the ITS and *nifD* restriction analysis (Fig. 3 and Fig. 4). If all the markers are included in the analysis a phenogram is built showing that among the strains 8 are *B. japonicum*, 3 *B. elkanii* and one strain has intermediate characteristics between these two species (Fig. 5)

In addition to this we confirmed the identity of the strains by measuring the ability to synthesize IAA (Gordon and Weber, 1950) as expected *B. elkanii* strains produced high levels of IAA (Fig. 6).

We are currently amplifying the 3.5 kb DNA fragment between *nifD* and repetitive sequences (Fig. 7) seeking to find structural differences within this region.

## CONCLUSIONS

The soils of Argentina contain naturalize strains of *B. elkanii* and *B. japonicum*.

These isolates differ in their ability to fix nitrogen.

A higher number of *B. japonicum* than of *B. elkanii* were found, which might be related with the fact that most inoculants in Argentina contain *B. japonicum*. Therefore *B. japonicum* does not appear to have an ecological advantage in the sampled soils.

One isolate share characteristics of *B. japonicum* and *B. elkanii* and the strain deserves further attention.

We are currently analyzing if there are structural differences within two 25 kb fragments containing the nodulation and nitrogen fixation genes.