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Comparative genomics as a tool for rapid identification of elite indigenous soybean nodulating Rhizobia in South Kivu, D.R. Congo

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ABSTRACT

The use of indigenous rhizobia as inoculants is the best option for improving soybeans productivity and Biological Nitrogen Fixation (BNF). The selection method to date of effective rhizobia nodulating legumes among indigenous population to be included in inoculants formula is time consuming. We sequenced 24 genomes of indigenous Soybean nodulating rhizobia (SNR) isolated from soybean's root nodules grown in South Kivu province of DRC in order to identify rapidly candidate elite strains. Full genomes sequences of 24 indigenous rhizobia were obtained on Miseq, libraries prepared using Nextera xt protocols and compared with genome of the commercial strain *Bradyrhizobium japonicum* USDA 110 (accession number CP011360.1). The genomic features were determined and the presence of nitrogen fixation genes detected. Out of 24 samples, we obtained 14 high quality genomes of indigenous SNR, of mean size of 8.383 Mb ±0.762 bp with mean GC content of 62%. These SNR belonged mostly to *Bradyrhizobium* (64%) genus and few to *Rhizobium*, *Microvirga* and *Kosakonia*. Their chromosomes comprised a mean of 8063±975 genes and 99% of these were protein-coding genes. The full set of nodulation genes (*nod and nif*) were detected in 11 out of 14 indigenous strains and eight genomes of indigenous strains (NAC53, NAC46, NAC22, NAC76, NAC37, NAC17, NAC28 and NAC42) were close to the commercial strains USDA110 and can be considered as candidate elite strains. We conclude that comparative genomics can be used is a tool for rapid identification of elite strains.

Key words: Genomics, *Glycine max*, indigenous rhizobia, rhizobia selection, South Kivu

RÉSUMÉ

L'utilisation de rhizobiums indigènes comme inoculants est la meilleure option pour améliorer la productivité du soja et la fixation biologique de l'azote (BNF). La méthode actuelle de sélection des légumineuses rhizobia nodulantes efficaces parmi la population indigène à inclure dans la formule des inoculants est longue. Nous avons procédé au

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séquencage de 24 génomes de rhizobium indigènes nodulant le soja (SNR) isolés à partir de nodules racinaires de soja cultivés dans la province du Sud-Kivu en RDC afin d'identifier rapidement les souches potentielles le plus performantes. Les séquences complètes des génomes de 24 rhizobiums indigènes étaient obtenues sur Miseq, à partir des bibliothèques préparées à l'aide du kit Nextera xt et étaient comparées au génome de la souche commerciale *Bradyrhizobium japonicum* USDA 110 (numéro d'accès CP011360.1). Les caractéristiques génomiques étaient déterminées et la présence de gènes de fixation de l'azote était détectée. Sur 24 échantillons, nous avons obtenu 14 génomes de SNR indigènes de haute qualité, de taille moyenne de 8,383 Mb ± 0,762 bp avec une teneur moyenne en GC de 62 %. Ces SNR appartenaient majoritairement au genre *Bradyrhizobium* (64%) et peu à *Rhizobium*, *Microvirga* et *Kosakonia*. Leurs chromosomes comprenaient en moyenne 8063 ± 975 gènes et 99 % d'entre eux étaient des gènes codant pour des protéines. L'ensemble complet des gènes de nodulation (*nod et nif*) a été détecté dans 11 des 14 souches indigènes et huit génomes de souches indigènes (NAC53, NAC46, NAC22, NAC76, NAC37, NAC17, NAC28 et NAC42) étaient proches des souches commerciales USDA110 et peuvent être considérées comme des souches potentielles élites. Nous concluons que la génomique comparative peut être utilisée comme outil d'identification rapide des souches élites.

Mots clés: Génomique, *Glycine max*, rhizobium indigène, sélection de rhizobium, Sud Kivu

INTRODUCTION

The Rhizobium–legume symbiosis, characterized by the formation of root nodules, is the most important bacteria–plant interactions (Hirsch *et al*., 2001). It is an important process in sustainable agriculture, as this symbiotic association is able to enhance soil nitrogen status and legume productivity (Alves *et al*., 2003). The symbiosis involving soybean is the most exploited in the world because it produces as much as 300 kg of N ha^{-1} in addition to the release, in the soil, of 20–30 kg N ha^{-1} (Hungria *et al*., 2013). This system is of more benefit in Developing World and particularly in South Kivu province (Eastern DRC) where most farmers are poor with very limited possibilities to improve soil fertility (Walangululu *et al*., 2011). Thus, yield of important crops including legumes remains very low (FAO, 2018). Soybean (*Glycine max*) is an important legume for its high protein and edible oil content; it has been considered for long time as "meat for the poor" (Hartman *et al*., 2011).

Successful Biological Nitrogen Fixation (BNF) by symbiosis legume-rhizobia depends on both good legume genotype and dominating nodule occupancy with highly and adapted efficient rhizobia strains (Alves *et al*., 2003; Checcucci *et al*., 2017). There exist numerous studies on selection of highly effective rhizobia among indigenous populations (O'Hara *et al*., 2002). The empirical approach of selecting highly effective and competitive rhizobia strains consists of 1) native rhizobia strains collection, isolation and authentication, 2) isolates screening against reference strains for symbiotic effectiveness, 3) competitiveness for nodules occupancy testing, and 4) isolates performance testing under varied field conditions (Yates *et al*., 2005). This selection method is time consuming and thus, there is need for an adequate and rapid selection approach.

Some studies have demonstrated recently the effectiveness of genomic approaches on detection of genetic component associated with nodules formation in rhizobia, nitrogenase regulation and other processes involved in BNF (Amadou *et al*., 2008). In this study, we sequenced and analysed the sequences of 24 indigenous Soybean Nodulating Rhizobia isolated from South Kivu in the DRC in order to identify genetic components associated with high N and high productivity in

these rhizobia and detect highly effective strains.

MATERIALS AND METHODS

Genomic DNA extraction, libraries preparation and sequencing. Rhizobia cultures were obtained from N2 Africa project of International Institute of Tropical Agriculture. Subquently, DNA was extracted using Qiagen Plant Mini kit following the manufacturer's instructions (Qiagen, Hilden, Germany) (Ghimire *et al*., 2010; Di Bella *et al*., 2013). The DNA quality check was performed on 0.8% agarose-buffer TAE and read on UV light using GelDoc-It2 imager (Batista *et al*., 2017). The concentration of DNA was measured using Qubit High Sensitivity (Batista *et al*., 2017). In brief, a mean of 450 base-pair libraries preparation was done by the Nextera™ XT Library Prep Kit following the manufacturer'instructions (Illunina, San Diego) (Ring *et al*., 2017). Genomes sequencing was conducted at the Bioscience Eastern and Central Africa of International Livestock Research institute (BecA-ILRI), Nairobi Kenya. Reads were generated on an Illunima MiSeq instrument.

Analysis of Sequences. Raw reads obtained from MiSeq sequencer were analyzed for quality using fastqc software (Leggett *et al*., 2013). Low quality reads were removed by Trimomatric and loaded in CLC main Workench version 7 for denovo assembling (Li *et al*., 2010). Assembled sequences were assembled first in contigs and then in scaffolds using SSPACE Basic software version 2.0.and Unicycler version 0.4.7. Scaffolds were mapped to references genomes *Bradyrhizobium diazoefficiens* USDA110 (accession number CP011360.1) (Sablok *et al*., 2017).

Genome annotation and data analysis. Improved scaffolds were submitted to Blastn program available in NCBI genebank (www.ncbi.nlm.nih.gov/blast) for strains identification and were used for gene prediction and annotation using Prokka bacteria annotation tool which uses Prodigal. Descriptive statistics were performed in XLstat version 2014. Genomes were compared to the commercial strain based on genome size, chromosome number and sizes, number of proteins-coding genes, C-G content and number of nitrogen fixation genes.

RESULTS AND DISCUSSION

Genomes quality. From 24 genomes sequences, 14 yielded higher quality genomes were considered for this study. The Figures 1 presents the per base quality scores of the sample NAC22. From this Figure the quality is scored using 0-38 scale, the acceptable quality scores starting from 20 to 38. From the Figure, most of the sequences were of highest quality. Quality control of sequences is very important for meaningful analysis. In a study conducted by Degnan and Ochman (2012), up to 85% of sequences were removed because they did not meet the treshhold accuracy.

General genomic features. Genomes characteristics are summarized in Table 1. Indigenous rhizobia strains nodulating soybeans from South Kivu belonged mostly to Bradyrhizobium (62%) and few to Rhizobium, *Agrobacterium, Kosakonian* and *Microvirga*. *Bradyrhizobium* genus was the most represented genus and this genus is mostly associated with soybean. These findings corroborate those of other authors who found that soybean is nodulated mostly by *Bradyrhizobium* genus in tropical soils (Wasike *et al*., 2009; Li *et al*., 2011; Chibeba *et al*., 2017; Gyogluu *et al*., 2018). Genomes size varied considerably (CV=14.6%) among indigenous strains and ranged from 5.669Mb and 9.963Mb with the mean genomes estimated at 8.383 Mb in size (Table 1). This size is consistent with other findings, for example those of Kaneko *et al*. (2011) and Bromfield *et al*. (2019). From this study, seven indigenous strains (NAC1, NAC22, NAC76, NAC37, NAC17, NAC28 and NAC42) hold a genome size closer to the commercial strain USDA110.

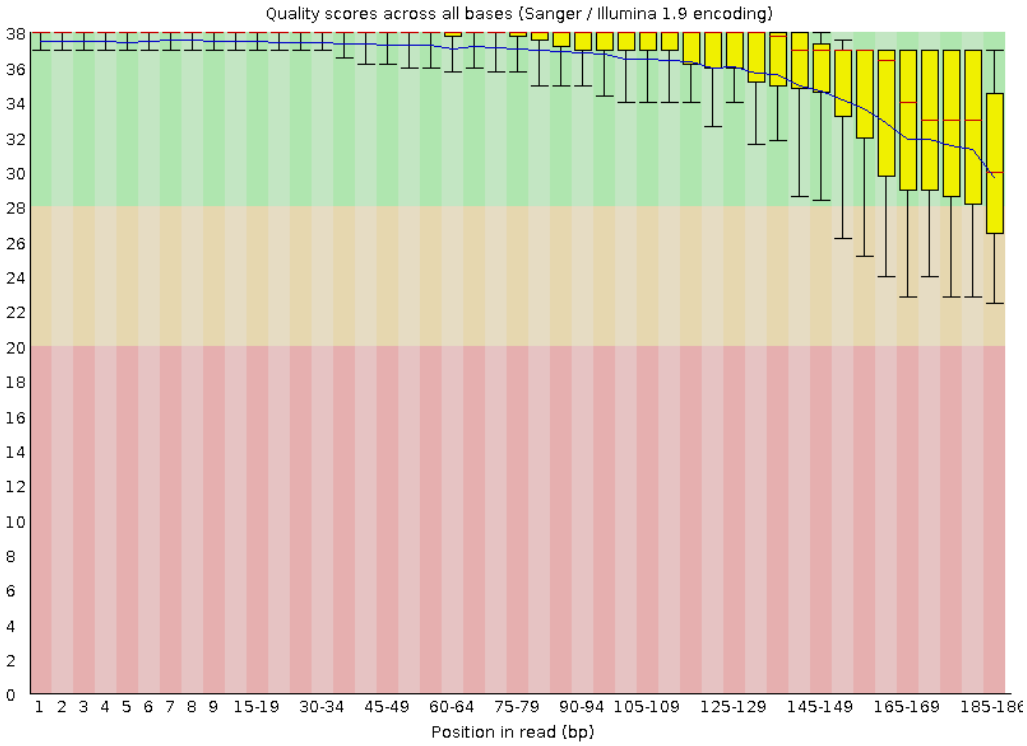

Figure 1. Quality score of sample NAC22

Identification of candidate elite strains by comparative genomics. The genes involved in nitrogen fixation such as nod, fix and nif were detected in genomes to identify candidates elites strains. The number of nod, fix and nif genes recorded in indigenous soybean-nodulating rhizobia and soybean-nodulating commercial strain USDA110 are presented in Table 2. The nif genes number recorded in indigenous strains varied from 0 to 2 while 2 to 11 were recorded for fix genes 4 to 11 for nod genes. In this study, 11 soybean-nodulating rhizobia strains (NAC53, NAC46, NAC37, NAC17, NAC28, NAC42, NAC69, NAC71, NAC72, NAC11, NAC94) out of 14 (Table 5) possess the full set of the nitrogen fixation and could be considered for a program of selection of effective rhizobia to be included in the inoculants commercial. The presence of the full set of nitrogen fixation genes is equivalent to higher capacity of both nodulation and nitrogen fixation. Many authors sustain that the presence of nitrogen fixation is essential for nodules formation and consequently for nitrogen fixation. For example, Okazaki *et al*. (2015) found *Bradyrhizobium* sp. DOA9 strain of particular biological interest because it possessed divergent nod genes compared with other bradyrhizobia and consequently a broader host range.

In addition, indigenous and commercial strains were compared based on their 16S rRNA region approximating 1400bp. Based on this phylogeny tree (Figure 2), indigenous soybean-nodulating rhizobia and the commercial strain USDA110 were partitioned into two main clusters. From this classification, six indigenous strains (NAC28, NAC42, NAC46, NAC76, NAC37 and NAC17) clustered together with the commercial strain USDA110 (98% bootstrap value) suggesting that they may have similar genomic features and thus could be considered as candidate elite strains. This finding corrobotes past studies that demonstrated that indigenous rhizobia are similar to commercial rhizobia in terms of legume grain yield improvement (Tena *et al*., 2016; Aboushanab *et al*., 2019) and genetically (Kawaka *et al*., 2018; Mwenda *et al*., 2018)

Table 1. Summary of genomics features

Strain	Identity (NCBI)	genome size (Mb)	G-C content (%)	number of genes	Protein-coding genes	tmRNA	tRNA
NAC1	*Agrobacterium* sp.	9.247	58.04	8747	8667	2	78
NAC53	*Bradyrhizobium diazoefficiens*	7.722	60.53	7376	7325	1	50
NAC46	*Bradyrhizobium diazoefficiens*	8.327	63.12	8200	8148	1	51
NAC22	*Bradyrhizobium elkani*	9.082	63.58	8567	8516	1	50
NAC76	*Bradyrhizobium japonicum*	9.963	63.47	9656	9597	1	58
NAC37	*Bradyrhizobium ottawaense*	9.567	63.68	9031	8973	1	57
NAC17	*Bradyrhizobium ottawaense*	9.312	63.27	9224	9174	1	49
NAC28	*Bradyrhizobium* sp.	9.469	63.90	9015	8962	1	52
NAC42	*Bradyrhizobium* sp.	9.024	63.95	8893	8840	1	52
NAC69	*Kosakonia oryzae*	5.640	54.25	5390	5314	1	75
NAC71	*Microvirga ossetica*	6.973	62.27	6830	6757	2	71
NAC72	*Microvirga ossetica*	7.019	62.28	6887	6847	2	71
NAC11	*Rhizobium jaguaris*	7.557	59.48	7170	7119	1	50
NAC94	*Rhizobium leguminosarum*	7.740	60.54	7390	7337	1	52
USDA110	*Bradyrhizobium diaazoefficiens* (Kaneko *et al*., 2002)	9.110	64.10	8571	8317	1	50
Min		5.640	54.25	5390	5314	1	49
Max		9.963	64.10	9656	9597	2	78
Mean		8.383	61.76	8063	7992	1,20	57.73
CV(%)		14.61	4.49	14.51	14.61	34.50	18.05

Table 2. Number of nif, nod and fix genes

Strain	nif	Nod	fix
NAC1	0	5	2
NAC53	1	8	7
NAC46	1	7	4
NAC22	0	11	5
NAC76	0	10	10
NAC37	1	7	10
NAC17	1	9	11
NAC28	1	8	6
NAC42	1	8	6
NAC69	1	4	2
NAC71	1	9	7
NAC72	1	10	7
NAC11	2	7	6
NAC94	1	8	7
Min	0	4	2
Max	2	11	11
Mean	0.85	7.92	7,57
CV	62.36	23.96	41.14

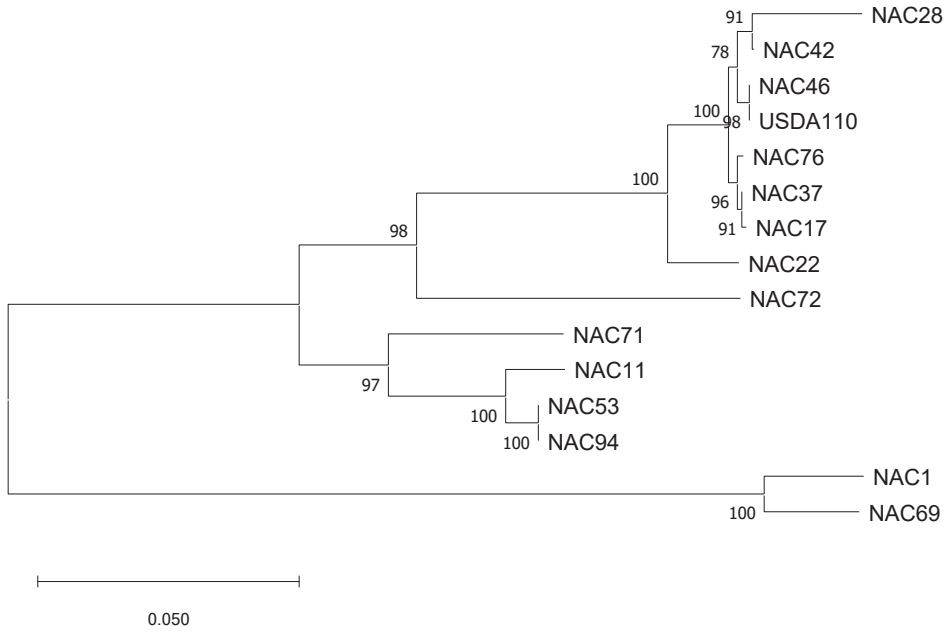

Figure 2. Phylogeny tree based on 16SrRNA constructed using Tamura-Nei model and Maximum Likelihood method. Bootstrap values are shown next to the branches

CONCLUSION AND RECOMMENDATIONS

This study has demonstrated the existence of indigenous soybean-nodulating rhizobia in South Kivu soils that have same genomics characteristics as the commercial rhizobia USDA110. These indigenous rhizobia strains exhibited comparable nitrogen fixation characteristics comparable to the commercial strain USDA110. We suggest further investigations and testing of these indigenous rhizobia under different environmental conditions to confirm their nitrogen fixation superiority. Finally, comparative genomics can be considered for rapid selection of effective rhizobia to be included in commercial formulation but must always be coupled with field testing.

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STATEMENT OF NO CONFLICT OF INTEREST

The authors declare that there are no competing interests in this publication.

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