Applied Soil Ecology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

Is there a need for *Bradyrhizobium yuanmingense* and *B. japonicum* reinoculation in subsequent cropping seasons under smallholder farmers' conditions?

Jacob Ulzen[a], Robert Clement Abaidoo[b,d,*], Cargele Masso[c], Emmanuel Degraft Johnson Owusu-Ansah[e], Nana Ewusi-Mensah[a]

[a] Department of Crop and Soil Sciences, Faculty of Agriculture, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana
[b] Department of Theoretical and Applied Biology, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana
[c] International Institute of Tropical Agriculture (IITA), Nairobi, Kenya
[d] International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria
[e] Department of Mathematics, College of Science, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana

ARTICLE INFO

Keywords:
Bradyrhizobium yuanmingense
Hyperbolic function
B. japonicum
Survival rate

ABSTRACT

Reliable information on the persistence of rhizobium in soil in the absence of host between growing periods is important in deciding whether inoculation on the same plot in subsequent seasons is necessary. This study determined the survival of introduced rhizobium strains and predominant factors that influence the declining rates of their populations. *Bradyrhizobium yuanmingense* (BR 3267) and *B. japonicum* (USDA 110) were manually incorporated into soils at four different locations (Kpalga, Tanina, Tunayilli and Busa) in northern Ghana at 2.5×10^8 ($\log_{10}$ 8.4) and 2.5×10^7 ($\log_{10}$ 7.4) cells g^{-1} peat, respectively, per 6 m^2. The populations of surviving cells were estimated at 0, 21, 42, 81, 142 and 296 days using the Most Probable Number (MPN) count technique. Several decline functions were applied to the data with hyperbolic regression function emerging as the option that provides the best fit for *B. yuanmingense* strain BR 3267 and *B. japonicum* strain USDA 110 at all locations. There was no significant difference in the declining rates between the different locations; however, there were differences in the declining rates for the sampling times. At 296 days, the numbers of surviving cells of *B. yuanmingense* strain BR 3267 and *B. japonicum* strain USDA 110 were $\log_{10}$ 1.9 and $\log_{10}$ 1.7, respectively. Native rhizobium population and soil moisture were the predominant factors that affected the survival of the introduced strains. It is evident from the studies that these strains can survive in sufficient numbers at least within a year; therefore, re-inoculation may not be necessary for a following season especially when using *B. yuanmingense* strain BR 3267.

1. Introduction

The need to re-inoculate legumes depends on resident rhizobia population, and more importantly the ability of the introduced rhizobia to survive in the absence of the host plant (Triplett et al., 1993). The survival rate is affected by many abiotic and biotic factors but the predominant ones are soil moisture, rainfall, soil temperature and native rhizobia population (Slattery et al., 2004). A desirable strain must be saprophytically competent in sufficient numbers after the growing period of the host plant. This is usually expected of the introduced strains, which must overcome the abiotic stress of their new environment to infect the host legumes (Vachot-Griffin and Thies, 2005). Native rhizobia are well adapted to local conditions and are widely perceived to have competitive advantage over introduced strains resulting in poor colonization and establishment of the latter. Therefore, introduced populations must be in numbers large enough to overcome the competitive advantage of the indigenous populations.

Most of the persistence studies in sub Saharan Africa have focused on greenhouse assessment of previously inoculated fields (Sanginga et al., 1996; Zengeni et al., 2006). Oves et al. (2017) demonstrated the survival of *Ensifer adharens* in the presence of heavy metals under laboratory conditions. However, it is known that conclusions from the works conducted in greenhouse or laboratory conditions do not always reflect strain performance in the field environment (Pitkajarvi et al., 2003). In parallel, few studies have addressed the persistence of introduced strains in the field (e.g., Woomer et al., 1992; Duodu et al.,

* Corresponding author at: Department of Theoretical and Applied Biology, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana.
E-mail address: rcabaidoo@knust.edu.gh (R.C. Abaidoo).

https://doi.org/10.1016/j.apsoil.2018.04.003
Received 15 February 2018; Received in revised form 27 March 2018; Accepted 4 April 2018

Table 1
Physical and chemical properties of the soils at the study locations.

Soil parameters	Locations			
	Kpalga	Tunayilli	Tanina	Busa
pH(1:2.5) (H_2O)	6.13 ± 0.25a[†]	6.3 ± 0.1a	6.0 ± 0.06a	6.0 ± 0.04a
Total N (%)	0.43 ± 0.02a	0.52 ± 0.022a	0.33 ± 0.012a	0.33 ± 0.003a
Available P (mg kg^{-1})	1.69 ± 0.23a	1.53 ± 0.22a	2.04 ± 0.025a	1.20 ± 0.18a
Exchangeable K (cmol (+) kg^{-1})	1.21 ± 0.09a	1.06 ± 0.1a	1.06 ± 0.021a	1.11 ± 0.05a
Organic C (%)	0.42 ± 0.02b	0.74 ± 0.05a	0.28 ± 0.01b	0.49 ± 0.01ab
Exchangeable Ca (cmol (+) kg^{-1})	3.15 ± 0.11a	4.41 ± 0.65a	2.66 ± 0.01a	2.93 ± 0.09a
Exchangeable Mg (cmol(+) kg^{-1})	0.38 ± 0.02a	0.60 ± 0.52b	0.62 ± 0.015b	0.62 ± 0.08b
Sand (%)	64.42 ± 1.50b	69.05 ± 7.04a	68.92 ± 0.02a	68.52 ± 1.60a
Silt (%)	27.74 ± 1.54a	24.08 ± 0.96b	12.88 ± 0.02c	24.64 ± 1.64ab
Clay (%)	7.84 ± 1.54b	5.84 ± 6.08c	18.2 ± 0.15a	6.84 ± 0.04bc
Texture	Sandy loam	Sandy loam	Sandy loam	Sandy loam

[†] Represent standard deviation of the means. Figures between columns with the same letters are not significant at 5% probability.

2005; Crozat et al., 1982; Corman et al., 1987; Narożna et al., 2015). For example, Woomer et al. (1992) predicted the persistence of introduced rhizobium in the field under varying climatic factors while Crozat et al. (1982) and Corman et al. (1987) studied the survival kinetics of rhizobium without considering the effects of the prevailing climatic conditions.

In recent times, there have been renewed interests in finding suitable inoculant strains for cowpea production in sub-Saharan Africa (SSA) since recent evidence suggests that the potential exists for improving biological nitrogen fixation in cowpea and groundnut cropping systems. The inoculant strain *B. yuanmingense* (BR 3267) from Brazil has shown a huge potential in increasing grain yields of cowpea in Ghana. Boddey et al. (2016) and Ulzen et al. (2016) reported of significant increases in grain yield of cowpea in response to inoculation with *B. yuanmingense* (BR 3267). Consequently, *B. yuanmingense* (BR 3267) has been recommended for inoculant production for smallholder farmers in Ghana. However, little is known about the persistence of the strain under smallholder farm conditions; an attribute of the strain needed for the decision on whether or not repeated inoculation would be needed in subsequent cropping seasons. The absence of such baseline data on the effects of environmental factors on the survival of introduced rhizobia in soils has made it difficult to predict the fate of introduced strains. The study hypothesized that the introduced strains have the same saprophytic competence as the native strains and therefore can survive the harsh environmental conditions in the Northern Ghana. The study specifically evaluated the saprophytic competence of *B yuanmingense* strain BR 3267 and *B. japonicum* strain USDA 110 and determined the major environmental factors (rainfall, soil moisture, temperature, relative humidity, sunshine and indigenous rhizobium population) affecting the survival rates in the study area. Such determinations will help rhizobiologists and farmers to make informed decision on the frequency of re-inoculation in subsequent seasons.

2. Materials and methods

2.1. Site characteristics

The experiments were set up in four different sites namely Kpalga (latitude 09°26′.447′ N and longitude 000°57′.575′ W with an elevation of 167 m above sea level), Taunayilli (latitude 09°20′.398′ N and longitude 000°59′.154′ W with an elevation of 177 m above sea level) in the Northern region of Ghana; and Tanina (latitude 09°53.126′ N and longitude 002°27.480′ W with an elevation of 353 m above sea level) and Busa (09°59.186′ N and longitude 002°20.370′ W with an elevation of 345 m above sea level) located in the Upper West region of Ghana (Suppl Fig. S1). The soils of the study locations are Acrisols (Kpalga and Tunayilli) and Lixisols (Tanina and Busa) (IUSS, 2006). The study sites have a unimodal rainfall distribution pattern with an average annual rainfall of 1000 – 1200 mm and mean temperature between 26 and 30 °C with little variation throughout the year. The fields had no known history of rhizobia inoculation and had previously been planted with sorghum.

2.2. Experimental setup

Each field was ploughed and harrowed to a depth of 15 cm. Twenty-five grams of *B. yuanmingense* strain BR 3267 and *B. japonicum* strain USDA 110 as peat-based inocula were manually introduced into an area measuring 2 m × 3 m as separate treatments. The *B. yuanmingense* and *B. japonicum* contained 2.5×10^8 ($\log_{10}$ 8.4) and 2.5×10^7 ($\log_{10}$ 7.4) cells g^{-1} peat, respectively. Each inoculum was manually incorporated into the soil using a hoe that was pre- sterilized with 95% ethanol. Proximate analysis of the carrier materials of *B. yuanmingense* strain BR 3267 and *B. japonicum* strain USDA 110 was carried out following the procedures of (AOAC, 1990). Measures were taken to ensure that there was no legume on the plots that could have influenced the persistence of the strains throughout the study period.

2.3. Soil sampling

Soil samples were collected from each site at 0, 21, 42, 81, 142 and 296 days after incorporation of the peat based inoculant using an auger (Eijkamp, Netherland). Five core soil samples were thoroughly mixed and composite samples taken for enumeration of rhizobium population. The auger was surface-sterilized with 95% ethanol between sites. The samples were kept in refrigerator at 4 °C before the cell count. Prior to the introduction of the strains, composite soil samples were collected and analyzed at the Kwame Nkrumah University of Science and Technology (KNUST) Soil Science Laboratory following standard laboratory procedures for particle size (hydrometer method), soil pH (1:2.5 soil to H_2O), organic carbon (Walkley-Black), total nitrogen (Kjeldahl method), available soil phosphorus (Bray No. 1 solution) and exchangeable potassium (ammonium acetate (NH4OAc) extract) (Table 1). Calcium and magnesium were determined in 1.0 M ammonium acetate (NH4OAc) extract.

2.4. Soil moisture measurement

Soil moisture was measured with a Time Domain Reflectometer (TDR) (Trase system 6050X1 Santa Barbara California 93105 USA) at each sampling time. The 15 cm probe of the TDR was inserted into the soil at five different spots, to measure the moisture, and the average recorded.