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Reducing spatial variability of soybean response to rhizobia inoculants in farms of variable soil fertility in Siaya County of western Kenya

M. Thuita*, Bernard Vanlauwe, E. Mutegi, C. Masso

International Institute of Tropical Agriculture, PO Box 30772-00100, Nairobi, Kenya

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ABSTRACT

Soybean grain yields in sub-Saharan Africa have remained at approximately 50% below those attained in South America despite numerous efforts. A study was conducted in Siaya County (western Kenya) involving 107 farms with soils of different fertility status. The main objective was to test combinations of two inoculants (Legumefix and Biofix) and nutrient sources (Minjingu and Sympal) to raise soybean grain yields. Inoculation used Legumefix or Biofix with and without Minjingu or Sympal in a factorial design. There was soil acidity and a widespread deficiency of potassium, nitrogen, and phosphorus. Inoculation and nutrient source led to increases in nodulation and nodule occupancy. For grain yields the response varied from nil in some sites to high increases in others. Highest grain yields (3000–4000 kg ha^{-1}) were obtained with Legumefix + Sympal (12% of the farmers testing it). The formulation of the nutrient source was important to meet other nutrient deficiencies in most of the soils. Farmers using Legumefix + Sympal require yield increases of 35% for profitability (Value cost ratio of 3) while farmers using Minjingu + inoculant require a yield increase of at least 68%. Inoculants used alone were most profitable but this is advisable only when farmers are too resource constrained to afford fertilizer. For sustainable yields Legumefix + Sympal or Biofix + Sympal were recommended.

1. Introductions

Soybean grain yields in most of sub-Saharan Africa (SSA) remain relatively low compared to those achieved in South America and USA (Mpepereki et al., 2000). In other regions, annual average yield increases have been reported of 31 kg ha^{-1} in the United States (Specht et al., 1999) and 28 kg ha^{-1} worldwide (Wilcox, 2004). To achieve their high yield potential, soybean must sustain high rates of photosynthesis and accumulate large amounts of nitrogen (N) in seeds (Salvagiotti et al., 2008). Nitrogen exists in leaves primarily as ribulose biphosphate carboxylase/oxygenase and there is generally a strong relationship between N per unit leaf area and photosynthesis (Sinclair, 2004). Soil is the main source for most plants but N remains a major plant growth limiting nutrient in SSA (Sanchez et al., 1997). The alternative source for soybean is biological nitrogen fixation (BNF) through symbiosis with rhizobia. Worldwide some 44–66 million tonnes (t) of N_2 are fixed annually by agriculturally important legumes with another 3–5 million t fixed by legumes in natural ecosystems, providing nearly half of all N used in agriculture (Smil, 1999; Graham and Vance, 2000). The contribution by BNF could be increased by improving the nutrition of legumes, attending to edaphic constraints such as soil acidity and drought, and breeding varieties that's target the symbioses with rhizobia (Graham and Vance, 2000).

Rhizobia inoculants have proven to be a more viable and sustainable approach to meet the its high N demand estimated at 80 kg N per 1000 kg of soybean grain (Hungria et al., 2006). Soybean often requires inoculation when introduced in new environments including SSA. Owing to challenges associated with inoculum production, handling, and storage, breeding for promiscuity was proposed (Kueneman et al., 1984). However, the use of promiscuous soybean did not yield the expected outcome as indigenous rhizobia are often not present in high enough numbers and nitrogen fixation efficiency is relatively low (Musiyiwa et al., 2005; Zengeni and Giller, 2007). Inoculation has been proposed and shown to be beneficial even on promiscuous varieties (Thuita et al., 2012).

In their review, Divito and Sadras (2014) demonstrated that nutrients such as potassium (K) and sulfur (S) play a major role in nodule function and BNF. This is in addition to the widely documented role of phosphorus (P) (Almeida et al., 2000; Olivera et al., 2004; Schulze, 2006). Sulieman et al. (2013) reported that legumes relying on BNF generally require more P, K, and S than those that do not. Even though in most of SSA, N and P are documented as the most limiting nutrients and most fertilizer inputs mainly contain them it is possible that limitations in other nutrients may be the reason why yields of soybean

* Corresponding author.
E-mail address: m.thuita@cgiar.org (M. Thuita).

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have remained relatively low with averages of about 2 t ha^{-1} (Mpepereki et al., 2000). Addressing these other possible constraints could be the key to increasing yields to a level nearer to the averages obtained in South America (Hungria et al., 2006). Approaches by way of integrated soil fertility management (ISFM) (use of fertilizer + organic inputs, improved varieties, and rehabilitation of non-responsive soils) have been proposed as a viable sustainable, and environmentally friendly approach (Vanlauwe et al., 2010) and must be used to increase agricultural productivity (Okalebo, 2009; Garnett et al., 2013). This study aimed at testing two ISFM components (2 inoculants and 2 nutrient sources) to reduce the spatial variability of soybean yields and the yield gap.

2. Material and methods

2.1. Field trial

The trials were conducted in five regions (Boro, Ugunja, Ukwala, Wagai, and Yala) of Siaya County in western Kenya. Siaya County is located at 00 08.468′N, 34°25.378′E, and 1336 m asl. The experimental sites were in the lower midland 1 (LM1), lower midland 2 (LM2), and upper midland 1 (UM1). These agroecological zones experience bimodal rainfall with long rains (LR) from March to July and short rains (SR) from late August to December (Jaetzold et al., 2007). Average annual rainfall is 1500 mm (GOK, 1997). The soils are mainly Ferralsols and Acrisols in the higher (hilly and elevated) areas and Vertisols in the lower areas (near river valleys and plains).

The farmers who participated in the trials were selected randomly First all the villages in each region were listed and random numbers were generated using Microsoft excel. From each selected village the names of all the farmers in the village were obtained and four were selected using random numbers. In total, 107 randomly selected farmers participated in the trials. Initial soil samples were taken for analysis before treatments were applied. Available P was determined using the Mehlich 3 method (Mehlich, 1984); pH (H_2O) was determined as described by Okalebo et al. (2002). Exchangeable K, Ca, Mg, Total N (%), and organic carbon (%) were determined as described by Tekaligu et al. (1991) and Anderson and Ingram (1993).

2.2. Treatment structure and application

Two soybean inoculants were tested. Legumefix® soya from Legume technology (UK) containing *Bradyrhizobium japonicum* strain 532c (Thuita et al., 2012) and Biofix® soya from MEA Ltd (Kenya) containing *Bradyrhizobium diazoefficiens* strain USDA110 (Delamuta et al., 2013). Random numbers were used to select farmers that were assigned to test one of the two inoculants so that each farmer tested only one. Each inoculant was tested alone, with acidulated and granulated Minjingu hyper phosphate (0–30–0 + 38CaO) or Sympal (0:23:15 + 10CaO + 4S + 1MgO + 0.1Zn) in full factorial multi-locational trials. Phosphorus rate of 30 kg P ha^{-1} was used. On each farm only one replicate was used; hence 6 plots were installed on each farm. No inputs were applied in the absolute control plots.

The plot sizes were 4.5 × 5 m and the treatments were completely randomized on each farm. Sweet potato was planted at 1 m inter-plot spacing to act as a buffer to prevent inter-plot contamination. Inoculation with Legumefix or Biofix was done at planting as a seed coating using the directions for use in the respective product labels. Each plot had 6 soybean lines of 5 m in length with 5 cm plant to plant within-row spacing and 50 cm between rows. Inoculation was done on all the rows. Soybean TGx1740-2F with medium maturity (95–100 days) (Kueneman et al., 1984) was used as the test crop. The experiment was repeated for three seasons; the location of plots and treatment allocations were kept unchanged.

2.3. Nodulation assessment

This was done at 50% podding when nodules are expected to be fully functional. In one of the inner inoculated rows about 50 cm from the beginning of the line, a length of 50 cm was cut and the roots and nodules were dug out. Data on the number of plants and the nodule biomass were taken. The nodules were surface sterilized and stored in glycerol (Thuita et al., 2012) for determination of nodule occupancy. Nodulation assessment was done for each of the three seasons for all treatments.

2.4. Harvesting

This was done at physiological maturity of the crop within the effective area, omitting the outer lines and the sampled row. Weights were taken at harvest and after oven drying to determine grain yields

2.5. Nodule occupancy

This was done for nodules obtained during biomass sampling at mid-podding. Before DNA extraction, the nodules were surface sterilized using 70% ethanol for 30 s and 3.3% Ca $(ClO)_2$ for 2 min, then rinsed three times with sterile distilled water. One nodule was crushed in 150 µl of sterile water and DNA was directly extracted (Thiao et al., 2004). Total genomic DNA was extracted separately from 10 nodules per treatment for 33% of the farms, i.e., a total of 10 nodules for each treatment per farm. Nodule occupancy was taken for two seasons (LR2014 and SR2014) in 12 farms selected randomly in the LR2014 season. The same farms were repeated for the short rains season. From the nodules sampled at biomass assessment stage, 10 nodules were picked randomly for each inoculant with and without fertilizer (Sympal or Minjingu) per farm.

2.5.1. DNA amplification and restriction

Genetic diversity was determined by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) amplification and restriction of the 16S-23S rDNA intergenic spacer region. A 930–1100 bp intergenic region between the 16S and 23S rDNA was amplified by PCR using rhizobia-specific primers derived from the 3′ end of the 16S rDNA (FGPS 1490-72; 5′-TGCGGCTGGATCCCCTC CTT-3′) (Navarro et al., 1992) and from the 5′ end of the 23S rDNA (FGPL 132-38; 5′-CCGGGTTTCCCCATTCGG-3′) (Ponsonnet and Nesme, 1994). The PCR amplification was carried out in a 25 µl reaction volume containing 2 µl of total DNA extract, 10 pmol of each primer, and one freeze-dried bead (puReTaq Ready-To-Go PCR beads, GE Healthcare UK Ltd) containing 2.5 U of Taq DNA polymerase, 200 µM in 10 mM Tris-HCl (pH 9 at room temperature) of each dNTP, 50 mM KCl, and 1.5 mM $MgCl_2$. The PCR amplification was performed in a Bio-Rad iCycler™ thermal cycler adjusted to the following program: initial denaturation for 5 min at 94 °C, 35 cycles of denaturation (30 s at 94 °C), annealing (30 s at 58 °C) and extension (30 s at 72 °C) and a final extension (7 min at 72 °C).

The PCR products were visualized by electrophoresis of 3 µl of the amplified DNA on 2% horizontal agarose gel in TBE buffer (1.1% Tris-HCl, 0.1% $Na_2EDTA{\cdot}2H_2O$, and 0.55% boric acid), pre-stained with 0.033 mg ml^{-1} of Ethidium Bromide. The gel was photographed under UV illumination with Gel Doc (BIO-RAD) Software (USA). Aliquots (10 µl) of PCR products were digested with the restriction endonucleases *Msp*I and *Hae*III (5 U) in a total volume of 15 µl for 2 h at 37 °C. The restriction fragments were separated by horizontal electrophoresis in 1X TBE buffer with 3% agarose gel prestained with 0.033 mg ml^{-1} of Ethidium Bromide. The gels were run at 100 V for 3 h and photographed under UV illumination with Gel Doc (BIO-RAD, USA) software. Strains with identical restriction fragment profiles (in individual fragment size and number) were classified into the same intergenic spacer (IGS) group.