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Selected indigenous Bradyrhizobium strains match or surpass elite exotic strains as soybean inoculants in Mozambique

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Abstract

Background and Aims

The potential of indigenous rhizobial strains to sustainably improve soybean production in Africa depends on environmental adaptation and background microbiome competition. Five indigenous *Bradyrhizobium* strains from Mozambique (*B. elkanii* Moz 4, Moz 19, and Moz 22; *B. japonicum* Moz 27 and Moz 61), previously selected under laboratory gnotobiotic conditions, were evaluated against three elite exotic commercial strains (*B. japonicum* SEMIA 5079, *B. diazoefficiens* SEMIA 5080, and USDA 110).

Methods

Multi-location field experiments were conducted during the 2017/2018 and 2018/2019 cropping seasons across four provinces in Mozambique. The study used a 10 × 2 factorial treatment arrangement in a randomized complete block design with four replications, utilizing promiscuous (Zamboane) and non-promiscuous (Safari) soybean varieties under variable background rhizobial densities (2.2×10^1 to 5.7×10^6 cells g^{-1} soil).

Results

Agronomic superiority was strictly strain-specific, with only Moz 4 and Moz 27 emerging as consistently preeminent. The two strains increased grain yields by 13% (161 kg ha^{-1}) and 19% (231 kg ha^{-1}), respectively, compared with the uninoculated control, matching or surpassing elite exotic strains and synthetic N-fertilization. While Zamboane yielded more vegetative biomass, Safari exhibited a 13% higher grain yield due to a drought-escape mechanism during seasonal water deficits.

Conclusion

Specific locally adapted strains possess the environmental plasticity needed to match or exceed elite global benchmarks. The robust performance of Moz 4 and Moz 27 validates their selection for regional commercial inoculant production to protect similar sub-Saharan agro-ecological zones against global supply chain disruptions.

Introduction

Soybean has the potential to become a major crop in Africa due to its diverse applications in human nutrition and animal feed (Sinclair et al., 2014; Siamabele, 2021). Furthermore, through symbiosis with bacteria known as rhizobia, soybean can convert highly stable atmospheric nitrogen (N_2) into biologically usable forms of nitrogen (NH_4^+, NH_3) (de Bruijn, 2015). This process minimizes reliance on

N-fertilizers, which are often prohibitively expensive and environmentally damaging if applied injudiciously. However, in areas where soybean has not been previously cultivated, inoculation is generally required to ensure that elite strains are present to meet the plant's N requirements (Chang et al., 2015; Hungria and Mendes, 2015).

In Africa, a practical alternative to the dependence on inoculants was suggested in the 1980s, largely due to a lack of personnel and facilities for their production and distribution (Pulver et al., 1985). Scientists at the International Institute of Tropical Agriculture (IITA) developed soybean TGx (Tropical Glycine cross) genotypes, known as promiscuous cultivars for their ability to establish effective symbiotic relationships with a broad range of rhizobia indigenous to African soils (Pulver et al., 1985; Abaidoo et al., 2007; Tefera, 2011). In many locations, however, these varieties often perform below their potential, presumably because indigenous rhizobial strains either occur in low numbers or lack sufficient effectiveness (Okogun and Sanginga, 2003; Abaidoo et al., 2007; Klogo et al., 2015). Currently, the inoculation of both promiscuous and non-promiscuous soybean varieties with exotic *Bradyrhizobium* strains is successfully promoted across the continent, contributing to significant grain yield enhancements (Gyogluu et al., 2016; Khojely et al., 2018; van Heerwaarden et al., 2018; Mburu et al., 2020).

Soybean inoculation success is dependent on several environmental factors, including soil pH (Collino et al., 2015; Leggett et al., 2017), available soil N (Regus et al., 2014; Santachiara et al., 2019), temperature (Collino et al., 2015; Jaiswal and Dakora, 2019; Santachiara et al., 2019), soil organic matter (Leggett et al., 2017), and rainfall (Collino et al., 2015; Leggett et al., 2017). Crucially, the presence of indigenous or naturalized host-compatible rhizobial strains influences inoculation outcomes (Streeter, 1994; Nishida et al., 2025). Inoculation failure is often attributed to indigenous populations that, while frequently exhibiting low N_2-fixing capacity, are highly competitive and well-adapted to the local environment (Ndusha et al., 2017; Jaiswal and Dakora, 2019). However, it is also well established that some indigenous rhizobia possess outstanding effectiveness and have the potential for development into commercial inoculants (Grönemeyer et al., 2014; Chibeba et al., 2017; Ndusha et al., 2019). Despite this evidence, comprehensive field validations demonstrating that elite, pre-selected indigenous strains can consistently match or surpass the performance of world-class exotic strains under diverse African agro-ecological conditions remain limited.

To address this knowledge gap, a systematic selection program was initiated in Mozambique. First, a greenhouse screening of 105 indigenous rhizobial isolates evaluated their N_2-fixation capacity on both promiscuous and non-promiscuous soybean varieties (Chibeba et al., 2017). These isolates were compared against five exotic reference strains commonly used in commercial inoculants: *B. diazoefficiens* SEMIA 5080 and USDA 110, *B. elkanii* SEMIA 587 and SEMIA 5019, and *B. japonicum* SEMIA 5079. From that evaluation, five indigenous strains—*B. elkanii* Moz 4, Moz 19, and Moz 22, and *B. japonicum* Moz 27 and Moz 61—demonstrated symbiotic performance similar or superior to the elite commercial strains, marking them as elite candidates for field testing.

The objective of this study was to evaluate the field performance of these five pre-selected indigenous strains in comparison with three the top-performing exotic commercial strains across diverse agro-climatic regions of Mozambique using both promiscuous and non-promiscuous soybean varieties. It was hypothesized that these elite indigenous strains would exhibit symbiotic effectiveness, biological yield, and grain yield comparable or superior to elite exotic reference strains under diverse field conditions.

Material and Methods

Description of the experimental locations

Field experiments were established in the 2017/2018 and 2018/2019 cropping seasons at five locations distributed across four provinces: Manica (Sussundenga), Nampula (Muriaze), Tete (Nkhame and Ntengo Umodzi), and Zambézia (Namarripe), representing the primary soybean production regions in Mozambique (Fig. 1). Under the Köppen-Geiger climate classification system, the climate types are Aw (savanna) in Nampula and Cwa (monsoon-influenced humid subtropical) in Manica, Tete, and Zambézia. According to FAO/UNESCO criteria, the soils are classified as Ferric Luvisols in Nampula, Orthic Ferralsols in Tete, and Rhodic Ferralsols at both Manica and Zambézia locations.

At each location, fields with no known history of soybean cultivation or inoculation were selected. Two months prior to sowing, 20 soil sub-samples were collected at a depth of 0–20 cm and divided into two parts: one for granulometric and chemical analyses, and the other for the enumeration of soybean-nodulation compatible rhizobia. Analyses were performed at the Soil and Plant Analysis Laboratory of the Agrarian Research Institute of Mozambique (IIAM) in Nampula. Soil samples were oven-dried for 48 hours at 60°C and sieved (2 mm). Particle size distribution was determined by the pipette method (Gee and Bauder, 1986). Soil pH was determined in deionized H_2O (1:2.5; soil:H_2O), 1 h after shaking. Available P and exchangeable K were determined using the Mehlich-1 extractant (0.05 mol L^{-1} HCl and 0.0125 mol L^{-1} H_2SO_4) (Sims, 1989),while exchangeable Ca and Mg were estimated using a 1.0 mol L^{-1} KCl extractant (Raij and Bataglia, 1991). Exchangeable acidity (Al + H) was determined in 0.5 mol L^{-1} calcium acetate ($C_4H_6CaO_4$) (Shaw, 1949). Soil organic carbon (SOC) was determined by the Walkley-Black method (Walkley and Black, 1934), and soil organic matter (SOM) was calculated as SOM = 1.724×SOC. Indigenous soybean-compatible rhizobial population were estimated at the Microbiology Unit of the IITA in Nigeria using the most probable number (MPN) counting method (Vincent, 1970) in air-dried, sieved (2 mm) soil. The promiscuous soybean variety TGx 1987-62F was employed as the trap host to ensure the detection of the widest possible range of indigenous rhizobia.

Treatments and management of the experiment

The experiment consisted of 20 treatments laid out in a randomized complete block design (RCBD) arranged in a 10 × 2 factorial (N sources × soybean varieties) with four replications. The first factor comprised 10 N-source treatments: five indigenous strains from Mozambique (Moz 4, 19, 22, 27, and

61); two exotic strains from Brazil (SEMIA 5079 and SEMIA 5080); one exotic strain from the USA (USDA 110); and two uninoculated controls (control and control + N). The uninoculated and N-fertilized control (control + N), represented an ideal condition where N is not limiting (Thies et al., 1991). For this treatment, urea [$CO(NH_2)_2$, 46.6% N] was applied at a rate of 200 kg ha^{-1} of N, split into two applications: 50% at sowing and 50% at the R2 reproductive stage (Fehr and Caviness, 1977). All strains are deposited at the NoduMax Factory of the IITA Business Incubation Platform (Nigeria) and in the Diazotrophic and Plant Growth-Promoting Bacteria Culture Collection of Embrapa Soja (WFCC #1213, WDCM #1054) in Londrina, Paraná, Brazil. Corresponding culture collection numbers are designated as follows: Moz 4 (= CNPSo 2895), Moz 19 (= CNPSo 2939), Moz 22 (= CNPSo 2944), Moz 27 (= CNPSo 2956), Moz 61 (= CNPSo 3088), SEMIA 5079 (= CNPSo 7), SEMIA 5080 (= CNPSo 6), and USDA 110 (= CNPSo 46).

The second factor consisted of two soybean varieties: Safari (non-promiscuous) and Zamboane (TGx 1904-6F, promiscuous for rhizobial nodulation). Safari is a late-maturing variety (120 days to maturity) (Sichilima et al., 2018; Seedco Zambia Limited, 2020) with a potential grain yield of 4,500 kg ha^{-1} (Seedco Zambia Limited, 2020). Zamboane is a medium-maturing variety (104–114 days to maturity), used for both grain and fodder, with a potential yield of 2,000–2,500 kg ha^{-1} (Eseigbe et al., 2024).

All strains were prepared as peat-based inoculants at NoduMax Factory. To ensure a uniform dose across all treatments, the Colony Forming Units (CFU) were verified to provide a final concentration of 1.2×10^6 cells $seed^{-1}$. Inoculation followed the two-step method: a sticker solution of 10% (w/v) arabic gum was applied to the seeds first, followed by the addition of the peat carrier. The seeds were vigorously shaken until uniformly coated and then left to air-dry in the shade for 10 minutes prior to sowing.

Plots consisted of eight 8-m long rows spaced 50 cm apart. Immediately prior to sowing, triple superphosphate (20.07% P) was applied in-furrow at a rate of 17.46 kg ha^{-1} of P across all plots. Three seeds per hole were sown at a depth of approximately 2 cm, with a final inter-row spacing of 50 cm and intra-row spacing of 10 cm. Ten days after sowing, plants were thinned to two per hole to achieve a target population of approximately 340,000 plants ha^{-1} accounting for a mean germination rate of 85%. To prevent cross-contamination between treatments, a 1.5 m alley with a physical ridge was maintained around each plot. Weeding was performed weekly with manual hoes until canopy closure, always beginning with the uninoculated plots. Across both cropping seasons, the crops relied solely on natural rainfall; precipitation data were recorded and mapped against the growth stages of the varieties at each location (Fig. 2).

Evaluation of nodulation, plant growth, relative effectiveness, N accumulation in shoots, biological yield, grain yield and 100-seed weight

At growth stage R2, ten plants were selected in pairs from the same hole—outside the 21 m^2 harvest area—and excavated to evaluate nodulation, plant growth, and shoot N accumulation. Plants were cut at the cotyledonary node to separate shoots from roots. Roots were rinsed over a 1-mm mesh sieve with a gentle stream of H_2O to remove soil debris and subsequently oven-dried at 60°C for 72 h. Nodule number

(NN, no. plant^{-1}) and nodule dry weight (NDW, mg plant^{-1}) were then determined. Shoots were also oven-dried (60°C for 72 h) to determine shoot dry weight (SDW, g plant^{-1}). Relative effectiveness (RE) was calculated as the percentage ratio between the SDW of a given treatment and that of the N-fertilized control (Simbine et al., 2021). Dried shoots were finely ground (1 mm) to quantify total N in shoots (TNS, mg plant^{-1}) via the Kjeldahl method (Bremner, 1960) at the IIAM Laboratory.

At the stage R8, when 95% of pods have reached their mature color (Fehr and Caviness, 1977), all plants within the central 21 m² area were harvested at the cotyledonary node to determine biological yield (BY, kg ha^{-1}). Grains were threshed manually to determine grain yield (GY, kg ha^{-1}) and 100-seed weight (SW, mg). GY was adjusted to a standard 13% moisture content, measured using an AgraTronix® MT–16 grain moisture tester.

Statistical analyses

Prior to statistical analysis, data were tested for homogeneity of variances (Bartlett's test) and normality of residues (Shapiro-Wilk test). Data that did not meet these assumptions were subjected to Box-Cox transformations. A two-way analysis of variance (ANOVA) was performed to evaluate the effects of N-sources, varieties, and their interaction. Whenever significant differences were detected ($p < 0.05$), treatment means were compared using Tukey's HSD test ($p < 0.05$). All analyses were conducted using R software version 4.4.1 (R Core Team, 2024) within the RStudio environment (version 2025.12.0).

Results

Soil properties, weather conditions, and biological factors

In general, soil fertility attributes across the experimental locations were less favorable during the 2017/2018 cropping season than in 2018/2019 (Table 1). As these trials were conducted in areas with no history of soybean cropping, the specific plots differed between the two seasons. For example, all experimental locations except Ntengo Umodzi had a sand content exceeding 700 g kg^{-1} in the first season, whereas three locations were below 700 g kg^{-1} in the second. Although the experiments were generally conducted in acidic soils, two locations (Muriaze and Ntengo Umodzi) were in the highly acidic pH 4.0–5.0 range in the first season, contrasting with all locations within the higher pH 5.0–6.0 range in the second. Based on the Hazelton and Murphy (2016) classification, three locations (Sussundenga, Muriaze, and Ntengo Umodzi) had low CEC ratings (< 5 $cmol_c$ dm^{-3}) in 2017/2018, while all evaluated areas showed moderate (5–15 $cmol_c$ dm^{-3}) or high (> 15 $cmol_c$ dm^{-3}) CEC ratings in 2018/2019.

However, in both seasons, certain soil and biological attributes remained consistently favourable across the distinct experimental areas. Two locations (Ntengo Umodzi and Namarripe in the first season; Muriaze and Namarripe in the second) maintained SOM content ≥ 1.72 g dm^{-3}, the critical threshold suggested for soybean (Cui, 2024). Additionally, all locations exhibited available soil P levels above the critical level of 3.5 mg dm^{-3} (Tiecher et al., 2023). Furthermore, indigenous rhizobial densities exceeded

10^1 cells g^{-1} of soil across all evaluated environments. Notably, densities exceeded 10^3 cells g^{-1} of soil at Ntengo Umodzi and Namarripe in the first season, and at Sussundenga and Namarripe in the second.

Rainfall improved markedly during the vegetative growth stages (Fehr and Caviness, 1977) between the 2017/2018 and 2018/2019 cropping seasons (Figure 2). At Sussundenga and Ntengo Umodzi, maximum rainfall during the V_3–V_5 stages increased from <50 mm in the first season to >150 mm in the second. Muriaze exhibited an identical trend during the V_3–V_6 stages, with peak rainfall increasing from less than 50 mm to greater than 150 mm. Meanwhile, at Namarripe, maximum rainfall during the VC–V_4 stages increased more modestly, shifting from <50 mm in the first season to ≥60 mm in the second. Paradoxically, the Safari variety reached physiological maturity in an average of 112 days, ahead of Zamboane, which matured in 118 days (Supplementary Table S1), despite their respective late- and medium-maturing genetic classifications (Section 2.2). Additionally, a 60% incidence of soybean rust (*Phakopsora pachyrhizi*) was recorded at Namarripe in the 2018/2019 cropping season when the Safari and Zamboane varieties were at the R7 and R6 growth stages (Fehr and Caviness, 1977), respectively. Furthermore, sporadic foraging by wild rabbits was observed at Sussundenga and Muriaze during the early vegetative stages (V2–V4) (Fehr and Caviness, 1977) of the 2017/2018 season, affecting approximately 20% of the experimental plots.

Nodulation (nodule number and weight)

In the 2017/2018 season, inoculation increased nodule number (NN) and dry weight (NDW) relative to the uninoculated and non-N-fertilized control (control) across all evaluated locations (Tables 2 – 6). *B. elkanii* Moz 4 and Moz 22 were the only strains that improved NDW compared with the control as well as the elite exotic strains *B. diazoefficiens* SEMIA 5080 and USDA 110 at Sussundenga (Table 2). Moz 22 and *B. japonicum* Moz 27 induced higher NN and NDW than the control and all exotic strains in the overall varietal evaluation at Muriaze (Table 3). Moz 4 exhibited the most pronounced performance, improving NN and NDW within and across varieties relative to the control and all exotic strains at Nkhame (Table 4). At Ntengo Umodzi, where the indigenous rhizobial population was 6.6 x 10^4 rhizobia $g^{-1}$of soil (Table 1), Moz 4 and Moz 61 induced higher NDW in the overall varietal evaluation than the control and all exotic strains except *B. japonicum* SEMIA 5079 (Table 5). Similarly, as shown in Table 6, at Namarripe, which had an indigenous rhizobial population of 3.2 × 10^5 rhizobia g^{-1} of soil (Table 1), Moz 4 and Moz 22 improved NDW in relation to the control and all exotic strains at a multi-varietal scale, whereas none of the exotic strains outcompeted the control.

In the 2018/2019 cropping season, inoculation responses were detected at only three locations (Tables 2–6). At Sussundenga, inoculation with indigenous strains, except for Moz 61, enhanced NDW relative to the control and all exotic strains specifically for the variety Zamboane and in the overall varietal evaluation (Table 2). Similarly, at Muriaze, Moz 4 and Moz 27 improved NDW at a multi-varietal scale compared with the control and all exotic strains (Table 3). Finally, at Namarripe, which had the highest

indigenous rhizobial population estimated at 5.7×10^6 rhizobia g^{-1} of soil (Table 1), Moz 4, Moz 61, and SEMIA 5079 were the only strains that outperformed the control across varieties (Table 6).

Overall, Moz 4 exhibited the most consistent performance at a multi-varietal scale, promoting a higher NDW than the control and the exotic strains SEMIA 5080 and USDA 110 at six of the ten location × cropping season combinations (Tables 2–6). Following this trend, Moz 22 elicited a higher NDW relative to the control and SEMIA 5080 and USDA 110 at four of the ten combinations, whereas Moz 27 enhanced NDW compared with the control and all exotic strains at three of the ten combinations. Among the exotic strains, SEMIA 5079 was the most effective, outperforming the control at four of the ten combinations, followed by *B. diazoefficiens* SEMIA 5080 and USDA 110 at three of the ten combinations. A pooled analysis across locations and cropping seasons indicated that while all strains enhanced NDW compared with the control at a multi-varietal scale, Moz 4, Moz 22, and Moz 27 were the preeminent strains, outperforming all exotic counterparts (Supplementary Table S3).

Inconsistent varietal effects were observed, with Safari recording higher NDW than Zamboane at three locations (Nkhame, Ntengo Umodzi, and Namarripe) in the 2017/2018 season, while Zamboane outperformed Safari at four locations (all except Nkhame) in the following season. In the first cropping season at Ntengo Umodzi (Table 5) and Namarripe (Table 6), mineral N-fertilizer application resulted in the suppression of nodulation within and across varieties, which was further confirmed by the pooled analysis across locations for the first cropping season (Supplementary Table S2) and by the overall analysis across locations and seasons (Supplementary Table S3).

Plant growth (shoot dry weight)

In the 2017/2018 cropping season, responses to inoculation in terms of shoot dry weight (SDW) were detected at three of the five locations (Tables 2–6). As shown in Table 3, at Muriaze, Moz 4, Moz 22, and Moz 27 stimulated higher SDW than the USDA 110 and the uninoculated and non-N-fertilized control (control), performing analogously to the uninoculated and N-fertilized control (control + N) in the overall varietal evaluation. At Nkhame, Moz 4 was the only strain that yielded higher SDW than the control, although its performance was equivalent to all other treatments (Table 4). At Namarripe, inoculation with the SEMIA 5080 promoted a higher SDW compared with the control, but the result did not differ from those of the indigenous Moz 4, Moz 22, and Moz 27 strains (Table 6).

During the 2018/2019 cropping season, inoculation responses were again observed at three locations. SEMIA 5079 elicited the highest SDW, outperforming the control, control + N, and all other treatments within the Zamboane variety and in the overall varietal evaluation at Muriaze (Table 3). At Nkhame, Moz 27 enhanced SDW compared with the control and SEMIA 5080, performing comparably to the control +

N treatment and USDA 110 in the overall varietal evaluation (Table 4). At Ntengo Umodzi, Moz 4 induced higher SDW than SEMIA 5079, the control, and control + N, exhibiting performance comparable to SEMIA 5080 and USDA 110 across varieties (Table 5).

Pooled analyses revealed inoculation effects across locations only in the second cropping season, in which Moz 4, Moz 27, and SEMIA 5079 promoted a higher SDW than SEMIA 5080, the control, and control +N at a multi-varietal scale (Supplementary Table S2). Highly consistent varietal responses were observed, with Zamboane achieving greater SDW than Safari in 70% of the location × cropping season combinations, a trend that was further confirmed across locations (Supplementary Table S2) and cropping seasons (Supplementary Table S3). N-fertilizer application promoted plant growth, improving SDW compared with the control in the overall varietal evaluation at three locations (Muriaze, Nkhame, and Namarripe) in the first season and at two locations (Muriaze and Nkhame) in the second season (Tables 2–6). This trend was corroborated by the pooled analysis across locations in the first cropping season (Supplementary Table S2).

Relative effectiveness

Inoculation enhanced relative effectiveness (RE) at Muriaze and Namarripe during the 2017/2018 cropping season (Tables 3 and 6). At Muriaze, Moz 4, Moz 22, Moz 27, and SEMIA 5080 achieved RE levels higher than USDA 110 and the uninoculated and non-N-fertilized control (control) in the overall varietal evaluation (Table 3). Similarly, at Namarripe, inoculation with Moz 4, Moz 27 and SEMIA 5080 resulted in higher RE relative to the control across varieties (Table 6).

In the following cropping season (2018/2019), inoculation effects were detected at three locations. At Muriaze, SEMIA 5079 outcompeted all other treatments except for the control + N specifically for the variety Zamboane and at a multi-varietal scale (Table 3). Inoculation with Moz 27 induced higher RE than the control, Moz 19 and USDA 110, although its performance was comparable to all other treatments in the overall varietal evaluation at Nkhame (Table 4). Analogously, at Ntengo Umodzi, Moz 4 elicited higher RE than Moz 19, SEMIA 5079, the control, and control + N within Zamboane, even though its performance was comparable to all other strains (Table 5).

Pooled analyses detected inoculation effects across locations within the Safari and Zamboane varieties, during the first and second cropping seasons, respectively. In the 2017/2018 cropping season, Moz 19 induced higher RE than the control and SEMIA 5080 but performed comparably to all other treatments specifically for the variety Safari (Supplementary Table S2). Similarly, in the following cropping season, Moz 27 promoted higher RE than the control and Moz 19, and SEMIA 5080 specifically for the variety Zamboane, eliciting a performance comparable to those of the other treatments (Supplementary Table S2).

Varietal effects were recorded at only one location in the first cropping season compared with five in the second. Zamboane attained higher RE than Safari in five of the ten location × cropping season combinations (Tables 2–6). In line with this, the pooled analysis revealed significant varietal effects on RE, with Zamboane outperforming Safari across locations in the second season (Supplementary Table S2). N-fertilizer effects were detected at a multi-varietal scale at Muriaze (Table 3) and Namarripe (Table 6) in the 2017/2018 season, and at Muriaze (Table 3) and Nkhame (Table 4) in the 2018/2019 season. Across locations, N-fertilizer effects occurred in the overall varietal evaluation only in the first cropping season (Supplementary Table S2).

Total nitrogen in shoots

Positive responses to inoculation on total nitrogen in shoots (TNS) were observed at two and three locations in the first and second cropping seasons, respectively (Tables 7–11). In the 2017/2018 season, Moz 27 increased TNS relative to SEMIA 5079 and USDA 110, and the uninoculated and non-N-fertilized control (control), at a multi-varietal scale, achieving levels indistinguishable from the uninoculated and N-fertilized control (control + N), Moz 4, Moz 22, and SEMIA 5080 at Muriaze (Table 8). At Namarripe, SEMIA 5080 elicited the highest TNS levels, outperforming all treatments except for Moz 4 and control + N across varieties (Table 11).

In the 2018/2019 season, SEMIA 5079 induced the highest TNS relative to all other treatments, excluding the control + N within the Zamboane genotype and across varieties at Muriaze (Table 8). At Nkhame, Moz 4 and SEMIA 5079 were the top performers, enhancing TNS compared with the control, Moz 19, Moz 22, SEMIA 5080, and USDA 110, while achieving similar levels to Moz 27, Moz 61, and the control + N in the overall varietal evaluation (Table 9). Notably, at Ntengo Umodzi, Moz 4, Moz 22, and SEMIA 5080 were the sole strains that outperformed the control and control + N in the overall varietal evaluation (Table 10).

Analysis across locations detected inoculation responses only in the 2018/2019 cropping season. Moz 27 attained the highest TNS, outperforming all treatments except for SEMIA 5079 within Zamboane (Supplementary Table S4). In the overall varietal evaluation, Moz 27, SEMIA 5079, and the control + N elicited higher TNS than all other treatments except for Moz 4, and USDA 110. N-fertilizer effects were detected at a multi-varietal scale at Muriaze (Table 8) and Namarripe (Table 11) in the 2017/2018 season, at Muriaze (Table 8) and Nkhame (Table 9) in the 2018/2019 season, and in the pooled analyses across locations for both seasons (Supplementary Table S4). Moreover, a highly consistent varietal response for TNS was observed, with Zamboane outperforming Safari in eight of the ten location × cropping season combinations, as well as in pooled analyses across locations for both cropping seasons (Supplementary Table S4).

Biological yield

Inoculation increased biological yield (BY) compared with the uninoculated and non-N-fertilized control (control) at all locations except Ntengo Umodzi (Tables 7–11). During the 2017/2018 cropping season, SEMIA 5080 was the top- performing strain at Muriaze, achieving an equivalent BY to the uninoculated and N-fertilized control (control + N) and Moz 27, while surpassing all remaining treatments in the overall varietal evaluation (Table 8). Moz 4 and SEMIA 5080 were the sole strains that attained higher BY than the control and control + N at Nkhame, (Table 9). At Namarripe, USDA 110 was the only strains that induced higher BY than the control, though its performance was not different from that of any other treatment when data were pooled across varieties (Table 11).

In the 2018/2019 cropping season, the indigenous strain Moz 19 outperformed all exotic strains and the control in the variety Safari at Sussundenga, achieving a BY equivalent to Moz 4 and the control + N (Table 7). At Muriaze, Moz 27 and USDA 110 outpaced SEMIA 5079 and the control in the overall varietal evaluation, even though their yields did not differ from any other treatment (Table 8). Meanwhile, at Nkhame, Moz 4 established preeminent performance, enhancing BY relative to all other treatments except Moz 27 at a multi-varietal scale (Table 9).

The pooled analysis across all locations and cropping seasons showed that the control + N treatment induced the highest BY, outpacing all treatments, except Moz 4, Moz 27, SEMIA 5080, and USDA 110 in the overall varietal evaluation (Supplementary Table S5). Additionally, a robust and consistent varietal effect was observed, with the promiscuous Zamboane variety outperforming Safari in total biomass in 80% of the location × cropping season combinations and across locations in both cropping seasons (Supplementary Table S4). At Namarripe, where soybean rust was recorded in the second cropping season (section 3.1), Safari had 33% higher BY than Zamboane (Table 11). N-fertilizer effects on BY were observed in the overall varietal evaluation at Muriaze (Table 8), Nkhame (Table 9), and Namarripe (Table 11), as well as across locations, exclusively in the first cropping season (Supplementary Table S4).

Grain yield

Grain yield (GY) enhancements attributed to inoculation were recorded at Muriaze, Nkhame, and Namarripe. During the 2017/2018 cropping season, inoculation with Moz 27 induced the highest GY, which matched the uninoculated and N-fertilized control (control + N) and Moz 4, outpacing all other treatments across both varieties at Muriaze (Table 8). At Nkhame, inoculation with SEMIA 5080 and USDA 110 resulted in higher yields than Moz 22 and control while matching the performance elicited by all treatments (Table 9). In the 2018/2019 cropping season, Moz 27 elicited the highest GY within Safari at Nkhame, outpacing the control, control + N, SEMIA 5080, and USDA 110, remaining on par with all indigenous strains and the exotic SEMIA 5079 (Table 9). Similarly, across varieties at this location, Moz 27 induced the greatest yields, surpassing all treatments except Moz 19 and Moz 61. At Namarripe,

where there was a dense native background population of 5.7 x 10^6 rhizobia g^{-1} of soil—the highest recorded across the experimental locations (Table 1) —inoculation with Moz 4, Moz 61, and SEMIA 5079 within Safari resulted in yield increases relative to Moz 19, SEMIA 5080, and the control, while at par with all other treatments (Table 11).

Across locations for the 2017/2018 cropping season, Moz 4 and Moz 27 enhanced GY by 18–20% (183–199 kg ha^{-1}) relative to the control, performing equivalently to control + N and all remaining strains (Supplementary Table S4). In the following cropping season, Moz 27 remained a preeminent strain, inducing a 23% (367 kg ha^{-1}) yield advantage relative to the control, and a 15–17% (257–283 kg ha^{-1}) increase compared with SEMIA 5079 and SEMIA 5080, while being commensurate with the other treatments within Safari (Supplementary Table S4). Analogously, in the overall varietal evaluation, Moz 27 promoted yield gains of 18% (263 kg ha^{-1}) relative to the control, and 12–19% (178–275 kg ha^{-1}) compared with Moz 61, SEMIA 5079, and SEMIA 5080, matching the GY levels of the remaining treatments (Supplementary Table S4. The comprehensive pooled analysis across locations and seasons indicated that Moz 27 improved GY relative to the control by 19% (231 kg ha^{-1}) and by 10–16% (134–212 kg ha^{-1}) compared with Moz 19, Moz 22, Moz 61, SEMIA 5079, and SEMIA 5080, attaining equivalent yields to Moz 4, USDA 110, and control + N (Supplementary Table S5).

Seasonal variation influenced varietal GY responses. Zamboane outperformed Safari at Sussundenga (Table 7), Muriaze (Table 8), and Nkhame (Table 9) during the 2017/2018 cropping season. In the subsequent season, however, Safari exhibited superior GY compared with Zamboane across all locations except Sussundenga (Tables 7–11). At Namarripe, where soybean rust was recorded (section 3.1), Safari achieved a 2.5-fold higher GY than Zamboane (Table 11). Overall, the pooled analysis across locations and seasons demonstrated that Safari yielded 13% (162 kg ha^{-1}) more than Zamboane, despite the latter's superior performance in biomass and shoot N accumulation (Supplementary Table S5). Prominent N-fertilizer responses were observed in the 2017/2018 season at Muriaze (Table 8), Namarripe (Table 11), and across locations (Supplementary Table S4). Conversely, in the following season, N-fertilizer effects were exclusively detected at Namarripe, with no significant trends emerging in the regional analysis (Supplementary Table S4).

100-Seed weight

Inoculation augmented 100-seed weight (SW) across all evaluated locations, with the sole exception of Muriaze (Tables 7–11). During the 2017/2018 cropping season at Sussundenga, Moz 27 emerged as the most outstanding strain, inducing SW levels greater than the uninoculated and non-N-fertilized control (control) and equivalent or superior to that of the uninoculated and N-fertilized control (control + N) both within and across varieties (Table 7). At Nkhame, Moz 4, SEMIA 5080, and USDA 110 were the sole strains that increased SW relative to the control, generating SW levels comparable or superior to the control + N treatment in the overall varietal evaluation (Table 9). An analogous trend was observed at

Ntengo Umodzi, where Moz 22 and SEMIA 5079 uniquely outpaced the control, although the performance was not different to any other treatment (Table 10). At Namarripe, Moz 27 and SEMIA 5080 were the only strains that generated higher SW than Moz 22 and the control at a multi-varietal scale, while matching SW levels promoted by all other strains (Table 11). During the 2018/2019 cropping season at Sussundenga, Moz 27 emerged as the preeminent strain, outpacing all other treatments specifically for the variety Safari and in the overall varietal evaluation (Table 7). At Ntengo Umodzi, Moz 27 surpassed Moz 19, Moz 61, SEMIA 5079, USDA 110, the control, and control + N, remaining on par with Moz 4, Moz 22, and SEMIA 5080 within Safari (Table 10). In the overall varietal evaluation at this location, Moz 27 also exceeded Moz 61, SEMIA 5079, the control, and control + N, matching Moz 4, Moz 19, Moz 22, SEMIA 5080, and USDA 110.

The prominence of Moz 27 was confirmed in the pooled regional evaluations. In the overall varietal evaluation, it outpaced all treatments except control + N during the first cropping season (Supplementary Table S4). Furthermore, in the subsequent season, Moz 27 matched the performance of Moz 4, SEMIA 5080, and control + N, while outpacing all remaining treatments. Ultimately, the comprehensive pooled analysis across locations and cropping seasons demonstrated that Moz 27 fell within the leading statistical group alongside SEMIA 5080 and control + N regardless of whether the evaluation is within or across varieties (Supplementary Table S5).

An exceptionally consistent varietal effect was observed, with Zamboane outstripping Safari at every location and in both cropping seasons (Tables 7–11), as well as in all pooled analyses (Supplementary Tables S4 and S5). N-fertilizer application improved SW compared with the control at Sussundenga (Table 7) and Namarripe (Table 11) within or across varieties in both cropping seasons. Corroborating to grain yield results, pooled analyses indicated that N-fertilizer effects on SW were detected in the first and not in the second cropping season (Supplementary Table S4).

Discussion

This multi-year, multi-location study provides critical field validation for previous laboratory research conducted under gnotobiotic conditions (Chibeba et al., 2017). In that study, five indigenous strains—*B. elkanii* Moz 4, Moz 19, and Moz 22, and *B. japonicum* Moz 27 and Moz 61—demonstrated symbiotic performance analogous to that of the exotic strains (*B. elkanii* SEMIA 587 and SEMIA 5019, *B. japonicum* SEMIA 5079, and *B. diazoefficiens* SEMIA 5080 and USDA 110). Strains SEMIA 587, 5019, 5079, and 5080 are the elite industry benchmarks extensively used in Brazil (de Souza et al., 2019), whereas USDA 110 is recognized as the reference inoculant strain widely adopted across many African countries (Chibeba et al., 2018). In this study, the indigenous candidate strains successfully matched or outcompeted these well-established exotic strains.

Promiscuous soybean varieties were historically bred in Africa during a developmental phase constrained by limited microbial inoculant production and distribution, under the premise that the genotypes would establish effective BNF with the abundant indigenous cowpea-type rhizobia (Pulver et

al., 1985; Tefera, 2011). Indeed, cowpea (*Vigna unguiculata*) was domesticated within the African continent (Panzeri et al., 2022) and naturally shares compatible microsymbionts with soybean (Pulver et al., 1985; Sanginga et al., 2000; Gyogluu et al., 2018). However, apart from the potential inefficiencies in fixing N_2, a primary limitation of relying solely on promiscuity is that indigenous rhizobial populations frequently fall below 10^3 rhizobia g^{-1} of soil (Okogun and Sanginga, 2003; Klogo et al., 2015) – the critical threshold density required to satisfy soybean N demand (Abaidoo et al., 2007). Corroborating this, 60% of locations in this study fell below this threshold (Table 1), reinforcing the argument that inoculation is a superior strategy over a passive reliance on native rhizobial populations of unknown density and effectiveness (Abaidoo et al., 2007; Chibeba et al., 2017; Akley et al., 2023).

Furthermore, while an indigenous rhizobial population density of ≥ 10 rhizobia g^{-1} of soil is often cited as a barrier to elite strains establishment (Thies et al., 1991; Streeter, 1994; Abaidoo et al., 2007; Jaiswal and Dakora, 2019), this study found that inoculation enhanced nodulation notwithstanding high (1.3 x 10^3 to 5.7 x 10^6 rhizobia g^{-1} of soil) indigenous densities (Tables 1, 5, 6). This corroborates observations from Ghana (Klogo et al., 2015), Mozambique (Chibeba et al., 2018) and Brazil (Hungria and Mendes, 2015; Hungria et al., 2017; Chibeba et al., 2018; Zilli et al., 2021) and supports the practice of re-inoculation to prevent often less effective, highly saprophytic established strains from dominating the soil rhizobial population (Zilli et al., 2021).

The pronounced seasonal variation in precipitation and soil textures encountered across the study locations establish stringent environmental filters that expose stark intra-species fitness variations among the tested rhizobia. Remarkably, despite evaluating multiple representatives from the same species, including three *B. elkanii* strains (Moz 4, Moz 19, Moz 22) and two *B. japonicum* strains (Moz 27, Moz 61), elite agronomic performance was strictly strain-specific, isolated only to Moz 4 and Moz 27. This divergence highlights that broad species-level traits are insufficient for environmental survival; instead, successful nodulation depends on high saprophytic competence to survive the free-living phase in the soil matrix before symbiosis can occur (Poole et al., 2018). This survival requirement is heavily pronounced in porous sandy soils (> 850 g kg^{-1} sand) and high native background populations (e.g., Namarripe), where recent genomic insights confirm that rhizobial reproductive fitness in the soil matrix is genetically independent from host-symbiosis traits and governed by complex, strain-specific loci (Gil-Polo et al., 2026). Consequently, it can be hypothesized that Moz 4 and Moz 27 possess distinct, minor genetic variations that grant them the saprophytic plasticity needed to outcompete both indigenous populations and their own genetic relatives.

The consistent inoculation success exhibited by Moz 4 and Moz 27 strains in terms of nodulation, plant growth, relative effectiveness and N-accumulation in shoots (Tables 3–6) supports earlier laboratory findings (Chibeba et al., 2017), suggesting robust ecological competence comparable or superior to that of exotic strains. Moreover, Moz 4 and Moz 27 generally induced shoot dry weight and relative effectiveness similar or superior to the N-fertilizer treatment (Tables 3–6), further supporting the deployment of these strains as a sustainable technology for smallholder farmers. At the host level, the promiscuous Zamboane variety demonstrated consistently higher shoot dry weight than the non-

promiscuous Safari, thereby validating the notion that promiscuous varieties optimize symbiosis with a wide range of rhizobia (Tefera, 2011) and underscoring Zamboane's utility as a dual-purpose variety (Eseigbe et al., 2024).

The comprehensive pooled analyses demonstrated that the indigenous strains Moz 4 and Moz 27 were consistently the top performers, exhibiting comparable effectiveness to synthetic N-fertilization in terms of biological and grain yields, as well as 100-seed weight (Supplementary Tables S4 and S5), thereby validating their potential for use in commercial inoculant production in Mozambique (Chibeba et al., 2017). This evidence suggests that smallholder farmers can sustain elevated productivity without incurring the environmental footprint associated with N-fertilizers, strengthening the contention that the use of N-fertilizers can be substituted in optimized biological nitrogen fixation systems (de Freitas et al., 2022; Yokoyama et al., 2022).

In the first cropping season – the period most severely impacted by drought – the uninoculated and N-fertilized control (control + N), outperformed the uninoculated and non-N-fertilized control (control), in shoot dry weight, relative effectiveness, grain yield, and 100-seed weight (Supplementary Tables S2 and S4), confirming that N was the primary limiting factor. In soybean, BNF is recognized as being more sensitive to drought stress than either respiration or photosynthesis (de Freitas et al., 2022). The physiological basis for this vulnerability lies in the tightening of the nodule's oxygen diffusion barrier and the buildup of ureides, whose combination triggers a feedback loop that suppresses nitrogenase activity during drought (Ladrera et al., 2007).

Interestingly, Moz 4, Moz 27, and SEMIA 5080 strains frequently outperformed the control across most variables at Muriaze and Namarripe, the locations most affected by drought during the first season (section 3.1). This suggests that while drought generally suppresses BNF, there is significant genetic variability in coping with edaphic water stress among the *Bradyrhizobium* strains. These strains may possess mechanisms such as improved osmotic adjustment or better nodule vascularity allowing them to maintain higher level of BNF under stress compared with indigenous populations (Cerezini et al., 2020). Consequently, while mineral N provided the most consistent rescue under drought, the performance of these specific strains demonstrates that selecting rhizobia capable of enduring water deficits is a viable strategy for mitigating yield losses in rain-fed soybean systems.

The paradoxical phenological shift wherein the late-maturing variety (Safari) reached physiological maturity ahead of the medium-maturing Zamboane presumably emanates from drought-induced forced senescence. This terminal stress response compels the plant to accelerate nutrient remobilization toward the seeds, effectively "escaping" deleterious water deficits (Desclaux and Roumet, 1996). In Safari, this "dry-off" phase was likely precipitated by an acute hormonal surge of abscisic acid (ABA) and ethylene. Conversely, Zamboane's superior vegetative biomass—a hallmark of its dual-purpose utility for grain and forage—suggests a distinct physiological buffering or water-use strategy that precluded such a rapid developmental acceleration.

The observed divergence in maturation directly dictated the impact of *Phakopsora pachyrhizi*, as Zamboane was caught at the highly susceptible R6 stage during the onset of the rust epidemic. At this full-seed phase, the pathogen functions as a competitive sink for carbohydrates and N, severely compromising final yield (Hartman et al., 1991). Conversely, Safari's forced senescence enabled it to reach the R7 stage before the infection peaked. Since dry matter accumulation is largely complete by this stage, Safari's yield was already physiologically partitioned (Board et al., 1994). Such a compressed life cycle effectively functioned as a mechanism for reducing plant susceptibility, demonstrating that under terminal drought, rapid maturation can provide an unintentional advantage in mitigating losses from late-season foliar pathogens (Kawuki et al., 2003; Kumudini et al., 2008)

Average soybean yields in Mozambique were 1,410 and 1,667 kg/ha for the 2017/2018 and 2018/2019 cropping seasons, respectively (Faostat, 2023), mirroring the incremental improvement in rainfall between the two periods. While the yields observed in this study followed a similar upward trend—increasing from 1,098 to 1,543 kg/ha—they remained 22% and 8% below the national averages, respectively (Supplementary Table S4). This yield gap in the first season is primarily attributable to suboptimal soil fertility at the experimental locations (Table 1), exacerbated by wild rabbit herbivory and more severe moisture stress during the vegetative growth stages (Section 3.1). Although soybean rust was recorded at one location during the 2018/2019 season, the overall yield gap narrowed as climatic conditions improved and biotic pressures from the previous year subsided.

While elite foreign strains are globally recognized for their high symbiotic effectiveness, relying on them for widespread use in Mozambique presents serious logistical and economic challenges. From a supply-chain perspective, importing these inoculants leaves local farming systems vulnerable to cold-chain breakdowns during international transit and exposes smallholder farmers to unpredictable market disruptions.

Ecologically, introducing large amounts of non-native commercial microorganisms acts like an invasive event that can permanently alter the balance of local microbial communities (Liu et al., 2022). These foreign biological introductions often disrupt native soil life by destabilizing critical keystone taxa that drive the foundational structure and nutritional functioning of local underground ecosystems (Banerjee et al., 2018). Given that our selected indigenous *Bradyrhizobium* strains successfully match the biological yield and grain output of imported reference strains, developing them provides a critical assurance for national biosecurity and input sovereignty. This approach offers a highly sustainable pathway for local soybean production, safeguarding Mozambique's native soil ecosystems without the systemic risks and logistical dependencies of imports.

This study demonstrates the high effectiveness of indigenous rhizobial strains relative to elite exotic counterparts, showing that the central hypothesis holds true for specific locally adapted strains. While response to inoculation occurred with both indigenous and exotic strains, Moz 4 and Moz 27 exhibited remarkable consistency, enhancing nodulation, plant growth, and grain yield—matching or surpassing the performance of exotic strains. These results underscore the vast potential for the selection and

utilization of indigenous rhizobia to maximize BNF. Adopting these locally adapted strains offers a sustainable strategy to increase soybean productivity in Mozambique without relying heavily on foreign microbial introductions, thereby safeguarding local agricultural systems against market fluctuations, high import costs, and supply chain vulnerabilities inherent to international trade.

Declarations

Competing Interests

The authors have no relevant financial or non-financial interests to disclose. The microbial strains utilized in this study (Moz 4, Moz 19, Moz 22, Moz 27, Moz 61, SEMIA 5079, SEMIA 5080, and USDA 110) are part of a public research collection and are subject to no commercial conflicts of interest.

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Author Contributions

All authors contributed to the study conception and experimental design. Material preparation, open-field trial management, and data collection were performed by AMC and SK-B. Statistical analyses, data curation, and interpretation of the Genotype × Environment interactions were conducted by AMC and JOO. The first draft of the manuscript was written by AMC, MAN and MH, and all authors commented on subsequent versions of the manuscript. All authors read and approved the final manuscript.

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Data Availability

The datasets generated during and/or analysed during the current multi-location field experiments are available from the corresponding author on reasonable academic request.

Tables

Tables 1-11 are available in the Supplementary Files section.

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Figures

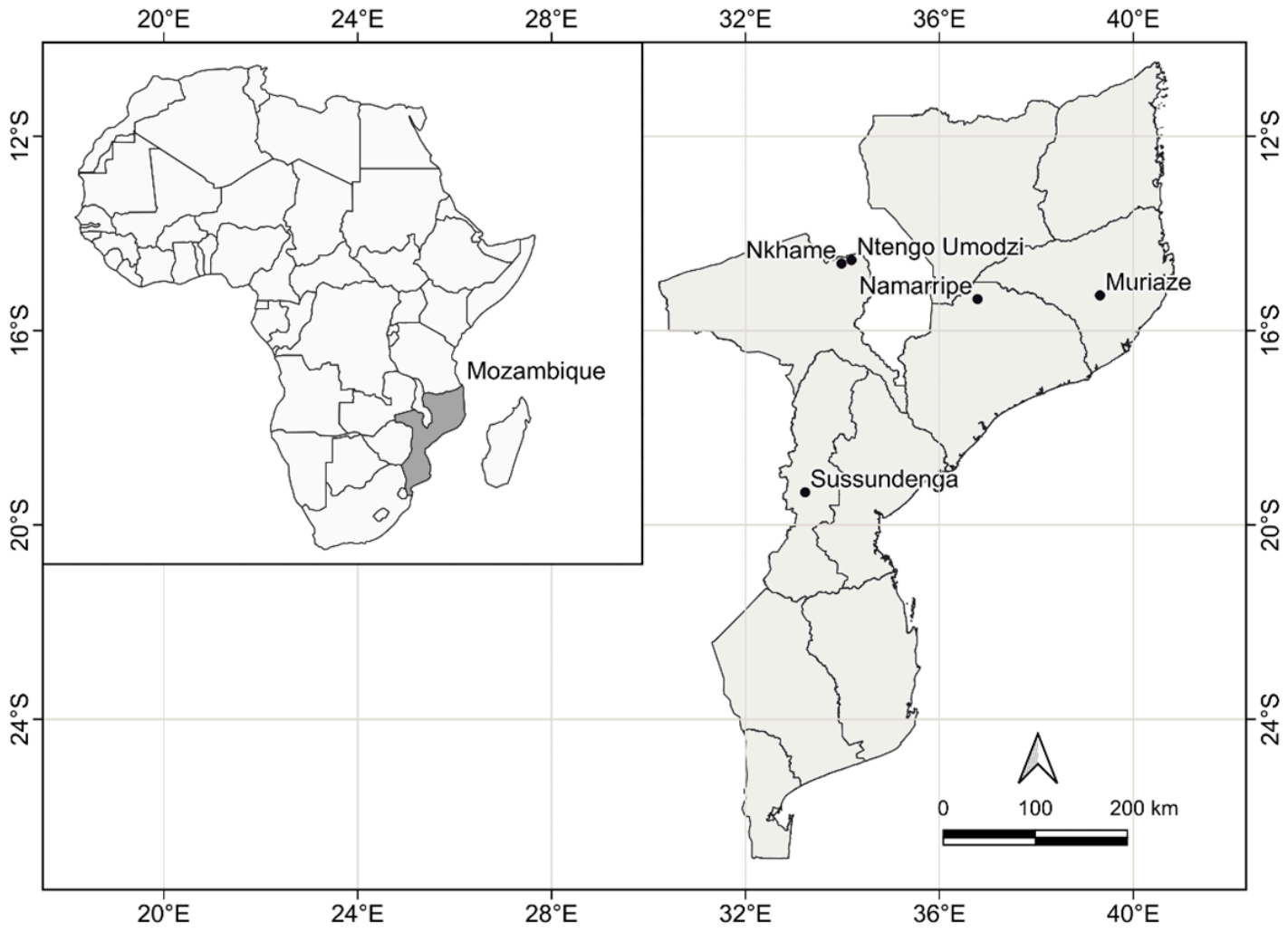


Figure 1

Legend not included with this version

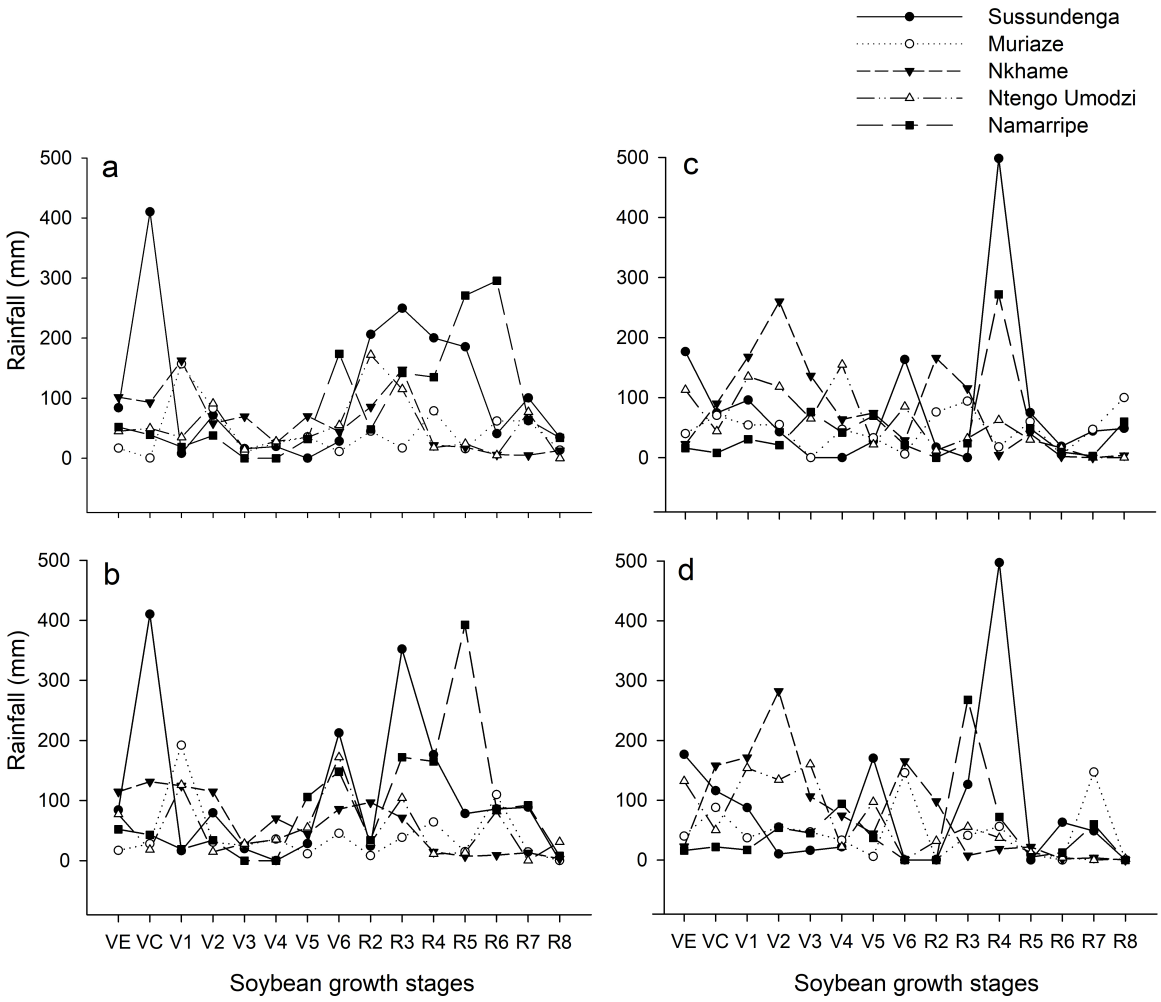


Figure 2

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