Towards sustainable yield improvement: field inoculation of soybean with Bradyrhizobium *and co-inoculation with* Azospirillum *in Mozambique*

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ORIGINAL PAPER

Towards sustainable yield improvement: field inoculation of soybean with *Bradyrhizobium* and co-inoculation with *Azospirillum* in Mozambique

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Abstract

The effects of sole inoculation of soybean (*Glycine max* L. Merrill) with *Bradyrhizobium* and co-inoculation with *Bradyrhizobium* and *Azospirillum* on nodulation, plant growth and yields were investigated in the 2013/2014 and 2014/2015 cropping seasons under field conditions in Mozambique. The treatments included (1) Control (non-inoculated control, with symbiosis depending on indigenous rhizobia), (2) Urea (non-inoculated, receiving 200 kg ha^{-1} of N), (3) Sole inoculation with *B. diazoefficiens* strain USDA 110, and (4) Co-inoculation with *B. diazoefficiens* strain USDA 110 and *A. brasilense* strains Ab-V5 and Ab-V6, evaluated in a randomized complete block design with five replications. Nodule number and dry weight, shoot dry weight, biological and grain yields, grain dry weight, and harvest index were evaluated. In general, both sole inoculation and co-inoculation enhanced nodulation in relation to control. Sole inoculation increased grain yield by 22% (356 kg ha^{-1}), the same enhancement magnitude attained under mineral N treatment, suggesting that *Bradyrhizobium* inoculation provides ecological and economic sustainability to the soybean crop in Mozambique or other countries with similar agro-climatic conditions. Co-inoculation did not increase grain yields in relation to neither the control nor sole inoculation, indicating that further research with adapted and high yielding soybean varieties along with effective rhizobial strains is required in Mozambique to attune the beneficial *Azospirillum*–plant cultivar–rhizobia interactions that have been reported in other countries for several legumes, including soybean.

Keywords Biological nitrogen fixation · Native rhizobia · PGPR · Non-promiscuous soybean

Introduction

The population on Earth has been predicted to grow beyond 10 billion by 2100 (Gerland et al. 2014; Sakschewski et al. 2014) and much of the growth is expected to occur in Africa (Cleland 2013; Gerland et al. 2014). To cope with the potentially enormous food demand, the priority in current agriculture should be on promoting and increasing the yield of multi-purpose crops such as soybean. With high protein

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(≈ 40%) and oil (≈ 20%) concentration in the seeds, soybean is an excellent source of protein for human and animal feeding. Soybean grains are also used in the pharmaceutical industry (Kusunoki et al. 2015) and, more recently, have been recognized as an alternative to fossil fuels (Zanetta et al. 2015).

Soybean cultivation away from the Southern East region of Asia, where it was first domesticated (Li et al. 2010), typically necessitates inoculation with effective bradyrhizobial strains (Abaidoo et al. 2007; Giller et al. 2011). In Africa, considering the logistic constrains for production and distribution of inoculants, the development of promiscuous soybean varieties, capable of fixing nitrogen with rhizobia indigenous to African soils, was suggested in the 1980s as a practical strategy (Pulver et al. 1985; Tefera 2011). In several locations, promiscuous varieties frequently yield poorly, presumably due to ineffective and/or low inocula density of indigenous rhizobial population in those soils (Abaidoo et al. 2007; Klogo et al. 2015). Thus, the use of inoculants containing elite bradyrhizobial strains is now being promoted for both promiscuous and non-promiscuous soybean varieties (Gyogluu et al. 2016; van Heerwaarden et al. 2018). Current research trends on the continent are oriented towards evaluating the potential of indigenous rhizobia for use as inoculant for soybean, with promising strains already identified (Chibeba et al. 2017; Nabintu et al. 2019).

Elsewhere, outside Africa, combined inoculation of rhizobial strains with plant growth-promoting rhizobacteria of the genus *Azospirillum* exerts beneficial effects in legumes including peanut (*Arachis hypogaea* L.) (Vicario et al. 2016), common bean (*Phaseolus vulgaris* L.) (de Carvalho et al. 2020) and soybean (Chibeba et al. 2015; Hungria et al. 2015). Among several effects, co-inoculation promotes higher rhizobial nodule occupancy (Aung et al. 2013), early nodulation (Chibeba et al. 2015; de Carvalho et al. 2020), increase in nodule dry weight (Aung et al. 2013; Chibeba et al. 2015), shoot dry weight (Chibeba et al. 2015; Ferri et al. 2017), and grain yield (Hungria et al. 2015; Ferri et al. 2017). In the context of global climatic changes, with alterations in patterns of temperature and rainfall, co-inoculation has potential to minimize the effects of drought and high temperatures, particularly if the unfavorable weather conditions occur in the early stages of the symbiosis (Chibeba et al. 2015; Cassán et al. 2020). Rather intriguingly, however, evidences of detrimental effects of *Bradyrhizobium*—*Azospirillum* co-inoculation on soybean grain yields have also been reported (Remans et al. 2008; Braccini et al. 2016), suggesting that further research to elucidate the effects is required.

In Mozambique, soybean is a recently introduced crop based mainly on promiscuous varieties with no use of inoculants (Gyogluu et al. 2016). However, the mounting demand for soybean grains to supply the poultry industry (Smart and Hanlon 2014) has ignited the research of higher-yielding non-promiscuous soybean cultivars, which generally require inoculation with elite rhizobial strains (van Heerwaarden et al. 2018). Inoculation technologies that increase the resilience of the crop to climatic changes are particularly important for Mozambique, a country with a recent history of frequent draught spells. Therefore, the objective of this study was to assess the effects of sole inoculation of a non-promiscuous soybean variety with *B. diazoefficiens* and co-inoculation with *B. diazoefficiens* and *A. brasilense* at locations that represent the major soybean production areas in Mozambique on nodulation, plant growth and grain yield.

Material and methods

Experimental locations: geographic, climate and soil characterization

This study is part of the research in which *B. diazoefficiens* USDA 110 was tested along with four other strains in Brazil and Mozambique (Chibeba et al. 2018). In Mozambique, *B. diazoefficiens* USDA 110 strain was tested as sole inoculation and co-inoculation treatments. The experiments were conducted in the 2013/2014 and 2014/2015 cropping seasons in Mozambique at five locations (Fig. 1), Muriaze (Nampula Province, 15°16′S, 39°19′E, Alt. 363 m), Nkhame (Tete Province, 14°38′S, 33°59′E, Alt. 1115 m), Ntengo Umodzi (Tete Province, 14°33′S, 34°11′E, Alt. 1225 m), Ruace (Zambézia Province, 15°08′S, 36°25′E, Alt. 673 m), and Sussundenga (Manica Province, 19°19′S, 33°15′E, Alt. 611 m). The climate types, based on the Köppen-Geiger's classification system, are *Aw (*Tropical monsoon climate) at Muriaze and *Cwa* (Dry-winter humid subtropical climate) at Nkhame, Ntengo Umodzi, Ruace and Sussundenga. Soil types, in accordance with the FAO's soil classification, are Arenosol (Muriaze, Ruace and Sussundenga) and Lixisol (Nkhame and Ntengo Umodzi). Two months prior to the installation of the experiments, 20 soil samples were taken at the 0–20 cm layer at each location to assess the soybean-nodulating rhizobial population, soil granulometry and chemical characterization. The most probable number per gram of soil (MPN, cells g^{-1}) (O'Hara et al. 2016) was used to estimate rhizobial population density, with soybean Storm variety employed as trap host. Soil granulometry was estimated by the hydrometer method (Kilmer and Alexander 1949) and soil pH was measured in water (1/2; soil/water). Exchangeable cations (Mg, Al, Ca, K) and P were analyzed through Mehlich-3 (Sims 1989) and then by inductively coupled plasma optical emission spectroscopy. Soil organic carbon (SOC) was estimated by the Walkley–Black chromic acid wet oxidation method (Walkley and Black 1934)

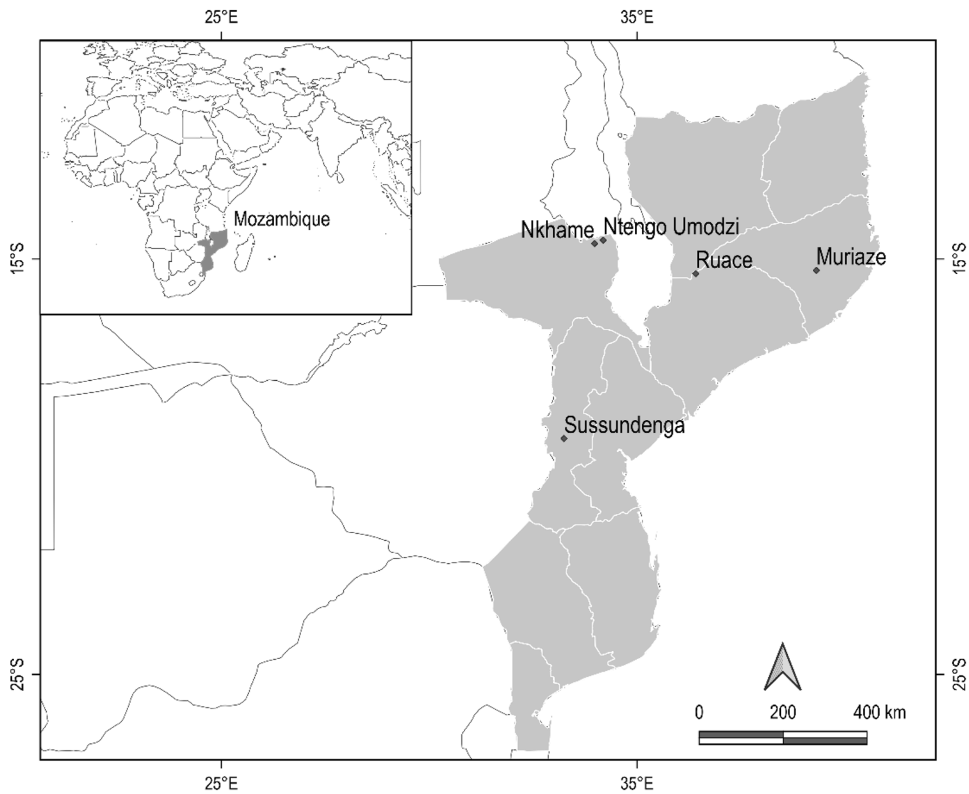

Fig. 1 Map of Mozambique highlighting the locations where the soybean inoculation field experiments were carried out in Manica (Sussundenga), Nampula (Muriaze), Tete (Nkhame and Ntengo Umodzi) and Zambézia (Ruace) provinces. The map was generated through QGIS® version 3.12.2 free software (https://qgis.org/en/site/)

and converted to soil organic matter (SOM) by the equation SOM = 1.724 × SOC.

At all locations, the experiments were carried out in areas with no known previous soybean cultivation history or rhizobia inoculation. The soil chemical and physical characterizations as well as soybean-nodulating rhizobial populations of the experimental locations are summarized in Table 1.

Treatments and conduction of the experiments

The treatments were: (1) C, control (non-inoculated control, with symbiosis relying on indigenous rhizobia); (2) U, urea (non-inoculated receiving 200 kg ha^{-1} of N), applied half at sowing and half at R2 stage (Fehr and Caviness 1977); (3) USDA 110, sole inoculation with *B. diazoefficiens* strain USDA 110; (4) USDA 110 + Azo, co-inoculation with *B. diazoefficiens* strain USDA 110 and *A. brasilense* strains Ab-V5 (= CNPSo 2083) and Ab-V6 (= CNPSo 2084). The experiments were arranged in a randomized complete block design with five replications. Storm, one of the high yielding and non-promiscuous soybean varieties mostly grown in Mozambique, Malawi, Zambia and Zimbabwe, when the study was carried out, was employed. *B. diazoefficiens* USDA 110 was selected for being the most widely used inoculant strain on soybean in Africa and our group has successfully co-inoculated *A. brasilense* strains Ab-V5 and Ab-V6 with *Bradyrhizobium* in previous studies (Hungria et al. 2013, 2015; Chibeba et al. 2015) in Brazil.

For the sole inoculation treatment, peat-based inoculant containing strain USDA 110 was applied to the seeds to provide a final concentration of 1.2 × 10^{6} cells seed^{-1} and 10% (w/v) sucrose solution was added as a sticker, whereas for the co-inoculation, strains Ab-V5 and Ab-V6 were additionally applied, as a liquid inoculant, to provide 1.2 × 10^{5} cells seed^{-1}. The inoculum was vigorously mixed with the seeds and allowed to dry in the shade for 2 h before sowing. Seeds received no pesticide treatment and all experiments were conducted under natural rainfall conditions.

Seven-row plots measuring 9 m in length and separated by 50 cm were sown to attain a population density of 300,000 plants ha^{-1}. At all locations, plots were separated by 1.5 m-wide terraces to avoid cross contamination. Fertilizer [0:20:20 ($N:P_2O_5:K_2O$)] was applied in-furrow at the rate of 300 kg ha^{-1} in all plots just prior to sowing.

Evaluation of nodulation, shoot dry weight, biological yield, grain weight, grain yield and harvest index

Five randomly selected plants were harvested from each plot with a manual hoe at R3 stage (Fehr and Caviness 1977) and taken to the laboratory, where shoots and roots were separated at the cotyledonal nodes for assessment of

Table 1 Rhizobial populations (MPN, cells g^{-1} soil), chemical (pH, $CaCl_2$; organic matter, g dm^{-3}; Available P, mg dm^{-3}; Exchangeable K, Ca and Mg, $cmol_c$ dm^{-3}; exchangeable acidity, $cmol_c$ dm^{-3}) and soil granulometric (silt, sand and clay, g dm^{-3}) properties of the soils at Muriaze, Nkhame, Ntengo Umodzi, Ruace and Sussundenga in the 2013/2014 and 2014/2015 cropping seasons

Soil characteristic[#]	Experimental locations[a]									
	2013/2014 cropping season					2014/2015 cropping season				
	Mur	Nkh	Nte	Rua	Sus	Mur	Nkh	Nte	Rua	Sus
Rhizobia (MPN, cells g^{-1} soil)	< 10	1×10^3	75	1×10^3	< 10	na	na	na	na	na
pH[b] ($CaCl_2$)	5.9	5.5	6.3	4.9	5.4	5.9	5.5	5.3	5.3	5.5
Organic matter (g dm^{-3})	41.38	12.41	22.80	13.53	11.38	27.41	25.17	21.90	18.10	16.21
Available P (mg dm^{-3})	13.20	27.60	7.96	22.40	4.12	3.94	19.10	2.17	28.50	16.50
K ($cmol_c$ dm^{-3})	0.65	0.22	2.02	0.27	0.22	0.56	0.31	0.56	0.38	0.16
Ca ($cmol_c$ dm^{-3})	9.45	3.11	8.70	2.12	3.38	7.25	3.67	6.20	3.61	2.45
Mg ($cmol_c$ dm^{-3})	1.38	1.13	3.57	0.49	0.83	1.44	1.10	1.95	0.97	0.62
EA[c] ($cmol_c$ dm^{-3})	1.02	0.78	0.82	1.30	0.83	0.85	0.99	2.19	1.30	0.66
SB[d] ($cmol_c$ dm^{-3})	11.48	4.45	14.29	2.88	4.44	9.25	5.08	8.71	4.96	3.23
CEC[e] ($cmol_c$ dm^{-3})	12.50	5.23	15.11	4.18	5.27	10.10	6.07	10.90	6.26	3.89
BS[f] (%)	91.88	85.07	94.57	68.88	84.17	91.62	83.74	79.92	79.17	83.01
Silt (g dm^{-3})	128	128	173	84	43	56	134	133	113	36
Sand (g dm^{-3})	542	682	420	842	861	664	719	537	817	897
Clay (g dm^{-3})	330	190	407	74	96	280	147	330	70	67

[a]Location of the experiments: *Mur* Muriaze, *Nkh* Nkhame, *Nte* Ntengo Umodzi, *Rua* Ruace, *Sus* Sussundenga

[b]pH ($CaCl_2$) = pH (H_2O) × 0.923 – 0.373 (Ahern et al. 1995)

[c]EA, exchangeable Acidity = (Al + H)

[d]SB, sum of Bases = (K + Ca + Mg)

[e]CEC, cation Exchangeable Capacity = (EA + SB)

[f]BS, bases Saturation = SB/CEC × 100

na due to logistic difficulties, rhizobial populations were not estimated in the 2014/2015 cropping season

[#]This is part of the study in which *B. diazoefficiens* USDA 110 was tested along four other strains in Brazil and Mozambique (Chibeba et al. 2018). In Mozambique, *B. diazoefficiens* USDA 110 strain was tested as sole inoculation and co-inoculation treatments

nodulation and shoot dry weight (SDW). Washed shoots were placed in paper bags and oven-dried at 50 °C for 72 h before determination of SDW. Roots were oven-dried at 50 °C for 72 h, then nodules were removed from the roots and counted for estimating nodule number (NN), and weighed for nodule dry weight (NDW), according to usual procedures.

At R8 stage [reproductive stage, 95% of pods have attained their mature pod color (Fehr and Caviness 1977)], the entire plant population within the middle area of 20 m^2 of each plot was used for determination of biological yield (BY), defined as total above-ground plant yield, grain yield (GY), and harvest index (HI). Plants were excised at the cotyledonal node, oven-dried at 50 °C for 72 h and weighed for assessing BY. For GY assessment, grains were weighed, and figures adjusted to 13% moisture content, based on the moisture status determined through a hygrometer. Grain weight (GDW), based on the mass of 100 grains, was also determined. HI was calculated as the ratio between GY and BY.

Statistical analysis

Prior to the statistical analyses, ANOVA assumptions, namely homoscedasticity of variances (with Bartlett test) and normally distributed residual variation (with Shapiro–Wilk test), were verified. After this, ANOVA was applied to the dataset, and whenever significant difference among treatments was detected, Tukey's Honestly Significant Difference Test (HSD) was employed for a *posteriori* multiple comparisons among the treatment means. Data from each cropping season and locations were also pooled and bulk analyzed. Differences were considered significant at $p < 0.05$. All statistical analyses were performed with the software SAS® 9.4 (SAS Institute, North Carolina, USA).

Results

While all the findings from this study are summarized in tables and graphs, variables whose treatment effects were significant are particularly highlighted in this section. In addition, emphasis in put on the comparisons between sole inoculation and co-inoculation treatments.

Indigenous rhizobial populations

Rhizobial population densities were only estimated in the 2013/2014 cropping season owing to logistic constraints. The population of indigenous rhizobia in the soil varied from < 10 (Muriaze and Sussundenga) to 1×10^3 rhizobial cells g^{-1} of soil (Nkhame and Ruace) (Table 1).

Nodule number and dry weight

Sole inoculation statistically increased nodule number (NN) in relation to control at Ntengo Umodzi (Table 4) and Sussundenga (Table 6) in the 2013/2014 cropping season, and at Muriaze (Table 2) in the 2014/2015 cropping season. Sole inoculation effect on NN across locations was negligible in the 2013/2014 but in the 2014/2015 cropping season sole inoculation statistically increased NN in relation to control (Table 7). Across locations and cropping seasons, sole inoculation significantly enhanced NN in relation to control (Table 8). Co-inoculation similarly enhanced NN in relation to control at Ntengo Umodzi (Table 4), Ruace (Tables 5) and Sussundenga (Table 6) in the 2013/2014 cropping season, and Muriaze (Table 2) in the 2014/2015 cropping season. Overall, co-inoculation also improved NN in relation to the control treatment (Table 8). However, contrasting results were observed on the effect of co-inoculation in relation to sole inoculation. While co-inoculation resulted in significantly lower NN in relation to sole inoculation at Muriaze in the 2014/2015 cropping season (Table 2) and Nkhame in the 2013/2014 cropping season (Table 3), opposite effects were observed at Ruace in the 2013/2014 cropping season (Table 5). The overall effect of co-inoculation in relation to sole inoculation on NN across locations and cropping seasons was non-statistically significant (Tables 7, 8).

Both sole inoculation and co-inoculation statistically enhanced nodule dry weight (NDW) at Ruace (Table 5) in the 2013/2014 cropping season but had lower NDW at Muriaze (Table 2) in the 2014/2015 cropping season, in relation to control. For the across locations analysis, sole inoculation and co-inoculation statistically enhanced NDW in relation to control in the 2013/2014 cropping season but both treatments failed to improve NDW in the 2014/2015 cropping season (Table 7). Positive and significant effects of co-inoculation in relation to sole inoculation were observed at Nkhame (Table 3), Ruace (Table 5) and across locations (Table 7) in the 2013/2014 cropping season, but no significant differences were observed between the two treatments in the 2014/2015 cropping season. Moreover, no significant differences were observed between sole inoculation and co-inoculation in terms of NDW in the analyses across locations and cropping seasons (Table 8). These analyses also revealed that N fertilization had a negative effect on nodulation as indicated by significant lower NN and NDW in relation to control (Table 8).

Table 2 Nodule number (NN, n° plant^{-1}), nodule dry weight (NDW, mg plant^{-1}), shoot dry weight (SDW, g plant^{-1}), biological yield (BY, kg ha^{-1}), grain dry weight (GDW, g 100 seeds^{-1}), and harvest index (HI) of soybean, cultivar Storm, grown in the 2013/2014 and 2014/2015 cropping seasons at Muriaze

Treatment[1#]	Muriaze, 2013/2014 cropping season						Muriaze, 2014/2015 cropping season					
	NN	NDW	SDW	BY	GDW	HI[2]	NN	NDW	SDW	BY	GDW	HI[2]
C	6.2^{a3}	27.40^{ns}	12.9^{ns}	5506^{ns}	15.6^{ns}	0.28^{ns}	18.5^{c3}	150.15^{a}	22.5^{ns}	3199^{ab}	15.8^{ab}	0.27^{ns}
U	3.4^{b}	27.60	19.8	5610	16.4	0.24	13.5^{d}	73.70^{b}	23.5	1955^{b}	15.9^{ab}	0.41
USDA 110	7.4^{a}	37.64	15.0	5575	15.8	0.31	35.4^{a}	89.20^{b}	21.4	3742^{a}	14.8^{bc}	0.44
USDA 110 + Azo	7.0^{a}	46.68	12.1	5007	16.2	0.29	30.7^{b}	55.91^{b}	17.5	3350^{ab}	16.9^{a}	0.37
p value	< 0.01	0.28	0.07	0.29	0.70	0.19	< 0.01	< 0.01	0.29	0.02	0.33	0.13
C.V. (%)	25.24	50.09	31.04	9.96	7.44	16.52	3.86	22.67	23.65	27.92	10.84	30.27

[1]*C* control, *U* urea (200 kg of N ha^{-1}), half applied during sowing and a half at R2; USDA 110*,* inoculation with *B. diazoefficiens* strain USDA 110; USDA 110 + Azo, same as before plus *A. brasilense* strains Ab-V5 and Ab-V6. *B. diazoefficiens* was applied at the rate of 1.2×10^6 cells seed^{-1} while *A. brasilense* strains were applied at 1.2×10^5 cells seed^{-1}

[2]Determined as a percentage ratio between GY and BY

[3]Means of five replicates and when followed by the same letter(s) in the same column are not statistically different ($p < 0.05$, Tukey HSD test)

[#]Same as Table 1

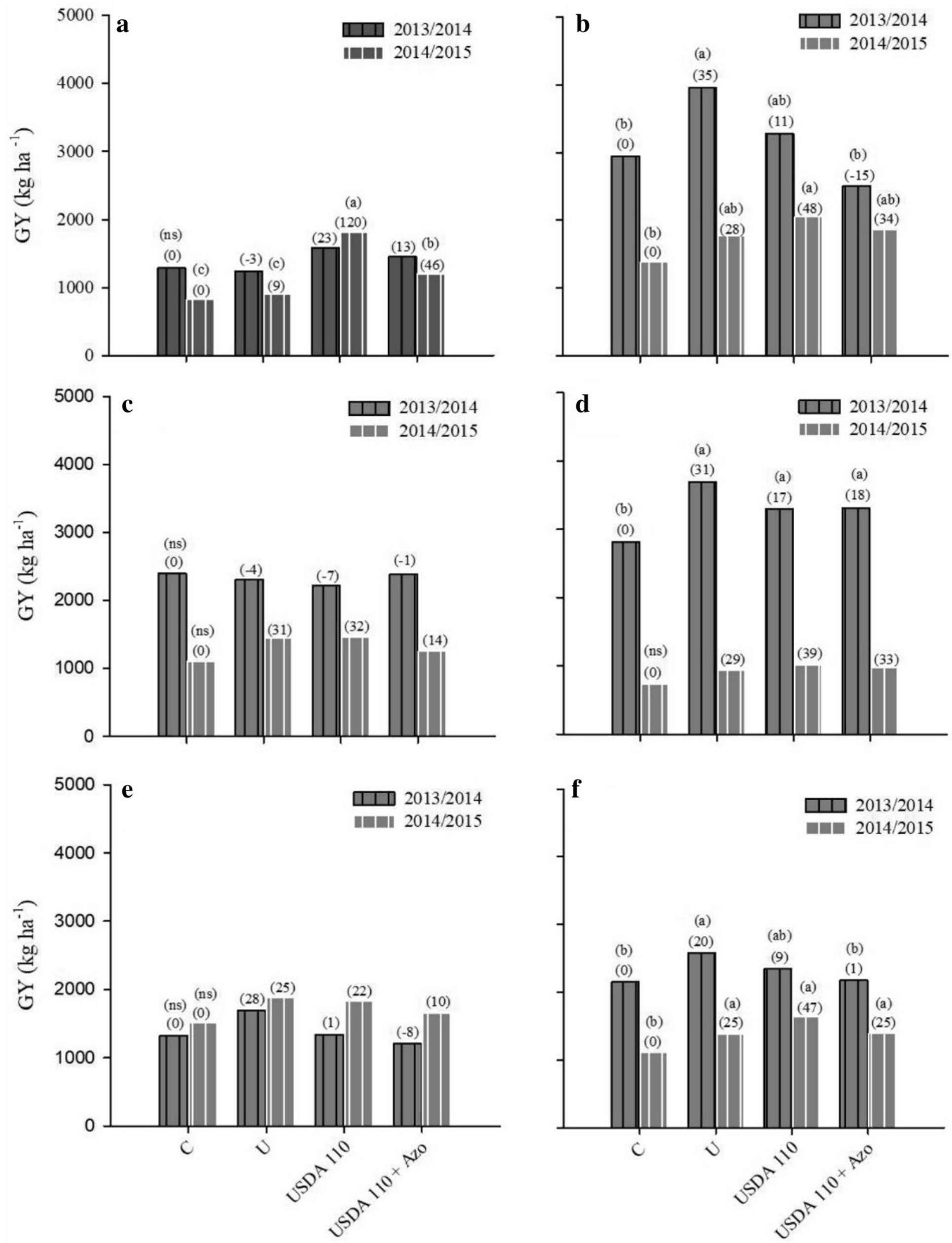

Fig. 2 Grain yield (GY, kg ha^{-1}) of soybean, cultivar Storm, grown in Mozambique at Muriaze (**a**), Nkhame (**b**), Ntengo Umodzi (**c**), Ruace (**d**), Sussundenga (**e**), and across all locations (**f**) in the 2013/2014 and 2014/2015 cropping seasons. *C* control, *U* urea (200 kg of N ha^{-1}), half applied during sowing and half at R2; USDA 110, sole inoculation with *B. diazoefficiens* strain USDA 110; USDA 110 + Azo, same as before plus *A. brasilense* strains Ab-V5 and Ab-V6. Bars are means of five replicates and when followed by the same letter at the same location and cropping season are not statistically different ($p < 0.05$, Tukey); ns—not statistically different ($p < 0.05$, Tukey). Number above each bar represents % of grain yield difference in relation to the control treatment. This is part of the study in which *B. diazoefficiens* USDA 110 was tested along four other strains in Brazil and Mozambique (Chibeba et al. 2018). In Mozambique, *B. diazoefficiens* USDA 110 strain was tested as sole inoculation and co-inoculation treatments

Grain yield and grain weight

In the 2013/2014 cropping season, sole inoculation resulted in grain yield (GY) enhancement of 17% (477 kg ha^{-1}) at Ruace, in relation to control (Fig. 2d). The effects of sole inoculation were more pronounced in the 2014/2015 cropping season as indicated by GY enhancements of 120% (989 kg ha^{-1}) at Muriaze (Fig. 2a), 48% (668 kg ha^{-1}) at Nkhame (Fig. 2b), and an average GY improvement of 47% (523 kg ha^{-1}) (Fig. 2f) was recorded across locations in relation to control. Overall, sole inoculation effect across locations and cropping seasons on GY was of 22% (356 kg ha^{-1}), a similar increase magnitude recorded when N-fertilizer was applied, in relation to the control treatment (Table 8).

Co-inoculation treatment resulted in GY gain of 18% (497 kg ha^{-1}), in relation to control, at Ruace (Fig. 2d) in the 2013/2014 cropping season. More pronounced co-inoculation effects were obtained in the 2014/2015 cropping season, as shown by GY gains of 46% (378 kg ha^{-1}) at Muriaze (Fig. 2a) and 25% (279 kg ha^{-1}) in the average across all the five locations (Fig. 2f), in relation to control. However, the overall co-inoculation effects across locations and cropping seasons were not significantly different from the control treatment (Table 8). Rather intriguingly, co-inoculation resulted in 34% (611 kg ha^{-1}) significantly

Table 3 Nodule number (NN, n° plant^{-1}), nodule dry weight (NDW, mg plant^{-1}), shoot dry weight (SDW, g plant^{-1}), biological yield (BY, kg ha^{-1}), grain dry weight (GDW, g 100 seeds^{-1}), and harvest index (HI) of soybean, cultivar Storm, grown in the 2013/2014 and 2014/2015 cropping seasons at Nkhame

Treatment[1#]	Nkhame, 2013/2014 cropping season						Nkhame, 2014/2015 cropping season					
	NN	NDW	SDW	BY	GDW	HI[2]	NN	NDW	SDW	BY	GDW	HI[2]
C	24.8[a3]	29.50[bc]	36.5[ns]	6995[ns]	15.6[ab]	0.28[b]	8.9[ns3]	91.63[a]	17.2[b]	3810[ns]	14.8[ns]	0.38[ns]
U	16.7[bc]	16.95[c]	58.6	8032	17.3[a]	0.33[a]	9.6	68.93[ab]	31.3[a]	4797	15.7	0.39
USDA 110	20.4[ab]	38.40[b]	52.4	7463	16.1[ab]	0.29[b]	16.4	52.00[ab]	24.3[ab]	4913	15.5	0.43
USDA 110 + Azo	12.8[c]	57.18[a]	47.8	7012	15.2[b]	0.25[c]	11.2	44.96[b]	29.5[ab]	4836	15.3	0.40
p—value	< 0.01	< 0.01	0.23	0.36	0.03	< 0.01	0.11	0.02	0.03	0.57	0.17	0.78
C.V. (%)	21.25	28.19	33.86	13.76	6.23	6.54	43.03	34.95	27.57	30.77	4.20	21.00

[1]*C* control, *U* urea (200 kg of N ha^{-1}), half applied during sowing and a half at R2; USDA 110; USDA 110, inoculation with *B. diazoefficiens* strain USDA 110; USDA 110 + Azo, same as before plus *A. brasilense* strains Ab-V5 and Ab-V6. *B. diazoefficiens* was applied at the rate of 1.2×10^6 cells seed^{-1} while *A. brasilense* strains were applied at 1.2×10^5 cells seed^{-1}

[2]Determined as a percentage ratio between GY and BY

[3]Means of five replicates and when followed by the same letter(s) in the same column are not statistically different ($p < 0.05$, Tukey HSD test)

[#]Same as Table 1

Table 4 Nodule number (NN, n° plant^{-1}), nodule dry weight (NDW, mg plant^{-1}), shoot dry weight (SDW, g plant^{-1}), biological yield (BY, kg ha^{-1}), grain dry weight (GDW, g 100 seeds^{-1}), and harvest index (HI) of soybean, cultivar Storm, grown in the 2013/2014 and 2014/2015 cropping seasons at Ntengo Umodzi

Treatment[1#]	Ntengo Umodzi, 2013/2014 cropping season						Ntengo Umodzi, 2014/2015 cropping season					
	NN	NDW	SDW	BY	GDW	HI[2]	NN	NDW	SDW	BY	GDW	HI[2]
C	6.0[b3]	96.40[b]	19.1[ns]	6081[ns]	14.9[ns]	0.39[ns]	29.0[ns3]	77.90[a]	18.8[ns]	2740[ns]	16.2[ns]	0.41[ns]
U	6.8[ab]	90.85[b]	24.0	5265	15.8	0.48	18.0	44.70[b]	18.6	3543	16.4	0.41
USDA 110	8.2[a]	118.84[ab]	22.4	5395	15.8	0.39	22.0	80.49[a]	16.8	3403	16.0	0.43
USDA 110 + Azo	8.8[a]	167.55[a]	20.0	5539	16.3	0.43	23.4	65.09[ab]	17.8	3197	15.4	0.41
p value	< 0.01	< 0.01	0.28	0.70	0.10	0.10	0.42	0.02	0.90	0.15	0.12	0.54
C.V. (%)	14.93	22.91	19.95	20.83	5.32	14.32	44.16	25.52	25.92	17.11	4.08	6.22

[1]*C* control, *U* urea (200 kg of N ha^{-1}), half applied during sowing and a half at R2; USDA 110, inoculation with *B. diazoefficiens* strain USDA 110; USDA 110 + Azo, same as before plus *A. brasilense* strains Ab-V5 and Ab-V6. *B. diazoefficiens* was applied at the rate of 1.2×10^6 cells seed^{-1} while *A. brasilense* strains were applied at 1.2×10^5 cells seed^{-1}

[2]Determined as a percentage ratio between GY and BY

[3]Means of five replicates and when followed by the same letter(s) in the same column are not statistically different ($p < 0.05$, Tukey HSD test)

[#]Same as Table 1

lower GY than sole inoculation at Muriaze in the 2014/2015 cropping season (Fig. 2a) and in general 10% (206 kg ha^{-1}) GY decrease across locations and cropping seasons was observed (Table 8).

Both sole inoculation and co-inoculation statistically improved grain dry weight (GDW) in relation to control at Ruace (Table 5) in the 2013/2014 cropping season. Co-inoculation also improved GDW across locations (Table 7) in the 2013/2014 cropping season. The analyses across locations and cropping seasons, however, revealed that neither sole inoculation nor co-inoculation significantly enhanced GDW in relation to control (Table 8). In general, N fertilizer application significantly improved GDW in relation to control, but the effect was not superior to that of the inoculants (Table 8).

Discussion

Soybean sole inoculation with *B. diazoefficiens* and co-inoculation with *B. diazoefficiens* and *A. brasilense* under field conditions was studied in two cropping seasons in Mozambique. The experiments were carried out in areas with no known history of soybean cropping or rhizobia inoculation, so that soybean plants in the control treatment fully relied on indigenous rhizobial strains established in

Table 5 Nodule number (NN, n° $plant^{-1}$), nodule dry weight (NDW, mg $plant^{-1}$), shoot dry weight (SDW, g $plant^{-1}$), biological yield (BY, kg ha^{-1}), grain dry weight (GDW, g 100 $seeds^{-1}$), and harvest index (HI) of soybean, cultivar Storm, grown in the 2013/2014 and 2014/2015 cropping seasons at Ruace

Treatment[1#]	Ruace, 2013/2014 cropping season						Ruace, 2014/2015 cropping season					
	NN	NDW	SDW	BY	GDW	HI[2]	NN	NDW	SDW	BY	GDW	HI[2]
C	9.9[b3]	100.00[c]	26.8[b]	8806[ns]	17.5[c]	0.32[b]	7.4[ab3]	48.68[ns]	7.8[b]	2267[ns]	14.2[ns]	0.40[ns]
U	2.4[c]	17.64[d]	32.8[a]	9167	18.3[bc]	0.37[ab]	6.2[b]	29.24	15.0[a]	2526	15.6	0.37
USDA 110	9.9[b]	139.92[b]	22.0[b]	8304	19.2[ab]	0.39[ab]	11.7[ab]	37.80	5.6[b]	2644	14.8	0.38
USDA 110 + Azo	17.0[a]	234.88[a]	26.8[b]	8160	19.6[a]	0.41[a]	12.6[a]	32.08	9.5[b]	2323	14.5	0.41
p value	< 0.01	< 0.01	< 0.01	0.05	< 0.01	0.04	0.01	0.26	< 0.01	0.83	0.22	0.39
C.V. (%)	21.60	17.72	9.95	6.75	3.24	12.63	33.01	43.01	24.04	29.77	7.00	10.23

[1]*C* control, *U* urea (200 kg of N ha^{-1}), half applied during sowing and a half at R2; USDA 110, inoculation with *B. diazoefficiens* strain USDA 110; USDA 110 + Azo, same as before plus *A. brasilense* strains Ab-V5 and Ab-V6. *B. diazoefficiens* was applied at the rate of 1.2×10^6 cells $seed^{-1}$ while *A. brasilense* strains were applied at 1.2×10^5 cells $seed^{-1}$

[2]Determined as a percentage ratio between GY and BY

[3]Means of five replicates and when followed by the same letter(s) in the same column are not statistically different ($p < 0.05$, Tukey HSD test)

[#]Same as Table 1

Table 6 Nodule number (NN, n° $plant^{-1}$), nodule dry weight (NDW, mg $plant^{-1}$), shoot dry weight (SDW, g $plant^{-1}$), biological yield (BY, kg ha^{-1}), grain dry weight (GDW, g 100 $seeds^{-1}$), and harvest index (HI) of soybean, cultivar Storm, grown in the 2013/2014 and 2014/2015 cropping seasons at Sussundenga

Treatment[1#]	Sussundenga, 2013/2014 cropping season						Sussundenga, 2014/2015 cropping season					
	NN	NDW	SDW	BY	GDW	HI[2]	NN	NDW	SDW	BY	GDW	HI[2]
C	8.9[b3]	118.98[ab]	8.2[b]	6056[ns]	9.1[ns]	0.23[ns]	5.5[ns3]	65.80[ab]	11.4[ab]	5519[ns]	13.5[ns]	0.28[ns]
U	4.1[b]	64.30[b]	23.3[a]	6728	9.5	0.25	5.8	53.04[b]	8.0[b]	6512	15.3	0.30
USDA 110	18.4[a]	246.38[a]	10.8[b]	7437	9.1	0.23	5.7	85.41[ab]	12.5[a]	6298	15.3	0.27
USDA 110 + Azo	15.9[a]	270.78[a]	10.8[b]	5222	9.1	0.24	6.3	89.48[a]	11.0[ab]	6983	14.9	0.28
p value	< 0.01	< 0.01	< 0.01	0.19	0.70	0.41	0.96	0.03	0.04	0.33	0.16	0.51
C.V. (%)	30.11	49.64	18.00	25.00	7.81	12.00	41.43	26.84	22.24	19.42	9.27	11.05

[1]*C* control, *U* urea (200 kg of N ha^{-1}), half applied during sowing and a half at R2; USDA 110, inoculation with *B. diazoefficiens* strain USDA 110; USDA 110 + Azo, same as before plus *A. brasilense* strains Ab-V5 and Ab-V6. *B. diazoefficiens* was applied at the rate of 1.2×10^6 cells $seed^{-1}$ while *A. brasilense* strains were applied at 1.2×10^5 cells $seed^{-1}$

[2]Determined as a percentage ratio between GY and BY

[3]Means of five replicates and when followed by the same letter(s) in the same column are not statistically different ($p < 0.05$, Tukey HSD test)

[#]Same as Table 1

the soil, in addition to the mineral N coming from the soil organic matter. Across the experimental locations and cropping seasons control plots recorded, on average, a grain yield of 1631 kg ha^{-1} (Table 8), which is greater than the national average of 1216 kg ha^{-1} reported in the same period in which these experiments were conducted (MASA 2015). The over 400 kg ha^{-1} grain yield difference can be explained by phosphorus fertilizer application in this study compared with low or no input applied by the predominantly smallholder farmers who grow soybean in Mozambique (MASA 2015; Gyogluu et al. 2016).

The fact that sole inoculation significantly increased grain yields in relation to the control treatment (Table 8) and yield enhancements were observed even in areas with an indigenous rhizobial population estimated at 10^3 cells g^{-1} (Table 1 and Fig. 2), corroborates abundantly reported responses to inoculation under similar conditions in Brazil (Hungria et al. 2013, 2015). Moreover, contrary to other reports (Streeter 1994; Vlassak et al. 1997), inoculation responses are still possible in the presence of a large indigenous or established rhizobial population, provided that the inoculant strains are competitive and highly effective (Giller 2001; Osunde et al. 2003). Consistent to this, *B. diazoefficiens* USDA 110 has been rated as highly effective (Abaidoo et al. 2007; Youseif et al. 2014; Chibeba et al. 2017) and competitive soybean microsymbiont (Ulzen et al. 2018). Overall, sole

Table 7 Nodule number (NN, n° plant^{-1}), nodule dry weight (NDW, mg plant^{-1}), shoot dry weight (SDW, g plant^{-1}), biological yield (BY, kg ha^{-1}), grain dry weight (GDW, g 100 seeds^{-1}), and harvest index (HI) of soybean, cultivar Storm, grown in the 2013/2014 and 2014/2015 cropping seasons at Muriaze, Nkhame, Ntengo Umodzi, Ruace and Sussundenga

Treatment[1#]	2013/2014 cropping season, across all locations						2014/2015 cropping season, across all locations					
	NN	NDW	SDW	BY	GDW	HI[2]	NN	NDW	SDW	BY	GDW	HI[2]
C	11.2[a3]	74.46[c]	20.7[b]	6689[ns]	14.5[b]	0.30[ns]	13.8[bc3]	86.83[a]	15.5[ns]	3506[ns]	14.9[ns]	0.35[ns]
U	6.7[b]	43.47[c]	31.7[a]	6960	15.4[a]	0.33	10.6[c]	53.92[c]	19.3	3866	15.8	0.38
USDA 110	12.8[a]	116.24[b]	24.5[b]	6835	15.2[ab]	0.32	18.2[a]	68.98[b]	16.1	4200	15.3	0.39
USDA 110 + Azo	12.3[a]	155.41[a]	23.5[b]	6188	15.3[a]	0.32	16.8[ab]	57.50[bc]	17.1	4138	15.4	0.37
p value	0.00	0.00	0.00	0.12	0.01	0.16	0.00	0.00	0.12	0.09	0.12	0.09
C.V. (%)	8.99	19.66	13.6	7.61	2.49	6.76	15.56	12.39	14.49	11.25	3.47	6.48

[1]*C* control, *U* urea (200 kg of N ha^{-1}), half applied during sowing and a half at R2; USDA 110, inoculation with *B. diazoefficiens* strain USDA 110; USDA 110 + Azo, same as before plus *A. brasilense* strains Ab-V5 and Ab-V6. *B. diazoefficiens* was applied at the rate of 1.2×10^6 cells seed^{-1} while *A. brasilense* strains were applied at 1.2×10^5 cells seed^{-1}

[2]Determined as a percentage ratio between GY and BY

[3]Means of five replicates and when followed by the same letter(s) in the same column are not statistically different ($p < 0.05$, Tukey HSD test)

[#]Same as Table 1

Table 8 Nodule number (NN, n° plant^{-1}), nodule dry weight (NDW, mg plant^{-1}), shoot dry weight (SDW, g plant^{-1}), biological yield (BY, kg ha^{-1}), grain yield (GY, kg ha^{-1}), grain dry weight (GDW, g 100 seeds^{-1}), and harvest index (HI) of soybean, cultivar Storm, grown in the 2013/2014 and 2014/2015 cropping seasons at Muriaze, Nkhame, Ntengo Umodzi, Ruace and Sussundenga

Treatment[1#]	2013/2014 and 2014/2015 cropping seasons, across all locations						
	NN	NDW	SDW	BY	GY	GDW	HI[2]
C	12.5[b3]	80.64[b]	18.1[b]	5098[ns]	1631[b]	14.7[b]	0.32[b]
U	8.7[c]	48.69[c]	25.5[a]	5413	1984[a]	15.6[a]	0.35[ab]
USDA 110	15.5[a]	92.61[ab]	20.3[b]	5517	1987[a]	15.2[ab]	0.36[a]
USDA 110 + Azo	14.6[a]	106.46[a]	20.3[b]	5163	1781[b]	15.3[ab]	0.35[ab]
p value	< 0.01	< 0.01	< 0.01	0.34	0.00	0.01	0.03
C.V. (%)	7.51	13.23	10.95	7.65	5.59	2.48	4.99

[1]*C* control, *U* urea (200 kg of N ha^{-1}), half applied during sowing and a half at R2; USDA 110, inoculation with *B. diazoefficiens* strain USDA 110; USDA 110 + Azo, same as before plus *A. brasilense* strains Ab-V5 and Ab-V6. *B. diazoefficiens* was applied at the rate of 1.2×10^6 cells seed^{-1} while *A. brasilense* strains were applied at 1.2×10^5 cells seed^{-1}

[2]Determined as a percentage ratio between GY and BY

[3]Means of five replicates and when followed by the same letter(s) in the same column are not statistically different ($p < 0.05$, Tukey HSD test)

[#]Same as Table 1

inoculation improved grain yields by 22% (356 kg ha^{-1}), the same enhancement magnitude recorded under urea treatment (Table 8), strengthening earlier evidences that soybean inoculation with effective rhizobial strains dismisses the use of N fertilizers (Hungria et al. 2015), thereby providing large ecological and economic sustainability to the agricultural systems.

Co-inoculation effects on nodulation were rather intriguing. While in the 2013/2014 cropping season positive and significant effects on nodule dry weight were observed in relation to sole inoculation across the five locations, the same was not observed in relation to nodule number (Table 7). Moreover, in the 2014/2015 cropping season, with less rainfall (Supplementary Fig. 1), at none of the five locations co-inoculation resulted in either increased nodule number or nodule dry weight in relation to sole inoculation (Tables 3, 4, 5, 6, 7), contradicting earlier reports that responses to co-inoculation with *Azospirillum* are more evident under moderately stressful conditions occurring at early growth stages (Dobbelaere et al. 2001; Cassán and Diaz-Zorita 2016). These observations suggest that further soybean co-inoculation research is required to elucidate the potential of this technology under the agro-ecological conditions of Mozambique.

From the 2013/2014 to the 2014/2015 cropping season there was a general grain yield decrease (Fig. 2), which can be ascribed to the lower rainfall recorded in the second in relation to the first cropping season (Supplementary Fig. 1). Although rhizobia populations were not estimated in the 2014/2015 cropping season, the occurrence of indigenous rhizobia can be inferred by the over 1000 kg ha^{-1} grain yield recorded in the control treatment across locations in this season (Fig. 2f). Therefore, the observed 47% (523 kg ha^{-1}) grain yield enhancement of sole inoculation in relation to control (Fig. 2f) confirms the previous reported high symbiotic effectiveness (Chibeba et al. 2017) and competitiveness (Ulzen et al. 2018) of *B. diazoefficiens* USDA 110 strain, this time under low rainfall conditions. Considering that both sole inoculation and co-inoculation improved grain yields in relation to control in the 2014/2015 cropping season and that the inoculation treatments were not significantly different (Fig. 2f), it can be inferred that the observed co-inoculation effect is mainly attributable to the *B. diazoefficiens* USDA 110 strain.

Robust evidences of co-inoculation superiority over sole inoculation treatment have been reported on soybean grain yields (Hungria et al. 2015; Ferri et al. 2017) ascribed to plant growth stimulation by *Azospirillum* through an array of favorable mechanisms (Bashan and de-Bashan 2010; Cassán et al. 2020). In this study, however, co-inoculation did not result in improved grain yields in relation to sole inoculation (Fig. 2 and Table 8). Lack of improvement of co-inoculation on nodulation, plant growth or grain yield in relation to sole inoculation have been reported in several legumes primarily attributed to *Azospirillum*–plant cultivar–rhizobia interactions (Remans et al. 2008; Cassán and Diaz-Zorita 2016). Indigenous rhizobial strains with promising symbiotic effectiveness have been identified in Mozambique (Chibeba et al. 2017) and are currently being tested in multilocation field experiments. These promising indigenous strains should be tested along with *Azospirillum* and the most adapted and high yielding soybean varieties to finetune the most beneficial *Azospirillum*–plant cultivar–rhizobia synergies in the country.

Conclusions

In general, soybean sole inoculation with *Bradyrhizobium* and co-inoculation with *Bradyrhizobium* and *Azospirillum* enhanced nodulation but none of the treatments increased plant growth when compared with control. Sole inoculation resulted in grain yield enhancement of 22% (356 kg ha^{-1}), the same magnitude increase attained with 200 kg ha^{-1} of N as urea, in relation to control, suggesting that soybean inoculation with *Bradyrhizobium* is a profitable and sustainable technology under the agro-climatic conditions of Mozambique. Co-inoculation did not increase grain yields in relation to the control or sole inoculation, indicating that further research involving adapted and high yielding soybean varieties along with effective rhizobial strains is required in Mozambique to finetune the beneficial *Azospirillum*–plant cultivar–rhizobia synergies that have been reported from other countries.

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Compliance with ethical standards

Conflict of interest Guimarães MF, Nogueira MA and Hungria M belong to the INCT-Plant-Growth Promoting Microorganisms for Agricultural Sustainability and Environmental Responsibility (465133/2014-2). The authors declare that they have no conflict of interest.

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