

# Potential of Inoculant and Phosphorus Application on Soybean Production in Mozambique

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**Abstract** Inoculation with effective *Bradyrhizobium* spp., and phosphorus application enhance atmospheric nitrogen fixation in soybean production. Soybean form symbiotic associations with the right rhizobium strain to incorporate atmospheric nitrogen into the plant tissues. The objective of this study was to evaluate responses of two soybean varieties to inoculation, phosphorus and starter-nitrogen, and their interactions on nodulation, growth, yield components and grain yield in different agroecologies of Mozambique. The study was conducted at three locations in Nampula, Tete and Zambézia provinces in Mozambique during 2012 and 2013 seasons. Two soybean genotypes (Storm and TGx 1904-6F) in split-plot design with phosphorus ( $P_2O_5$ ) rates as main plot, inoculation application as subplots and nitrogen rates as sub-sub plots with four replications were used. Nodulation, plant growth, biomass nutrient content at R3 stage, yield and yield components were evaluated. Data analyzed for combined and individual locations in Statistical Analysis System<sup>®</sup> 9.4 indicated that inoculation increased nodulation and yield (37% to 95%) in both soybean genotypes but the effect of phosphorus on nodule formation was not consistent across sites and varieties. Inoculants have a potential to supply required nitrogen for soybean production in Mozambique because farmers seldom use mineral fertilizers due to its high cost.

**Keywords** Biological Nitrogen Fixation, Legumes, Nodulation, Promiscuous, Non-Promiscuous and Yield

## 1. Introduction

Soybean (*Glycine max* L.) has potential to become a major crop in Africa owing to its many uses including human food, animal feed and in industry (Hartman et al., 2011; Sinclair et al., 2014). Additionally, soybean has the ability of fixing nitrogen from the atmosphere obviating the

need to apply large amounts of nitrogen fertilizers. This advantage is particularly important in Africa where the predominantly subsistence farmers cannot afford the high prices of mineral fertilizers (Gyogluu et al., 2016). The successful introduction of soybean into regions abroad of Southeast Asia, where it was first cultivated (Solidaridad 2014), is dependent on inoculation with exotic rhizobia (Pereira 2013; Ulzen et al., 2016). To overcome this limitation, researchers from the International Institute of Tropical Agriculture (IITA) developed soybean TGx (Tropical Glycine cross) breeding lines, known as promiscuous varieties, owing to their ability to establish effective symbiotic partnerships with naturally occurring rhizobia in African soils (Majengo et al., 2011; Lamptey et al., 2014). Numerous studies conducted across Africa have shown that, in many locations, indigenous rhizobia are either not effective, or do not occur in adequate numbers to meet the N demand of promiscuous varieties (Sanginga et al., 2002). This signifies that inoculation is a less risky option than the reliance on costly commercial fertilizers or indigenous rhizobia of unknown potential (Solidaridad 2014; Singleton et al., 1985). Since 1977 until the early 2000 the selection for promiscuity was primarily based on the number of nodules formed and the varieties were not tested for their response to inoculation (Singleton et al., 1985). Accumulated evidence indicates that promiscuous varieties respond significantly to inoculation with rhizobial strains in the field and laboratory (Sanginga et al., 2002; Ahiabor et al., 2014).

Phosphorus (P) is an essential macronutrient that plays a crucial role in seed germination, shoot growth, flower initiation, seed formation and seed yield. The role of phosphorus in biological nitrogen fixation (BNF) is well established in nodule formation and functioning (Weisany et al., 2013). Biological nitrogen fixation imposes a high P demand, therefore it can only occur when the nutrient is sufficient (Tefera et al., 2010). Phosphorus has a key role in the energy metabolism of all plant cells, particularly in N fixation as an energy-requiring process. As a result, nodule

numbers and specific nitrogenase enzyme activity increase with the addition of P, signifying more efficient and effective N fixation process (Israel 1987). In addition, P must be supplied to N fixing plants to maintain nodule tissue and for the energy consuming biochemical processes involved in the nitrogenase system which makes nodules an important P sink in N fixing plants (Weisany et al., 2013). Therefore, inoculating soybean with *Bradyrhizobium* spp., can increase yield especially on fields with no recent history of soybean production for both the non-promiscuous and promiscuous genotypes. Promiscuous soybean varieties form nodules and fix atmospheric N with diverse indigenous rhizobia strains in the soil, whilst non-promiscuous genotypes require specific rhizobia strains to fix N from the air (Tefera et al., 2010).

Soybean is a relatively new crop in Mozambique but is becoming popular driven by the poultry industry and export market (Gelfand and Robertson 2015; Valinejad et al., 2013). More recently, soybean utilization has expanded to include nutrition of children, lactating mothers and the sick, increasing the demand for the crop (Gyogluu et al., 2016). Soybean farmers have the challenge of increasing production by over 40,000 metric tons, from 50,000 metric tons in 2015, and to 80,000 metric tons in 2020 to meet the country's cake demand (Solidaridad 2014; Pereira 2013). However, this increase does not account for the soybean oil consumption that is mostly imported in Africa. Managing interventions on soil nutrient supply resources are necessary to increase soybean production among smallholder producers who seldom use fertilizers. Unlike in Mozambique where some farmers only apply inoculant with no further nutrient supplementation, a substantial number of farmers' worldwide use soybean inoculants that increases yields (Ulzen et al., 2016), plus addition of P (Gyogluu et al., 2016). Inoculating soybean is the process of applying *Bradyrhizobium* spp., strain to seed or the soil before planting in order to enhance nodulation on the soybean roots and increase nitrogen (N) fixation. The amount of N fixed through the symbiotic relationship between the soybean plant and the bacteria is affected by the availability of other nutrients such as P (Majengo et al., 2011; Lamptey et al., 2014). Singleton et al., (1985) found that high nodulation is possible under conditions of adequate P while maximum responses to P occur when there is adequate mineral N or supplied through effective BNF process.

Biological N fixation meets about 50 - 60% of the soybean N demand and could be enhanced through inoculation with the appropriate *Bradyrhizobium* spp., to

improve soybean yields (Sanginga et al., 2002). However, many environmental factors affect the BNF process. High soil N concentration limits BNF (Gelfand and Robertson 2015), but low initial soil N affects crop establishment; hence, addition of relatively small amount of fertilizer N may improve the initial plant growth and development before nodulation and nitrogen fixation process begins (Valinejad et al., 2013). Thus, when applying supplemental N either in combination with inoculant or as top dress, proper timing becomes key as it could have counteractive effect on nodulation (Gelfand and Robertson 2015) or leach beyond the rooting zone before soybean establish a root system to utilize it. Other factors that affect the BNF process and should be optimum include soil acidity, moisture and supply of molybdenum. To realize optimum benefits from BNF process soil pH should be above 6.0 although soybean can tolerate pH lower than 5.0 especially with inoculation (Mubarik and Sunatmo 2014). Therefore, inoculation plus other good management practices lead to enhanced efficiency of the BNF process in soybean. Combination of inoculant and P or N and P increase soybean yields especially in slightly acid soils (Sharma 2011). However, for resource poor farmers like the large population in Mozambique use of inoculant alone could go a long way to improve soybean yields. More so, the economic incentive of using inoculant and the yield returns can be high in Mozambique (Kyei-Boahen et al., 2017).

Application of P, N and inoculants promotes accumulation of nutrients in both reproductive and vegetative portions of the plant. The content of these nutrients can be determined to ascertain the quality of the grain as well as the biomass (Morshed et al., 2008). Nutrients contained in the biomass can be recycled to improve soil conditions such as organic matter level, fertility, water holding capacity, infiltration and supply of elements to the subsequent crop. Rotaru (2010) reported that both biomass production and nutrient contents in soybean increased with higher P application level. On the contrary, Hanway and Weber (1971) reported that N, P, and K proportions in some plant parts did not vary with fertility treatments. Therefore, nutrient content in plant parts will depend on the growth stage as some of them are translocated along the development continuum. Since no information on fertilization and inoculation regimes exist in Mozambique, we investigated the effects of P, N, inoculant and their interaction on soybean production. The objective of this study was to evaluate the responses of two soybean varieties to inoculation, P and starter-N application, and their interactions on nodulation, growth, yield components, and grain yield.

**Table 1.** Soil chemical characteristics of the experimental locations

Location	pH	P±	K	Ca	Mg	Na	EC	CEC	N
		-----ppm-----					dS/cm	cmolc/kg	%
Ntengo	5.3	6.7	233.5	1135.0	277.5	17.2	0.18	15.1	0.10
Muriaze	6.4	7.6	156.5	1009.0	107.1	19.1	0.04	7.5	0.12
Ruace	5.9	26.1	221.0	803.5	112.8	13.2	0.07	7.2	0.05

<sup>±</sup>Phosphorus determined using the Olsen method

## 2. Materials and Methods

### 2.1. Site Description

The study was conducted during the 2012 and 2013 growing seasons at three sites, Ntengo (14° 32' S, 34° 11' E), Ruace (15° 23' S, 36° 67' E), and Muriaze (15° 17' S, 39° 19' E), in Tete, Zambézia and Nampula provinces of Mozambique respectively. The soil types were Sandy clay (Ntengo and Muriaze), and Loamy sand (Ruace). Parcels of land that had been under maize (*Zea mays* L.) in the previous season and with no history of inoculant application were used in each growing season. Similar cultural practices were used to manage the trials, although scheduling differed among sites depending on climatological conditions. Pre-season soil samples were collected and analyzed (Table 1). Two soybean varieties, Storm (determinate and non-promiscuous) and TGx 1904-6F, locally known in Mozambique as Zamboane (indeterminate and promiscuous), were sown at all sites for both seasons. The sowing dates varied by location depending on the onset and distribution of rains. Ntengo was sown on 29 December 2011 and 28 December 2012, Ruace on 15 December 2011 and 12 December 2012, for the 2012 and 2013 growing seasons, respectively. In Muriaze, soybean was sown on 17 December 2012 in the 2013 growing season.

### 2.2. Experimental design, Plot Management and Data Management

The experiment design was a split-plot arranged in a randomized complete block with four replications. Phosphorus (P) levels of 0 and 40 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> served as the main plot. The subplots consisted of a factorial combination of the two varieties (Storm and TGx 1904-6F), 40 kg N ha<sup>-1</sup> as starter-N, a peat-based inoculant with *Bradyrhizobium diazoefficiens* strain USDA110 formerly known as *Bradyrhizobium japonicum* (Delamuta et al., 2013) applied to the seed at sowing and a combination of 40 kg N ha<sup>-1</sup> and inoculant.

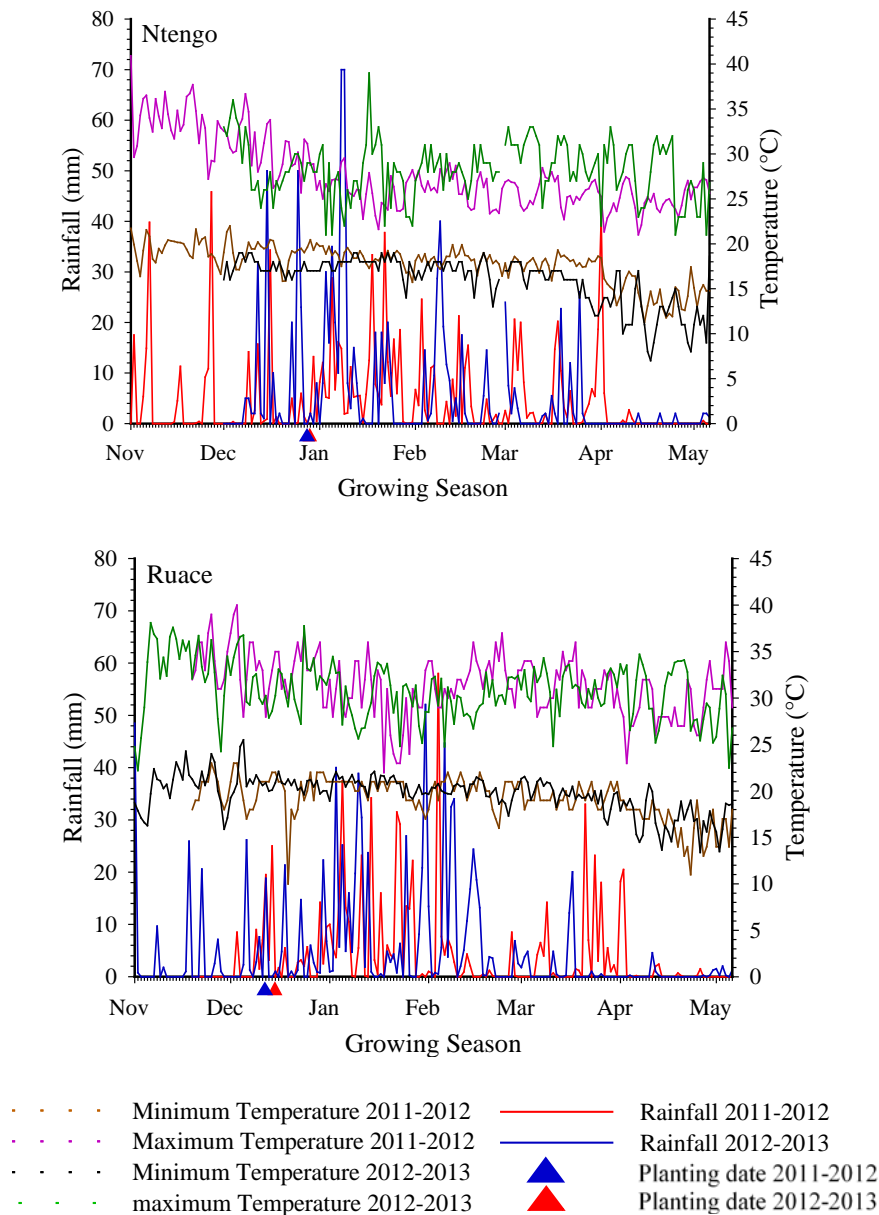
Five plants were sampled at R3 stage (beginning pod) and nodules were removed and counted while the above ground biomass was retained for tissue nutrient analysis. Nodules were oven-dried at 60 °C for 24 hours before

being weighed. Above ground biomass was dried at 60 °C for 72 hours or until a stable weight was attained. The samples were then ground to determine the P and N concentration in the above ground tissues. Due to logistic constraints tissue nutrient analyses were only conducted for Ntengo and Ruace. Plant height was measured at maturity. Ten plants from each plot were randomly selected for determination of pods density per plant. Forty pods from the 10 plants were randomly selected to determine the number of seeds per pod. Whole plot harvest was used to calculate the grain yield and biomass production per hectare. Hundred seeds from the plot harvest batch were counted to determine the seed weight. The grain yield and 100-seed weight were corrected to 13% moisture content.

Analyses of variance (ANOVAs) were performed using PROC GLM in Statistical Analysis System (SAS)<sup>®</sup> 9.4. Varieties and treatments were considered as fixed effects and environment (season and sites/location) and replication were random effects (Moore and Dixon 2015). Means were determined for treatments, and comparisons done at the p ≤ 0.05 significance level using the least significant difference (SAS Institute 2004; Littell et al., 2006). First, a combined balanced-factorial ANOVA was performed, to evaluate the effects of environment, variety, treatment and their interactions. Secondly, ANOVA for each variety was performed for all the tested variables for each site.

## 3. Results

Data collected and analyzed indicated that the measured variables responded variedly to the treatment depending on the differences in the nitrogen fixing promiscuity of the varieties and applied nutrients. There were distinct differences among the variables evaluated for both varieties tested in Ntengo and Ruace than Muriaze. Ntengo and Ruace are the high soybean production potential regions in Mozambique. The yield components that were significantly different among the tested variables did not directly explained obtained yield since their interactions are quite complex. Climatic conditions such as rainfall and temperature affected soybean development and were also different among the experimental sites (Figure 1).



**Figure 1.** Rainfall and temperature conditions during growing seasons in the environments of Ntengo and Ruace for 2011/2012 and 2012/2013 growing seasons

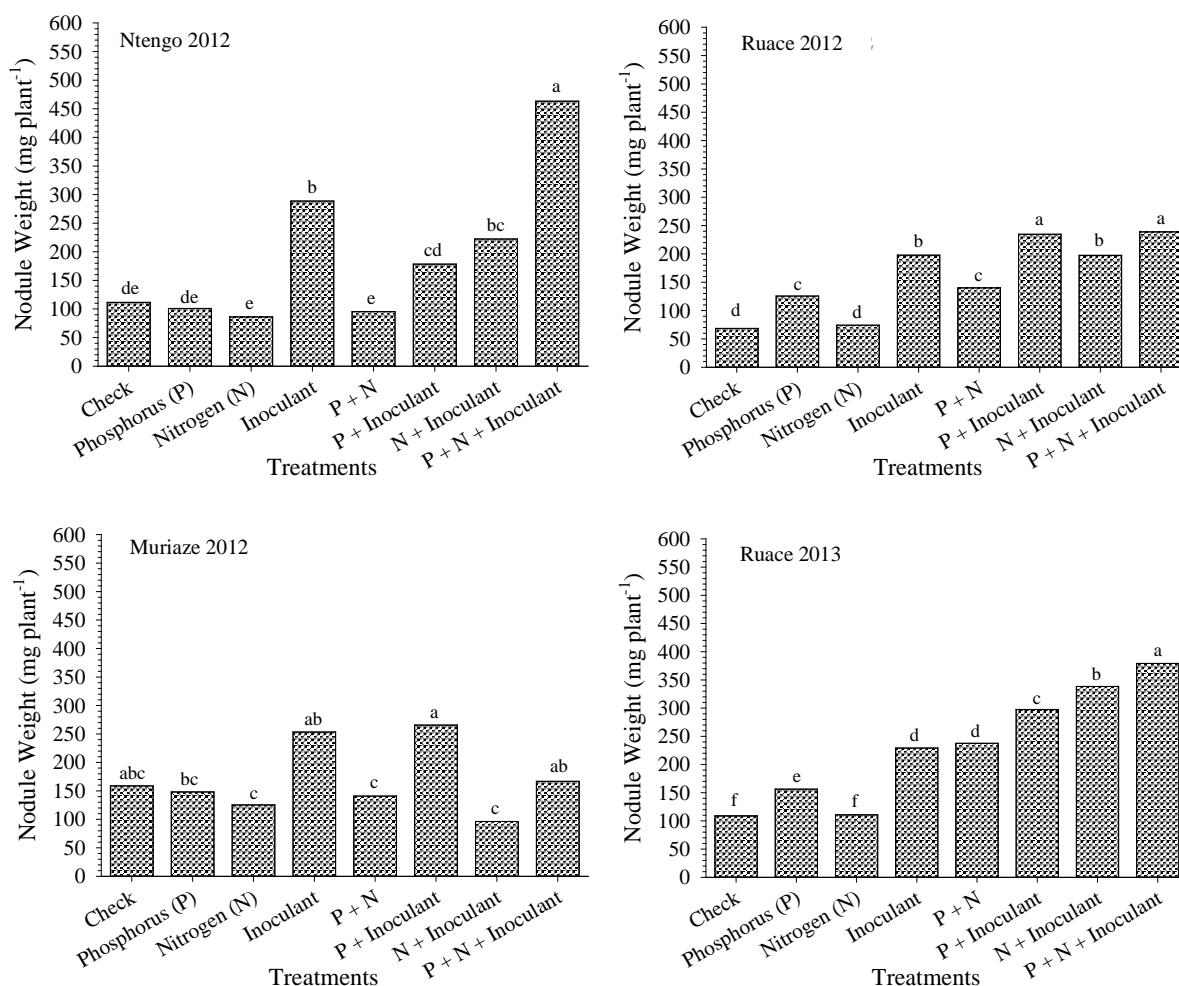
**3.1. Nodulation**

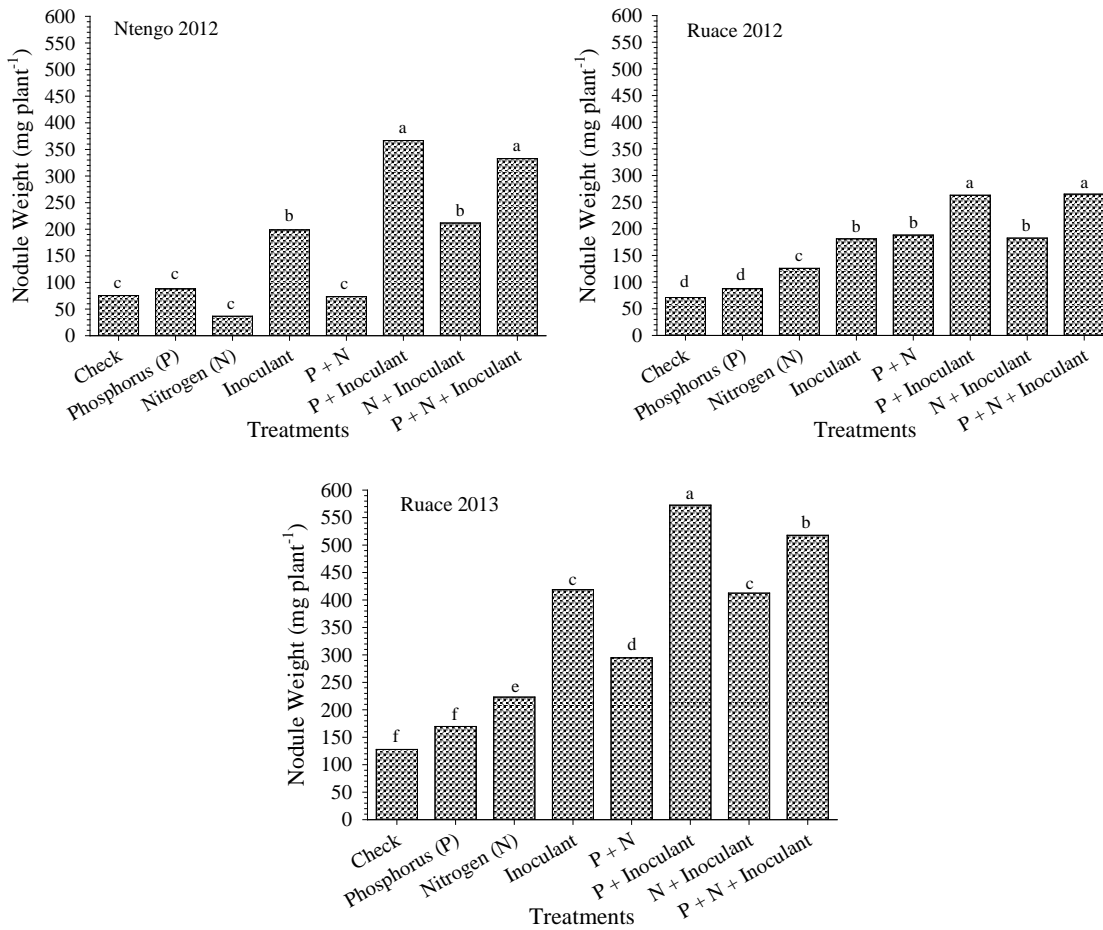
Inoculation consistently increased while starter-N variably depressed nodule number and dry weight in both soybean varieties across sites and growing seasons (Table 2, Figure 2 and 3). For example, applying N to inoculated plants did not affect the nodule dry weight of TGx 1904-6F in both seasons across sites (Figure 3). Nodule number correlated better with dry weight for TGx 1904-6F ( $r=0.83$ ,  $p<0.0001$ ) than for Storm ( $r = 0.64$ ,  $p <0.0001$ ). Contrarily, correlation of nodule number and yield ( $r = 0.20$ ,  $p =0.012$ ;

$r = 0.26$ ,  $p <0.0011$ ) and nodule dry weight ( $r = 0.33$ ,  $p<0.0001$ ;  $r = 0.25$ ,  $p <0.0013$ ) was poor for Storm and TGx 1904-6F respectively. Unlike inoculation, the effect of P on nodulation was not consistent across sites and between varieties. For example, in Ruace where soil P status was relatively high (Table 1), P application alone did not affect nodule formation of both Storm and TGx 1904-6F in 2013. However, application of P together with inoculation significantly increased the number and dry weight of nodules more than applying either P or inoculant alone in all environments (Figure 2 and 3).

**Table 2.** Effects of inputs application on number of nodules produced per plant at various locations in Mozambique during the 2012 and 2013 growing seasons

Treatments	NTengo 2012	Ruace 2012	Ruace 2013	NTengo 2012	Ruace 2012	Ruace 2013
	-----Storm-----			-----TGx 1904-6F-----		
Check	18.3	9.4	20.2	13.6	9.4	22.8
Phosphorus (P)	18.9	11.5	20.7	16.6	10.6	23.7
Nitrogen (N)	14.9	5.6	14.4	7.5	14.3	24.1
Inoculant (I)	23.2	23.2	31.4	21.0	21.8	56.6
P + N	9.5	11.6	21.8	15.5	25.9	29.5
P + I	28.1	24.3	43.5	31.8	31.2	77.2
N + I	29.4	11.5	41.9	27.2	21.0	46.7
P + N + I	31.3	22.4	52.3	34.8	31.3	76.1
LSD <sub>0.05</sub>	4.4	0.6	0.6	4.4	0.6	1.0
%CV	26.8	5.7	4.4	28.1	5.0	5.5

**Figure 2.** Effects of inputs application on nodule dry weight of soybean variety Storm in 2012 (Ntengo and Ruace) and in 2013 (Muriaze and Ruace). Means were separated using LSD at  $p \leq 0.05$



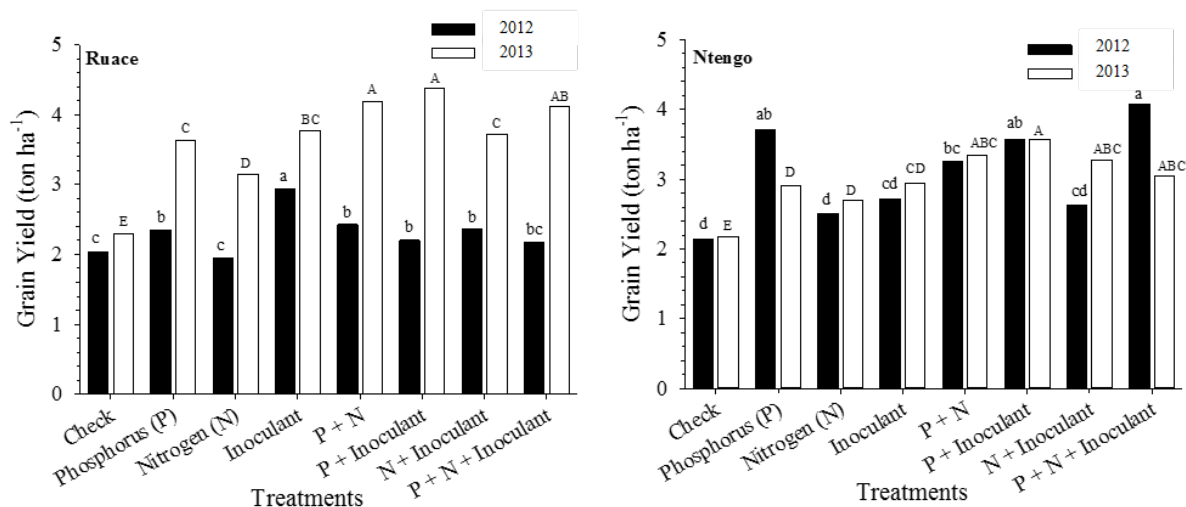
**Figure 3.** TGx 1904-6F nodule weight in mg plant<sup>-1</sup> from three environments in 2012 (Ntengo and Ruace) and in 2013 (Ruace). Means separated by LSD at p≤0.05

### 3.2. Grain yield

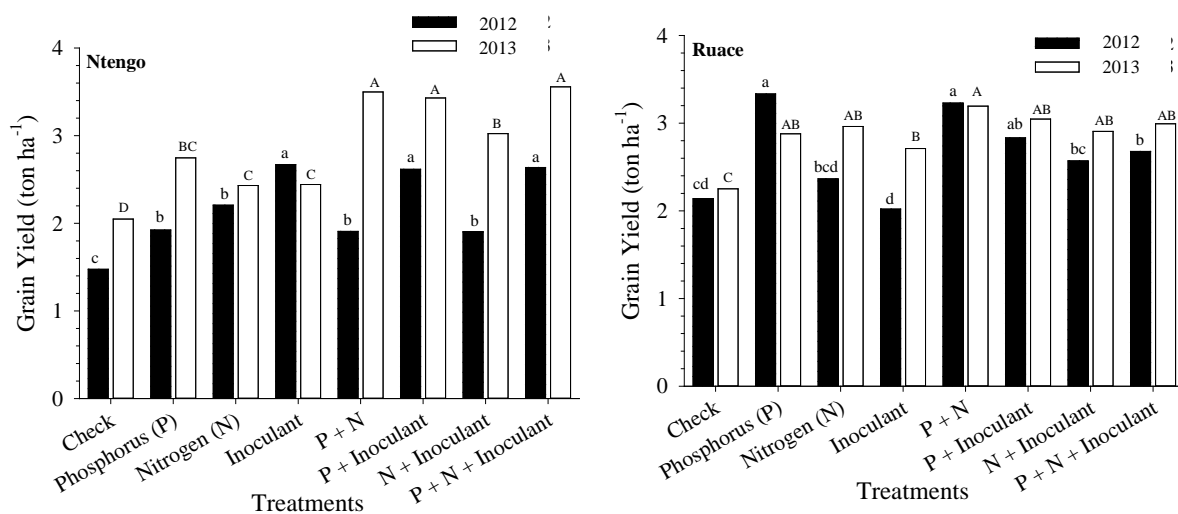
The trends observed in grain yield in respect to the different treatments were distinct across locations and varieties. Storm gave higher yields than TGx 1904-6F in all the environments (Figure 4 and 5). Application of P, N and inoculant resulted in higher Storm grain yields in all the environments except in Ntengo and Ruace 2012 when N was applied (Figure 4). In Mozambique, soybean planting starts in the month of December in Ntengo and Ruace. Unlike Ntengo and Ruace, Muriaze is a drier ecology that impedes soybean production in some seasons; hence, only the combination of the three inputs responded significantly. In the 2012 growing season, grain yield of Storm was relatively higher at Ntengo than Ruace which experienced severe early season drought (Figure 1). The responses to treatments were not consistent across environment for Storm. In Ntengo 2012, applying a combination of P + N + inoculant produced the highest grain yield of 4.1 t ha<sup>-1</sup> compared with the yield of the control plot which was 2.1 t ha<sup>-1</sup> representing over 95% increase (Figure 4). At the same location in 2013 season, P + inoculant gave the highest grain yield of 3.6 t ha<sup>-1</sup> equivalent to 64% yield increase over that of the control plot (2.2 t ha<sup>-1</sup>). At Ruace, yields

were lower in 2012 than 2013. Inoculation alone resulted in the highest yields (2.9 t ha<sup>-1</sup>) compared with 2.0 t ha<sup>-1</sup> for the check in 2012. In the second season, P + inoculant produced the highest yield of 4.4 t ha<sup>-1</sup> compared with 2.3 t ha<sup>-1</sup> for the check. The highest yield increase over the control for Storm at Ruace in 2012 and 2013 were 45% and 91%, respectively.

In contrast, 2013 season, P + N and N + inoculant application significantly increase yield of TGx 1904-6F at Ntengo and Ruace (Figure 5). Similar to Storm, TGx 1904-6F did not respond to the treatments consistently across environments. At Ntengo, the inoculant alone gave the highest yield of 2.7 t ha<sup>-1</sup> in 2012, equivalent to 80% more than that for the check at 1.5 t ha<sup>-1</sup>, whereas at Ruace, in the same season, application of P had produced the highest yield of 3.3 t ha<sup>-1</sup>, 57% over the check with 2.1 t ha<sup>-1</sup>. In the 2013 growing season, P + N + inoculant had the highest yield Ntengo of 3.6 t ha<sup>-1</sup>, which was 80% higher than that of the check plot (2.0 t ha<sup>-1</sup>). In the same season at Ruace, the highest TGx 1904-6F yield was from the P + N treatment (3.2 t ha<sup>-1</sup>) compared with the lowest yield of 2.2 t ha<sup>-1</sup> for the check representing 45% yield increase following use of combined nutrients.



**Figure 4.** Grain yield of Storm in from two locations Ntengo and Ruace in t ha<sup>-1</sup>. Means separated by LSD at  $p \leq 0.05$  in the 2012 (lower case) and 2013 (upper case) growing seasons



**Figure 5.** Grain yield of TGx 1904-6F in from two locations Ntengo and Ruace in t ha<sup>-1</sup>. Means separated by LSD at  $p \leq 0.05$  in 2012 (lower case) and 2013 (upper case) growing seasons

### 3.3. Yield Components

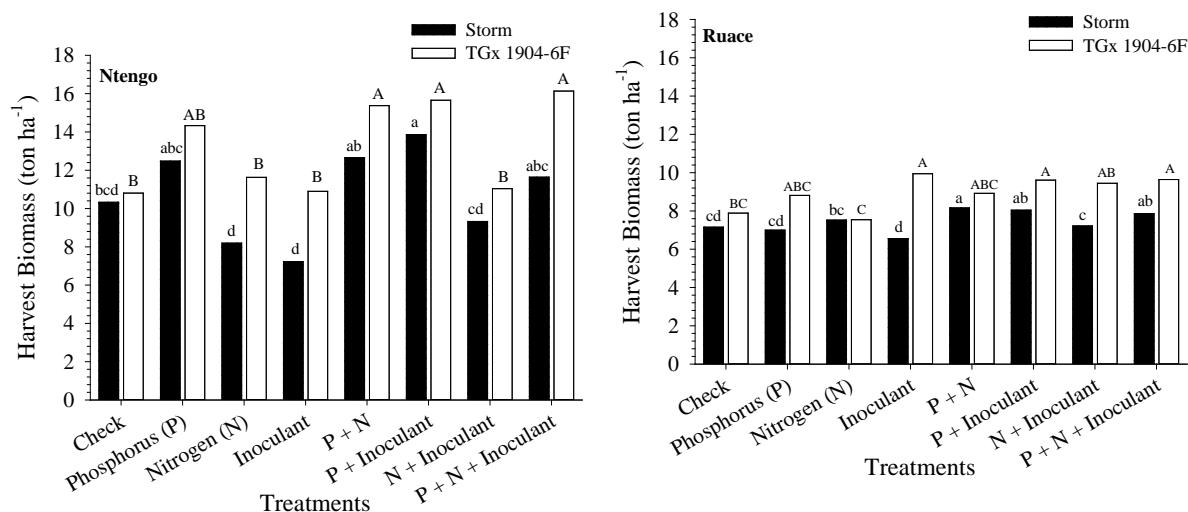
Two yield components, 100-seed weight and pods per plant responded differently to N, P and inoculant application. Generally, Storm (a large seeded variety) had higher 100-seed weight than TGx 1904-6F in all environments, although seeds from Ruace were larger than those from Ntengo for both varieties for the respective treatments (Table 3). There was a significant increase in Storm seed weight at Ntengo 2013, following application of a combination of P + N (15.6 g), P + inoculant (15.6 g), N + inoculant (15.7 g) and P + N + inoculant (16.3 g) over the check treatment and when the nutrients were applied singly ( $\leq 15.2$  g) (Table 3). Similar to seed weight, the higher the number of filled pods per plant contribute to increase in grain yield. Plants at Ruace produced relatively more pods (42 – 66 per plant) than those at Ntengo (17 – 42

per plant) and Muriaze (30 – 64 per plant) in 2013 for Storm (Table 3). The difference in the number of pods per plant could be due to better rainfall distribution at Ruace at the time of flowering and pod development (Figure 1) or the branching of the varieties at each location. The rainfall distribution and amount were poor in 2012 than 2013 during the time of flowering (mid-February to early-March) leading to the differences in the number of pods per plants for Storm between the seasons at Ruace (Figure 1). The number of pods produced as a result of the various treatments was not consistent across the different environments. The genotype TGx 1904-6F exhibited comparable pods per plant trend like Storm where the numbers were higher in Ruace than Ntengo, while P + N + Inoculant treatment was significant across varieties and locations (Table 3).

**Table 3.** Plant growth characteristics and yield components of two soybean genotypes treated with inoculant and fertilizers in the 2013 growing season at three locations (Muriaze, Ntengo and Ruace) in Mozambique

Treatments	Ntengo	Ruace	Ntengo	Ruace	Ntengo	Muriaze	Ruace	Ruace	Ntengo	Ruace	Ntengo	Muriaze	Ruace
	-----100-seed weight (g)-----				-----Number of pods per plant-----				-----Plant height (cm)-----				
	---Storm---		TGx 1904-6F		-----Storm-----			TGx 1904-6F	---Storm---		-----TGx 1904-6F-----		
Check	14.9	17.6	11.1	12.5	26.9	36.8	43.3	53.3	59.0	51.3	67.1	67.5	88.2
Phosphorus (P)	15.2	18.3	12.2	13.5	31.8	64.0	42.0	52.1	76.7	54.6	85.3	58.3	85.8
Nitrogen (N)	14.5	18.8	11.2	13.7	21.6	47.5	54.1	69.4	62.7	53.2	65.1	61.4	98.0
Inoculant	15.1	19.3	11.0	14.3	17.3	30.3	47.8	70.0	67.5	54.5	65.7	70.8	90.7
P + N	15.6	18.9	12.1	13.4	31.8	31.5	65.7	70.0	59.3	61.3	95.7	56.0	94.7
P + Inoculant	15.6	20.1	12.1	14.9	32.0	43.1	55.4	61.0	73.8	52.5	81.8	65.8	86.0
N + Inoculant	15.7	19.6	11.1	14.2	34.8	42.6	65.7	59.1	68.8	54.0	65.4	68.7	94.2
P + N + Inoculant	16.3	20.6	12.1	14.8	41.7	48.2	66.3	66.8	76.5	57.2	87.8	65.8	93.9
LSD0.05	0.7	0.7	0.6	0.9	9.9	18.9	3.6	6.0	16.4	3.4	15.1	6.4	4.3
%CV	3.1	2.4	3.8	4.2	22.7	29.8	4.5	6.6	12.4	4.2	3.3	6.8	3.2





**Figure 6.** Harvest biomass ( $t\ ha^{-1}$ ) produced by soybean varieties in two environments of Ntengo and Ruace in the 2013 growing season. Means were separated using LSD at  $p \leq 0.05$  for Storm (lower case) and TGx 1904-6F (upper case)

**Table 4.** Phosphorus and nitrogen content in plant tissue ( $g\ kg^{-1}$ ) at R3 stage for two soybean varieties (Storm and TGx 1904-6F) grown at Ntengo and Ruace in the 2012 and 2013 growing seasons

Treatments	Ruace 2012	Ruace 2012	Ntengo 2012	Ntengo 2013	Ntengo 2013	Ruace 2013	Ruace 2012	Ruace 2013	Ntengo 2013
	-----Phosphorus-----				-----Nitrogen-----				
	Storm	-----TGx 1904-6F-----			-----Storm-----		-----TGx 1904-6F-----		
Check	1.3	1.2	1.8	1.8	18.6	27.8	20.7	34.6	20.0
Phosphorus (P)	1.6	1.5	1.6	2.7	31.3	35.9	26.8	36.9	20.3
Nitrogen (N)	2.5	1.8	2.3	3.5	28.7	38.3	28.7	43.6	24.7
Inoculant	2.1	1.7	3.5	5.1	31.7	47.1	30.1	46.0	31.1
P + N	1.8	2.0	3.5	3.9	39.6	43.7	32.6	49.2	27.3
P + Inoculant	3.2	1.7	3.1	3.7	34.5	45.0	27.8	45.0	35.2
N + Inoculant	2.7	3.8	3.7	4.2	38.0	42.8	38.5	48.5	41.1
P + N + Inoculant	2.3	2.5	5.9	3.1	40.9	41.7	33.6	48.8	34.4
LSD <sub>0.05</sub>	0.7	0.5	0.6	1.0	11.0	7.0	7.0	8.0	11.0
%CV	18.3	10.7	26.8	14.1	18.6	9.6	12.9	10	21.9

Plant height can be an indicator of the number of node pods produced by each crop, which in turn affect yield. At Ruace, TGx 1904-6F had taller plants than Ntengo for both seasons perhaps due to the upsurge in rainfall amount during the active vegetative growing period. Despite the plants for both varieties being taller in Ruace than Ntengo, there was more branching in the latter site as evident by the harvest biomass produced (Figure 6).

### 3.4. Nitrogen and Phosphorus Content in Biomass

The phosphorus content in Storm plant tissue for all

treatments at R3 stage in Ruace were significantly higher than the check plot except that for the P and P + N in 2012 (Table 4). From the Storm tissues analyzed, the P content at Ruace in 2012 for P + inoculant treatment ( $3.2\ g\ kg^{-1}$ ) was almost 2.5 times higher than that for the check ( $1.3\ g\ kg^{-1}$ ). At Ntengo 2013, the amount of P in the inoculated TGx 1904-6F tissues ( $5.1\ g\ kg^{-1}$ ) at R3 was almost three-fold more than that contained in the check ( $1.8\ g\ kg^{-1}$ ). Similar trends as in P plant tissue content were observed for N where treated plots had either numerical or significantly higher amounts than the check in both locations and varieties in 2013 but only for TGx 1904-6F at Ruace in

2012 (Table 4). In both varieties, the amount of N in the plant tissue for treated than those from the control plots ranged from about 1.0 (P application) to 2.2 times (P + N + inoculant). Both these extremes were observed in plant tissue N content at Ntengo in 2013. Biomass at Ruace 2013 generally contained more plant tissue N than in the other environments.

#### 4. Discussion

Inoculation increases nodule population but nodule dry weight is a better variable to estimate nitrogen fixation (Kyei-Boahen et al., 2017). Fixed nitrogen has a higher potential to be retained as residual nutrient in plant biomass unlike fertilizer that is prone to leaching. However, in soils with low inherent nitrogen, starter-N should be applied judiciously to improve the crop vigor without detrimental effects on nodulation before the BNF process begins. The decrease in nodulation by starter-N application alone or in combination with P and/or inoculant was not consistent among the varieties. Population is a number of nodules that vary in sized that should be qualified by the activity of the resident rhizobia. Biological nitrogen fixation is increased with application of P which plays a role in biological functions related to phosphorylation in roots. The roots and especially the nodules are a great sink of P while in shoots it remains relatively stable in N fixing legumes (Tsvetkova and Georgiev 2003). Therefore, application of P in combination with inoculants improves nodulation and yield of soybean particularly in P deficient soils.

Nitrogen fertilizer is vital for crop development and yield improvement yet in some cases there is no response to its application. Perhaps the lack of response to application of N in 2012 season was because of the high rainfall at these sites (Figure 1) that led to leaching since the crop had not established its root system. Because of leaching, the soils in the N treated plots possibly remained with the inherent N like the check plots. Some studies have reported that starter-N did not contribute to increased yield in soybean especially under conventional cultivation (Valinejad et al., 2013). These confirms our finding that supplementing soybean inoculation with phosphorus application in P deficient soils does improve grain yield (Tsvetkova and Georgiev 2003). This finding could be attributed to combined effect of N fixation and P availability to TGx 1904-6F for relatively longer period as this is a late maturing variety. It is evident that combination of N with P did significantly increase grain yield of TGx 1904-6F in Ntengo 2012 but no differences when the nutrients were applied singly (Figure 5). Although yield increases following application of a combination of P + N were relatively higher than that for the application of P alone at Ruace for both seasons, the differences were not significant. Several researchers earlier reported that combining inoculation with P increases yield of TGx

1904-6F (Kamara et al., 2008; Kamara et al., 2007; Tairo and Ndakidemi, 2013) and cowpea (Kyei-Boahen et al., 2017). Despite the yield increases due to application of nutrients either singly or as combinations in different environments, use of inputs should consider the economic advantage in the selection of suitable nutrient management regimes for specific locations.

Soybean yield obtained is dependent on complimentary and contrasting effects of several yield components response to management interventions along the development continuum. Inoculation and availability of N and P led to a complementary effect on Storm seed weight when applied in combination of two or three inputs. Supplying N and P in soybean production at the right time regardless of the source will promote growth of some yield components such as seed weight that has a positive impact on grain yield (Aniekwe and Mbah 2014). Nitrogen and inoculant provide N needed for seed cells building blocks while P is important in vital biological functions such as in the Adenosine triphosphate (ATP) cycle within cells for metabolism. At Ruace in 2013, the seed weight of Storm significantly increased with application of P, N and inoculant either alone or in combination. The seed weight of TGx 1904-6F variety increased significantly for all the treatments over the check that had 12.5 g per 100-seeds. Similar to seed weight, the higher the number of filled pods per plant for various treatments was not consistent across the different environments. For instance, at Muriaze 2013, application of P resulted in the highest number of pods per plant (64) that declined when the nutrient was combined with N or inoculant. This suggests that P was the most limiting in the development of pods in Muriaze and addition of N either as fertilizer or inoculant led to a dense leafy canopy and less flowers that reduce the number of pods developed (Bing and De-Ning 2015). Generally, development of soybean-cropping systems that enhance efficient use of P and N especially through BNF leads to availability of N to the crop for a prolonged period.

Another yield component, plant height, increases the biomass production especially for the promiscuous branching soybean varieties like TGx 1904-6F. This might not always be true as is the case observed at Ruace. At times, excessive vegetative growth could compromise pod and seed development because of the thick canopy that limits light penetration to the lower parts of the plants due to shading (Bing and De-Ning 2015). The variety TGx 1904-6F produced more biomass when P, N and inoculant were applied compared with that for Storm (Figure 6). Similar findings were reported in a study that evaluated the effect of phosphorus on soybean production in Kenya (Verde et al., 2013). Nitrogen and P are required for the development and biological functions of soybean plant cells. Soybean biomass production is essential in soil fertility improvement. Nitrogen and P among other elements contained in the biomass are recycled back into the soil when the residues are incorporated. The following

seasons' crops then utilize the contained nutrients. The tissue P content was higher at Ntengo in the plants that were inoculated or received nutrient supplementation because they could have formed a better P scavenging root system or mycorrhizal associations. Application and better plant uptake of P in soybean production leads to its accumulation in seeds, leaves, roots and nodules (Rotaru 2010). Also, supplementing soil N in soybean production either through application of fertilizers or inoculation led to more plant tissue N content. Similar to our findings, application of fertilizer N and inoculation resulting in higher amounts of the mineral N in soybean and other legumes plant tissue has earlier been reported (Tefera et al., 2010). The N and P that accumulate in the biomass become beneficial to subsequent seasons' crops as the materials decompose leading to mineralization of the nutrients to plant utilizable forms.

## 5. Conclusions

Application of inoculant in combination with P led to increased nodulation in both varieties. Therefore, farmers can improve soybean nodulation by applying both inoculants and P fertilizers, which in turn contribute to higher yields. In soils where N levels are very low, then it is necessary to apply starter-N too, although the risk of leaching exists in seasons with early-elevated rainfall. The other growth and yield variables followed different trends within the environments, which gives farmers options to choose from, depending on their conditions and resource endowment. Grain yields of inoculation or P treatments alone were higher than those that received starter-N. The challenge is timing of starter-N application either at or a few days after planting in order to synchronize with the crop demands. To improve soybean yields and quality in Mozambique and sub-Saharan Africa as a whole, farmers need to use inoculants either singly or in economically viable combination with starter-N or P. More studies are required to understand better timing of starter-N, P application and inoculation that would enhance more effective nodulation in soybean to optimize yields in Mozambique through the BNF process. Also, there is need to document the economic returns to investment for either inoculant, P or N application that will provide additional information on the adoption potentials of these soybean production inputs.

## Conflict of Interest

The authors have affirmed that there is no conflict of interest regarding the publication of this paper.

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