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Original Article

SOYABEAN RESPONSE TO RHIZOBIAL INOCULATION UNDER CONTRASTING SMALLHOLDER AGRO-ECOLOGICAL CONDITIONS IN ZIMBABWE.

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Abstract

Soyabean is grown widely with rhizobial inoculants on commercial farms in Zimbabwe, but its inoculation response in smallholder cropping environments is poorly characterized. Symbiotic interactions of six rhizobial strains (MAR 1305,1306,1326,1494, 1497 and 1515) from the Grasslands Rhizobium Collection were evaluated under contrasting soils and rainfall zones (natural region) in Zimbabwe. The rhizobial strains were evaluated on one specific and three promiscuous soyabean varieties (SC Saga and TGX (1740-2F/ 1987-628/ 1987-11E). The study was conducted over two rainfall seasons namely 2011-2012 and 2012-2013. Field experiments were set up as a randomized complete block design with two factors (soyabean variety and rhizobial strain), a negative control without inoculation and a positive control inoculated with commercial inoculant strain MAR 1491. All treatments were replicated three times. Nodulation was significantly ($P< .0001$) influenced by rhizobial strain inoculation with nodulation ranging from 28 to37 nodules per plant. Over the two seasons, soyabean inoculated with MAR 1305, 1306, 1494 and 1515 consistently gave higher grain yield higher (average of 600kg/ha) compared to that inoculated with commercial inoculant strain MAR 1491 (average of 400kg/ha). Nodulation of both promiscuous and specific varieties decreased with annual rainfall amounts in the order: Natural Region (NR)II> NRIII> NRIV. Rhizobial strains MAR 1305, 1306 and 1494 were superior in NR II and NRIII while rhizobial strain MAR 1515 was superior in NR IV. Our results show that identification of superior strains best suited to smallholder cropping environments and inoculation response are crucial for increasing soyabean productivity in these environments.

Keywords: crop productivity, inoculation response, rainfall zone, smallholder, rhizobial strain

Introduction

In Sub-Saharan Africa, soil fertility decline in smallholder cropping environments continues to threaten food, nutrition and income security (Mpepereki et al., 2000; Giller, 2008). Nitrogen (N) remains the most limiting nutrient in many cropping environments, and it is a high input cost to farmers. This is because it is added as a synthetic fertilizer whose price is dependent on the energy cost for its production, among other accessibility challenges (Peoples et al., 1989; Rothstein, 2007). Nitrogen is an integral component of all plant and animal proteins and is mainly responsible for growth and chemical processes. However, N fertilisers are seldom within the reach of most smallholder farmers as they are expensive or poorly distributed.

Legume biological nitrogen fixation has been identified as a system that could address the constraints of the limiting nitrogen in smallholder cropping systems, as it is cost effective and eco-friendly (Mpepereki et al., 2002). Biological nitrogen fixation (BNF) is a natural process in which a legume or non-legume host plant forms a symbiotic relationship

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with a nitrogen fixing micro-organism to convert atmospheric nitrogen into ammonium salts that can be used by the host plant (People et al., 1989). One such symbiotic system that has been identified is that between soyabean and rhizobia. Soyabean is a grain legume that has been identified to have multiple benefits when compared to other grain legumes. It is one of the richest plant protein sources with a protein content higher than most animal protein sources and has a high cash price which makes it a good source of income (Kolapo, 2011; IITA, 2012).

Soyabean also has a high potential of producing N rich biomass which when incorporated into the soil, provides organic matter helping to condition the soil and supply N to successive crops (Kolapo, 2011; Mpepereki et al., 2000). Because soyabean-rhizobia symbioses produce their own nitrogen supply, soyabean requires no ammonium nitrate top dressings which make its production costs lower than most crops (Seed Co, 2010). Also because soyabean produces its own N, it reduces the need for excessive use of nitrogen fertilizers which lead to ground water pollution as nitrates are leached making it significant in ecological function (Berrada and Fikri-Benbrahim, 2014). Increased and improved soyabean production relies on several factors which include good seed germplasm, effective and elite inoculant rhizobial strains, proper management and good soil and climatic conditions (Giller et al., 2011). Formation of effective symbioses between the rhizobial strains and the seed is a critical and important process in successful soyabean production (Hardson and Atkins, 2003; Douglas, 2010). Symbiosis can only occur effectively, if the seed and soil rhizobial strains are compatible and the soil conditions are conducive for rhizobial population multiplication nodule formation. Also the seed bed must present optimum conditions for good seed germination coupled with optimum climatic conditions (Rainfall and temperature) (Giraud et al., 2007; Jones et al., 2007).

Despite the knowledge of the potential and multiple benefits of soyabean-rhizobia symbioses, characterization of varieties and rhizobial strains best suited to smallholder cropping environments in Zimbabwe, and similar areas, remains poor. This may be attributed to several factors such as climate, differential soil management practices among smallholder farmers and improper storage and use of rhizobial inoculants (Zahran, 1999). Hence more information is needed to guide the targeting of soyabean production domains and associated inoculants in smallholder cropping environments if increased and improved soyabean productivity is to be achieved.Several studies were carried out in Goromonzi, Guruve and Hurungwe districts of Zimbabwe where most of the soils were sandy and sandy loams and the average annual rainfall ranging from is 700 to 1050 mm. Soils in smallholder farming areas have been found to have low rhizobial populations and inoculation has become a critical aspect in the success of soyabean production (Zingore et al., 2005). However, the success of inoculation depends on the rhizobial strain's compatibility with the host soyabean plant and its saprophytic competence under prevailing environmental conditions. Kasasa's studies (1999) suggested that use of promiscuous varieties in most low input smallholder cropping systems where rhizobial inoculants are not readily available could help improve nodulation and hence soyabean yields. However, soyabean varieties produced by commercial breeders in Zimbabwe at present are all specific requiring commercial inoculants. Although rhizobial inoculant distribution points have increased, the proper use and storage of these inoculants is still limited by insufficient knowledge. Previous soyabean inoculant promotions have not been sustained due to economic challenges.Chirinda (2003) also carried out a study to assess the yield response to rhizobial inoculation rate and concluded that soyabean yields increased steadily up to five times the recommended commercial rate. This study suggested that there was need to identify better adapted strains to the local environmental conditions to ensure economical use of the rhizobial inoculants.

Rhizobial inoculation makes soyabean production cost effective compared to maize production in that it is way cheaper to inoculate. A rhizobium sachet priced at US$5 has the potential to supply an equivalent amount of N to a crop as that supplied by three top dressing bags priced at US$ 35 each. However, because only two commercial rhizobial strains are available on the market there is need for identification of other strains to cater for the diverse soil management and climatic conditions experienced in smallholder cropping environments. Zengeni (2003) sought to determine whether introduced rhizobia persisted after inoculation in smallholder cropping environments and concluded that populations were still high enough to sustain adequate nodulation a year or two after inoculation. However, reinoculation was recommended three years after inoculation as rhizobial populations gradually declined to levels too low to provide adequate nodulation.

Musiyiwa (2005) determined the host ranges and symbiotic effectiveness of indigenous rhizobia isolates and suggested that knowledge on these aspects was very limited and needed further study. Symbiotic effectiveness between the host soyabean and rhizobia is a crucial process in soyabean production. Hence there is need for sufficient information on which rhizobia- soyabean combinations best suited to specific environments to ensure sustainable soyabean productivity.

In (2006) Zengeni investigated the capacity of cattle manure to improve soil conditions for survival and persistence of rhizobial populations. Results showed that cattle manure application improved the rhizobial populations potentially improving soyabean yields through symbiotic N fixation. Organic matter is important for the saprophytic survival of rhizobia when the legume crop symbiont is not in the field. Farming practices that add organic materials in the form of cattle manure and composts to the soil create a suitable soil environment for the proliferation and persistence of soyabean nodulating rhizobia. Mapfumo and Mtambanengwe (2006) investigated the contribution of legumes to soil fertility and incomes of households and identified improved seed varieties and legume BNF technologies as key factors.

In spite of several studies, potential of soyabean to fix N and give significant yields to improve food security in the smallholder sector has remained low. This may be attributed to the little knowledge on rhizobia N fixation potential with indigenous and commercial varieties under field conditions in smallholder cropping environments. Little research has been done in smallholder farming areas on identifying elite rhizobial strains best suited to selected varieties and the prevailing climatic and soil conditions in these areas. Hence more research is needed to improve knowledge increasing information to guide deployment of soyabean into smallholder cropping environments.

In view of the prevailing constraining conditions in smallholder cropping environments that affect the effectiveness of legume – rhizobium symbioses this study therefore sought to evaluate superior soyabean - rhizobial strain combinations best suited to varying soils and rainfall zones in smallholder farming areas of Zimbabwe.

Materials and Methods

Study Site Selection and Characterization

The study was conducted in Murewa, Chegutu, Guruve and Mudzi districts during 2011/2012 and 2012/2013 cropping seasons. Three field sites were selected in each of the 2011/2012 and 2012/2013 cropping seasons. One field site was selected per district each season giving a total of six sites (Table 1). The fields were selected where there was no history of soyabean cultivation and/ or rhizobial inoculation. Size of available land suitable for establishment of experimental trials and the willingness of the farmer to avail their land for use during the two seasons were additional factors considered during site selection.

Table 1: Study sites location and agro-ecological characteristics in the soyabean inoculation response study during the 2011/2012 and 2012/2013 cropping seasons in Zimbabwe.

District	**Location**	**Agro-ecological Region**	**Rainfall Per Annum**
Murewa	17° 43’16” S 31°41’53” E 1361 m.a.s.l	II	700-1050 mm
Chegutu	18°06’48.65” S 30°44’30.50” E 1356 m.a.s.l	II	700-1050 mm
Chegutu	18° 07’ 36.83” S 30°43’12.21 E 1344m.a.s.l.	III	500-800mm
Guruve	16°S 30°E 1100-1600 m.a.s.l	III	500-800mm
Mudzi	(a)17°05’14.46” S 32°46’30.29” E682 m.a.s.l (b)17°09’54” S 32°77’67” E694 m.a.s.l	IV	450-650 mm

Table 2: Soil characteristics at the rhizobial inoculation response study sites in Zimbabwe (2011/2012).

Soil Parameter	**Site**		
	Murewa (NRII)	**Chegutu (NRIII)**	**Mudzi (NRIV)**
pH (0.01M CaCl$_2$)	4.89	4.7	6.14
Exchangeable Ca (cmol$_c$/kg)	0.741	0.594	3.353
Exchangeable Mg (cmolc/kg)	0.327	0.372	2.03
Exchangeable Na (cmolc/kg)	0.166	0.177	0.533
Exchangeable K (cmolc/kg)	0.07	0.054	0.091
Total N%	0.07	0.10	0.06
Available P (mg/kg)	49.08	11.97	42.9
Soil Organic Carbon (SOC) %	1.95	0.5	0.81
Texture	coarse loamy sand	coarse sand	medium loamy sand
Cropping History	maize monocrop	maize-round nut-maize	maize-finger millet-round nut

Available P was measured using the Bray 1 method

During the first season, experimental trials were established in Murewa (NRII), Chegutu (NRIII) and Mudzi (NRIV) districts (Table 1). The natural regions (agro-ecological zones) are mainly differentiated according to their rainfall and temperature patterns. However different sites were used during the second season. This was because either farmers wanted to use their field or that some had removed the permanent pegs we had left which would result in experimental contamination. Sites selected during the second season were in Chegutu (NRII), Guruve (NRIII) and Mudzi (NR IV) districts. These sites had similar characteristics in many aspects to those used in the first season and were in the same natural regions (Table 1).

Table 3: Soil characteristics at the rhizobial inoculation response study sites in Zimbabwe (2012/2013).

Parameter	**Site**		
	Chegutu (NRII)	**Guruve (NRIII)**	**Mudzi (NRIV)**
pH (0.01M CaCl$_2$)	4.17	5.46	5.45
Exchangeable Ca (cmol$_c$/kg)	5.74	4.45	3.24
Exchangeable Mg (cmolc/kg)	3.38	1.5	0.9
Exchangeable K (cmolc/kg)	0.45	0.16	0.13
Total N%	0.08	0.06	0.04
Available P (mg/kg)	8	6.13	22.38
Soil Organic Carbon (SOC) %	2.42	2.53	1.68
Texture	Medium sandy clay	Fine loamy sand	Medium sand
Cropping History	Maize-paprika-paprika	Maize-sugar bean-maize	Sorghum-maize-maize

Available P was measured using the Olsen method

Table 5: Soyabean variety origins and characteristics in the soyabean inoculation response study in Zimbabwe (2011/2012 and 2012/2013).

Seed Variety	Origin	Characteristics
SC Saga	Zimbabwe, bred by Seed Co, Zimbabwe	Indeterminate suitable for middleveld (600- 1200 m.a.s.l) and lowveld (150-600 m.a.s.l) areas, high seed yield potential, long shatter free period, good resistance to lodging, good pod clearance, good standibility.
TGx 1740-2F	IITA/DARS‡/NCRI‡	Indeterminate, high grain yield potential, ability to smother weeds and pod load per plant. Early maturity, good resistance to lodging, bacterial blight and *Cercospora* leaf spot
TGx 1987-628	IITA/DARS/NCRI	Indeterminate, high grain yield, medium maturity, good resistance to lodging, bacterial blight and *Cercospora* leaf spot
TGx 1987-11E	IITA/DARS/NCRI	Indeterminate, high seed yield potential, good pod load and pod clearance, late maturity, good resistance to lodging, bacterial blight and *Cercospora* leaf spot

Adopted from the Seed Company Agronomy Guide (2010), and the International Institute of Tropical Agriculture (IITA), (2008).
‡ DARS - Department of Agricultural Research Services.
‡ NCRI - Nigerian National Cereal Research Institute.

Experiments were mainly researcher managed with limited farmer management. For initial characterization, soil sampling was done according to Warrick *et al* (1986) and one composite sample was collected for the whole field for uniform recommendation. The composite samples were air-dried and sieved for laboratory characterization. The soil pH was determined using the calcium chloride method (Anderson and Ingram, 1993), soil organic carbon using the Walkley black method (Nelson and Sommers, 1982), total N using the Kjeldahl method (Hesse, 1971; Bremner and Mulvaney, 1982), available P using the Bray 1 method (Olsen and Sommers,1982), exchangeable bases using the ammonium acetate method and texture using the hydrometer method as is described in Anderson and Ingram (1993). The initial soil properties are shown in Tables 2 and 3.

Land preparation and planting

Land at the selected experimental sites was ploughed soon after the first effective rains using an ox-drawn mouldboard plough. Forty-eight plots, each measuring 3m by 4m, were pegged on each field/. Planting rows with inter and intra-row spacings of 0.07m and 0.5m, respectively were marked. Basal compound L (5% N: 18% P_2O_5: 10% K_2O: 0.25% B: 8% S) fertilizer was applied by placing in the planting furrows at 150 kg ha^{-1}. Soyabean varieties planted were: SC Saga and one of the three TGx varieties (TGx 1740-2F in NR II, TGx 1987-628 in NR III and TGx 1987-11E in NR IV) (Table 5). Three different TGx varieties were used as only limited quantities of the seed could be imported from Malawi due to national restrictions and also because of the need to match the plant growth and maturity rates to the prevailing conditions of the three natural regions under study. The three promiscuous varieties had the same growth habit but different maturities (early, medium and late). Seven *Bradyrhizobium* strains from the Grasslands Research Rhizobia Collection were used (Table 4). Six of the strains were the test strains and one was the positive control. Seed inoculation was done according to inoculant manufacturer's recommendation but at 5 times the manufacturer's rate (Zengeni et al., 2003; Chirinda et al., 2003). This was in light of previous studies that had shown that rhizobial cell mortality increased in sandy soils hence increasing the number of rhizobial cells introduced into the soil would increase the number of effective cells. Care was taken to avoid cross contamination and plasmolysis of inoculant rhizobial cells Planting was carried out from late January to early February as test strains were released late, at all the sites during the 2011/2012 cropping. During the 2012/2013 cropping season planting was done from mid-December to early January. Seeds were hand dropped into planting furrows and thinned three weeks after germination to give plant population of 285, 000 plants ha^{-1}.

Table 4: Rhizobial strain origins and characteristics in the soyabean inoculation response study in Zimbabwe (2011/2012 and 2012/2013).

Strain	**Origin**	**Characteristics**
MAR 1305	Beltsville, USA (126/CRB)	Highly effective on *G.max* cvv, Bragg, Duiker, Oribi, Roan and Sable, poorly effective on cv Nyala; 1993
MAR 1306	Beltsville, USA (138/CRB)	Highly effective on *G.max* cvv, Bragg, Duiker, Buffalo, Hernon 147, Impala, Kudu, Oribi, Rhosa and Sable, effective on Roan; 1985
MAR 1326	Isolated at Marondera, Zimbabwe, from peat inoculant from Illinois, USA	Highly effective on *G.max* cvv, Bragg, Buffalo, Geduld, Hernon 147, Kudu, Oribi, Rhosa and Roan, effective on Sable; 1985
MAR 1491†	Beltsville, USA, (110/CRB)	Grasslands' inoculant strain. Highly effective on all *G.max* cvv. Tested. Heat tolerant and therefore suitable for both high and lowveld areas of Zimbabwe; 1993
MAR 1494	Beltsville, USA, (184/CRB)	Highly effective on *G.max* cvv Duiker, Gazelle, Oribi and Sable with above average stover nitrogen content; 1993
MAR 1497	Beltsville, USA, (142/CRB)	Acid Tolerant
MAR1515	Brazil, BR 29	Acid Tolerant

Glycine max cultivars: Bragg was introduced from the USA, Geduld was introduced from South Africa and Rhosa was introduced from South Africa as a breeding line and named and released in Zimbabwe. Buffalo, Duiker, Impala, Kudu, Nondo, Nyala, Oribi, Roan and Sable were all bred in Zimbabwe.

†-refers to the reference strain used in the experiment.

Source: In-house communication compiled by staff at the Soil Productivity Research Laboratory at The Grasslands Research, Marondera, Zimbabwe

Experimental design and trial management

The experiment was set up as a randomized complete block design with two factors, soyabean variety (SC Saga and TGX (1740-2F/ 1987-628/ 1987-11E) and rhizobial strain (MAR 1305,1306,1326,1494, 1497 and 1515). Included was a negative control with no inoculation and a positive control inoculated with reference commercial strain MAR 1491. The experiment was replicated three times. Weeding was done manually using a hand hoe two weeks after germination and thereafter when necessary. Daily precipitation at each site was recorded by farmers trained to use rain gauges. Each farmer was assisted in installation of the gauge and recording of the rainfall data. . At the end of each season monthly rainfall figures for each site were calculated by simple addition using Microsoft excel.

Determination of biomass productivity, total N and nodule assessment at mid-flowering.

At mid-flowering plants were cut at the cotyledonary node on one random border row on a random position measuring 1m. Fresh weight of all biomass was measured and a weighed sub-sample collected. All sub-samples were air dried for two weeks and oven dried at 60°C for 24 hours and biomass yield was calculated. The dried samples were ground and used to determine the total N using the Kjeldahl method (McDonnell and Murphy, 1952). The roots of the cut plants were removed taking care not to damage roots and nodules and the nodules on each plant counted and recorded and two nodules cut open to check for nodule internal colour. For nodule scoring and ranking, a 0 to 5 scoring scale adapted from Corby et al., 1977 modified by the N2Africa project (Bala et al., 2011) was used.

Determination of grain productivity

The middle four rows of each plot were harvested by cutting above ground biomass and pods were removed and shelled. Shelled grain was weighed and sun dried to 12% moisture content (measured using a moisture meter), reweighed and grain yield per ha calculated using direct proportion.

Data Analyses

Analysis of variance (ANOVA) to separate grain, biomass and total N yield treatment means as well as nodule numbers was conducted using SAS version 9.3 (SAS, 2010). Standard errors of differences (SED) of means were used to compare and separate the treatment means as post hoc using the Fisher’ s least significant difference.

Results

Rainfall distribution

Rainfall during the 2011-2012 cropping season was evenly distributed although a few mid- and early season dry spells were experienced. The rainfall data collected from all farmers’ fields fell within the average annual rainfall of the natural regions in which the fields fell in. Distribution was poor during the 2012-2013 season with the rains coming late in December and episodes of flooding and prolonged dry spells being experienced throughout the season.

Biomass and total nitrogen response to rhizobial inoculation at mid-flowering

At mid-flowering (6-8 weeks after emergence) biomass yields and total nitrogen responded significantly to the six test rhizobial strains ($p=0.0239$ and $p=0.0001$ respectively). The natural region significantly influenced the biomass yields ($p<0.0001$) and total nitrogen ($p=0.0138$). Biomass yields of up to 3550 kg ha^{-1} and 2500 kg ha^{-}were obtained in the 2011/12 and 2012/13 seasons, respectively. Total nitrogen yields ranged from 80 to150 kg ha^{-1}. Biomass yields were in the order NRII sites> NRIII sites> NRIV sites (Figures 2 and 3). Overall, soyabean biomass yields were significantly higher in the 2011/2012 than 2012/2013 cropping season (Figures 2 and 3). The cropping season also significantly influenced the total nitrogen fixed ($p<0.0001$) (Figures 4 and 5). The variety grown, however, had no significant influence on the biomass at mid-flowering and total nitrogen fixed yields (Figures 2-5).

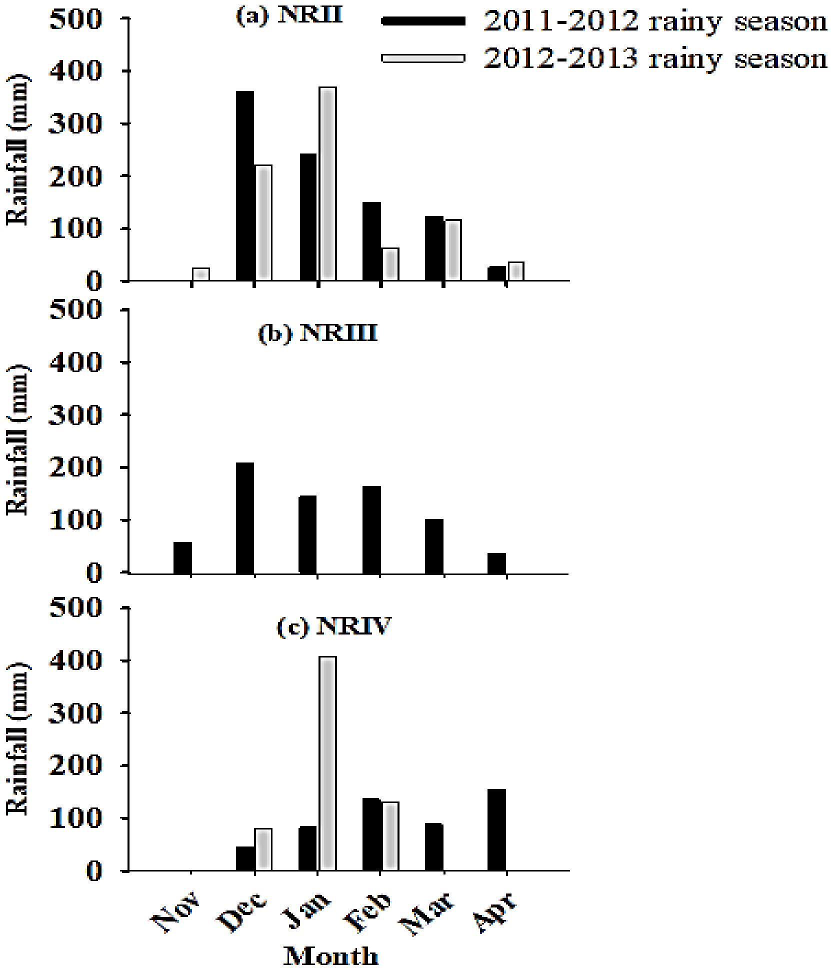
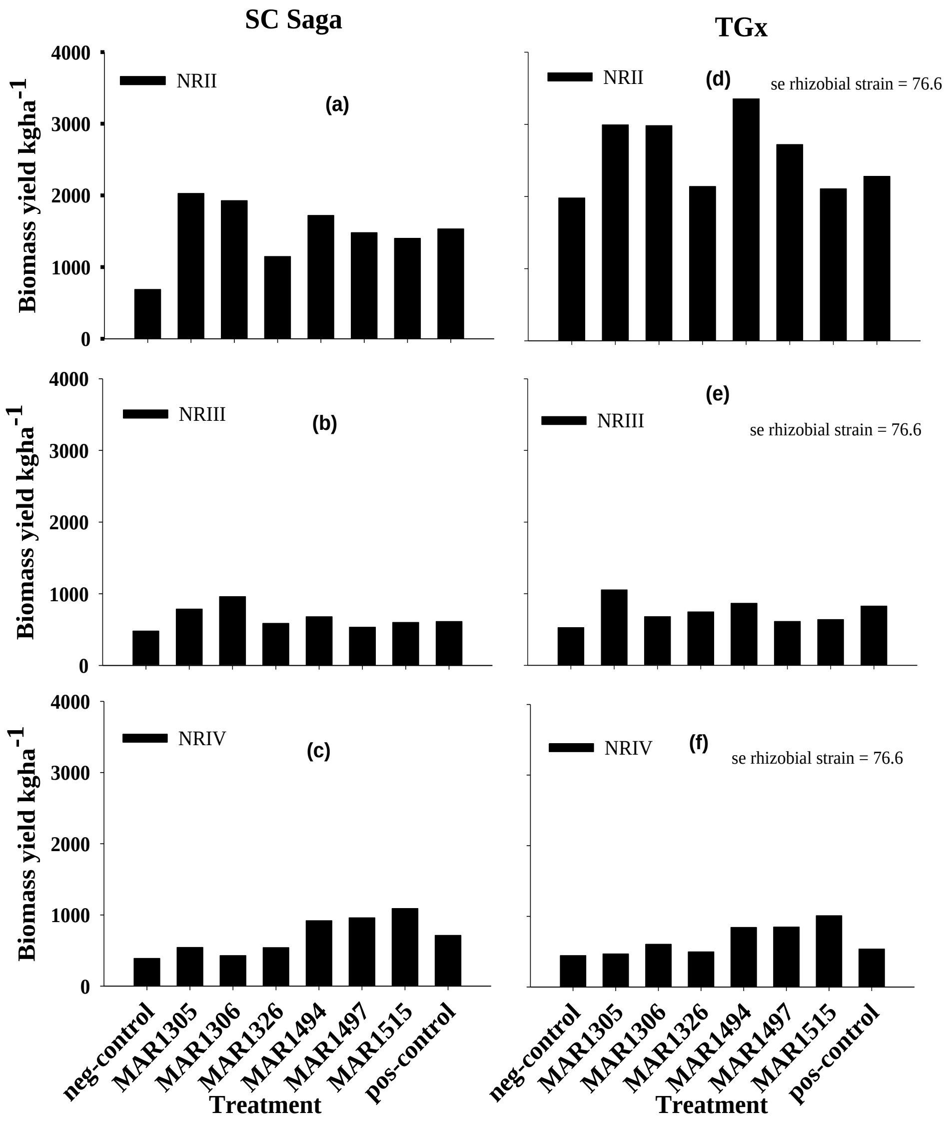
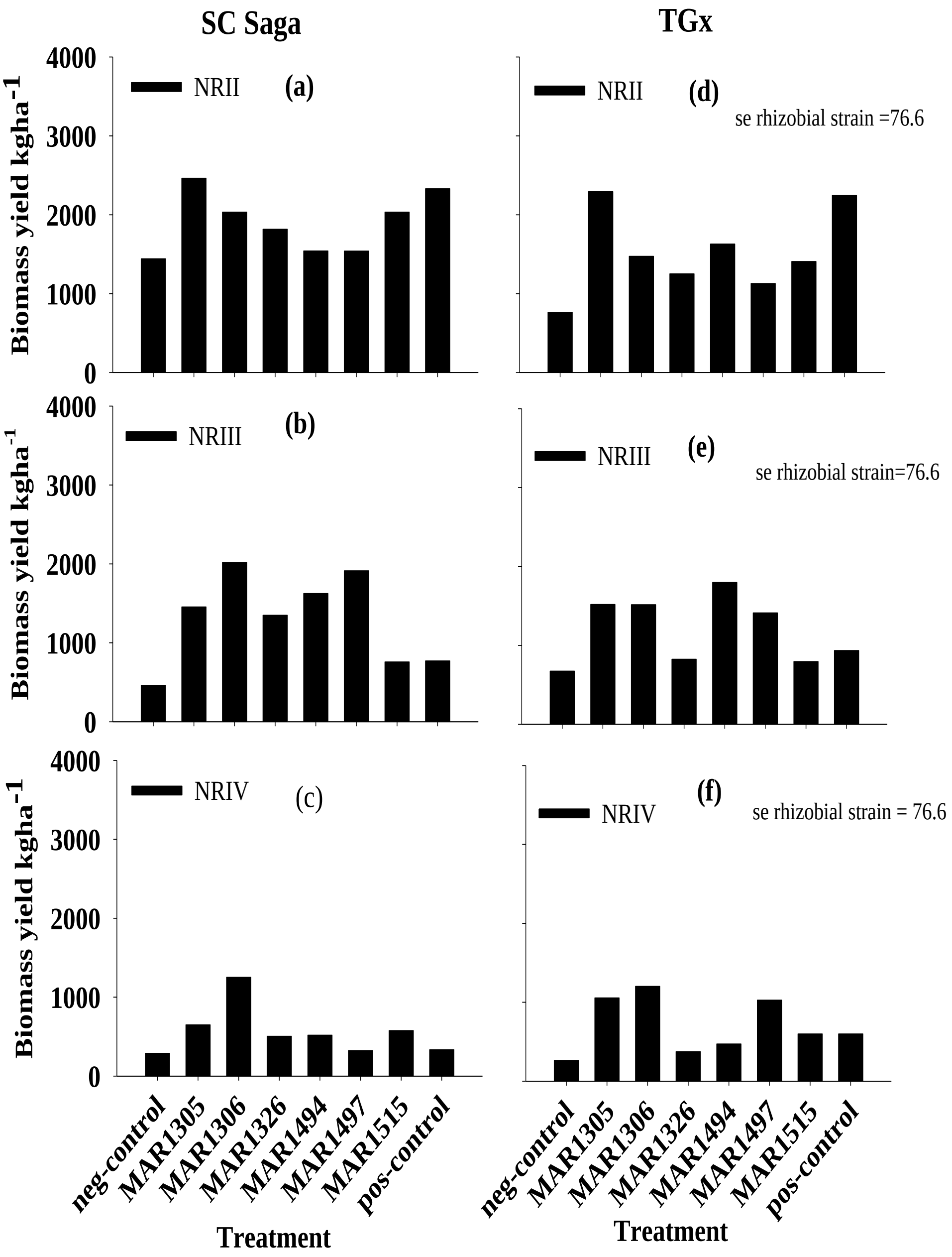


***Figure 1:** Monthly rainfall distribution at the inoculation response study sites in Zimbabwe (2011/2012 and 2012/2013).*



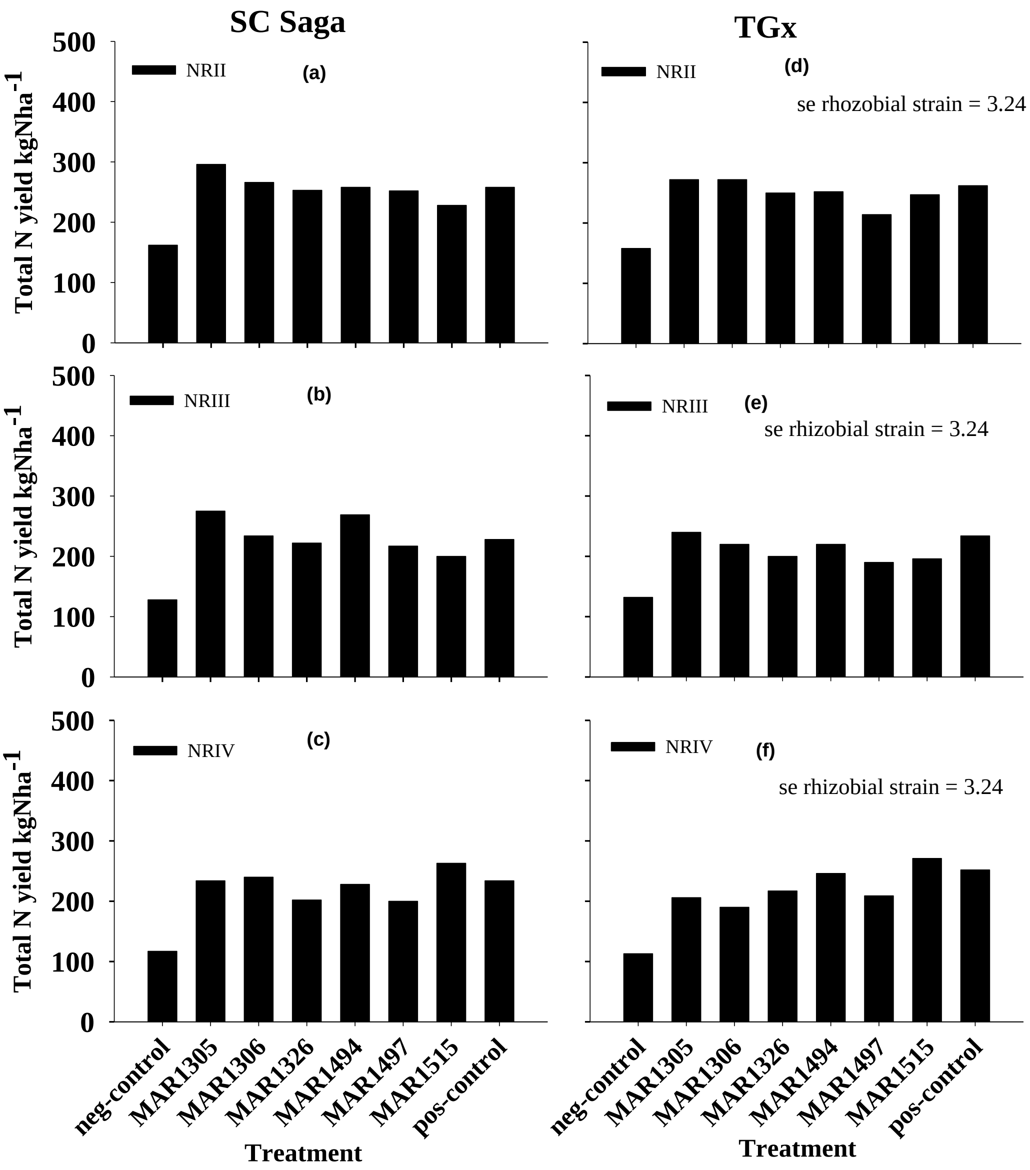
#Soyabean varieties- SC Saga and TGx

Figure 2: ***Soyabean biomass yield response to rhizobial inoculation at three sites located in 3 NRs in smallholder farms in Zimbabwe (2011/2012***



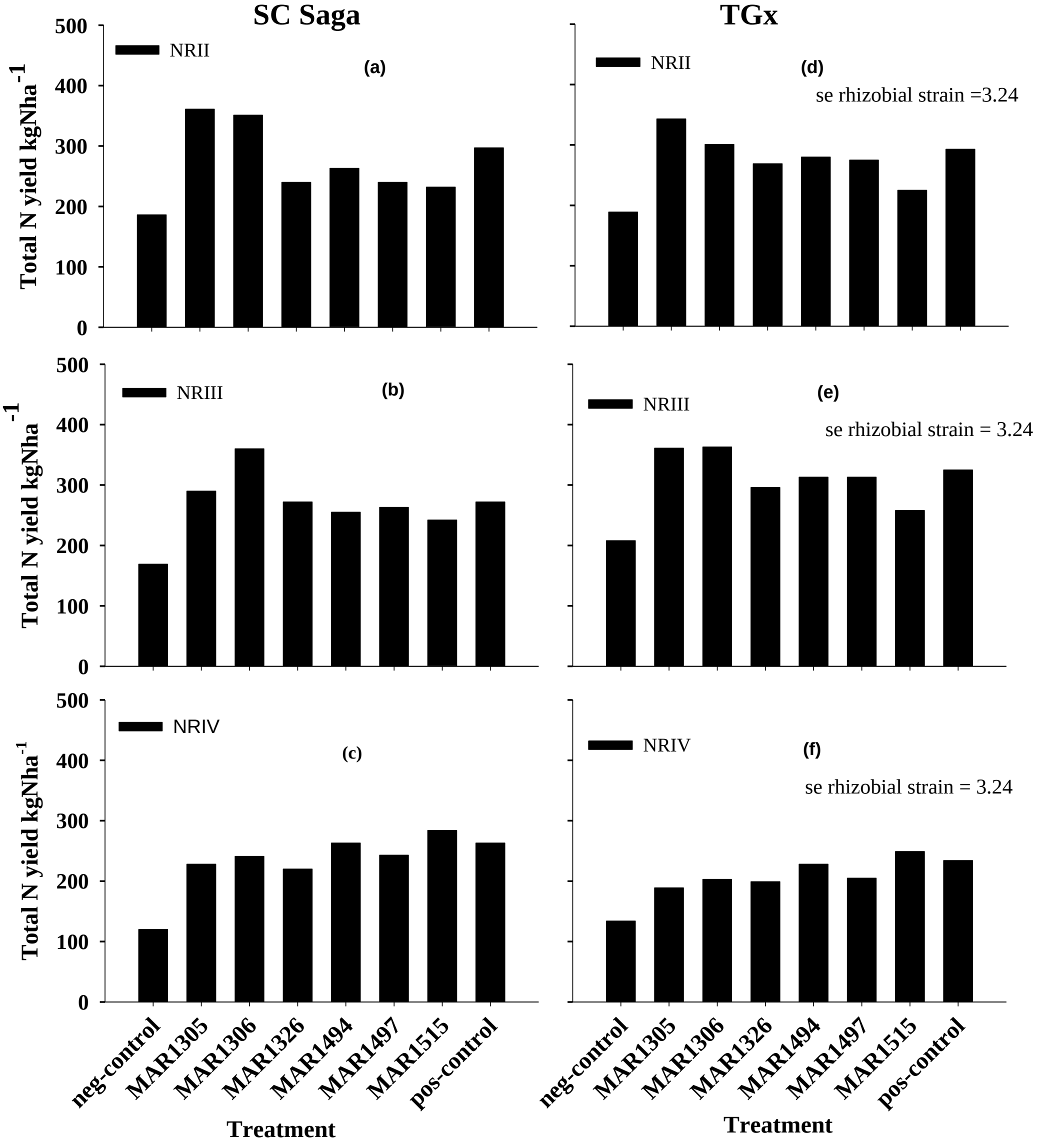
#Soyabean varieties- SC Saga and TGx

Figure 3: ***Soyabean biomass yield response to rhizobial inoculation at three sites located in 3 NRs in smallholder farms in Zimbabwe (2012/2013).***



#Soyabean varieties- SC Saga and TGx

Figure 4: Soyabean total nitrogen yield response to rhizobial inoculation at three sites located in 3 NRs in smallholder farms in Zimbabwe (2011/2012).

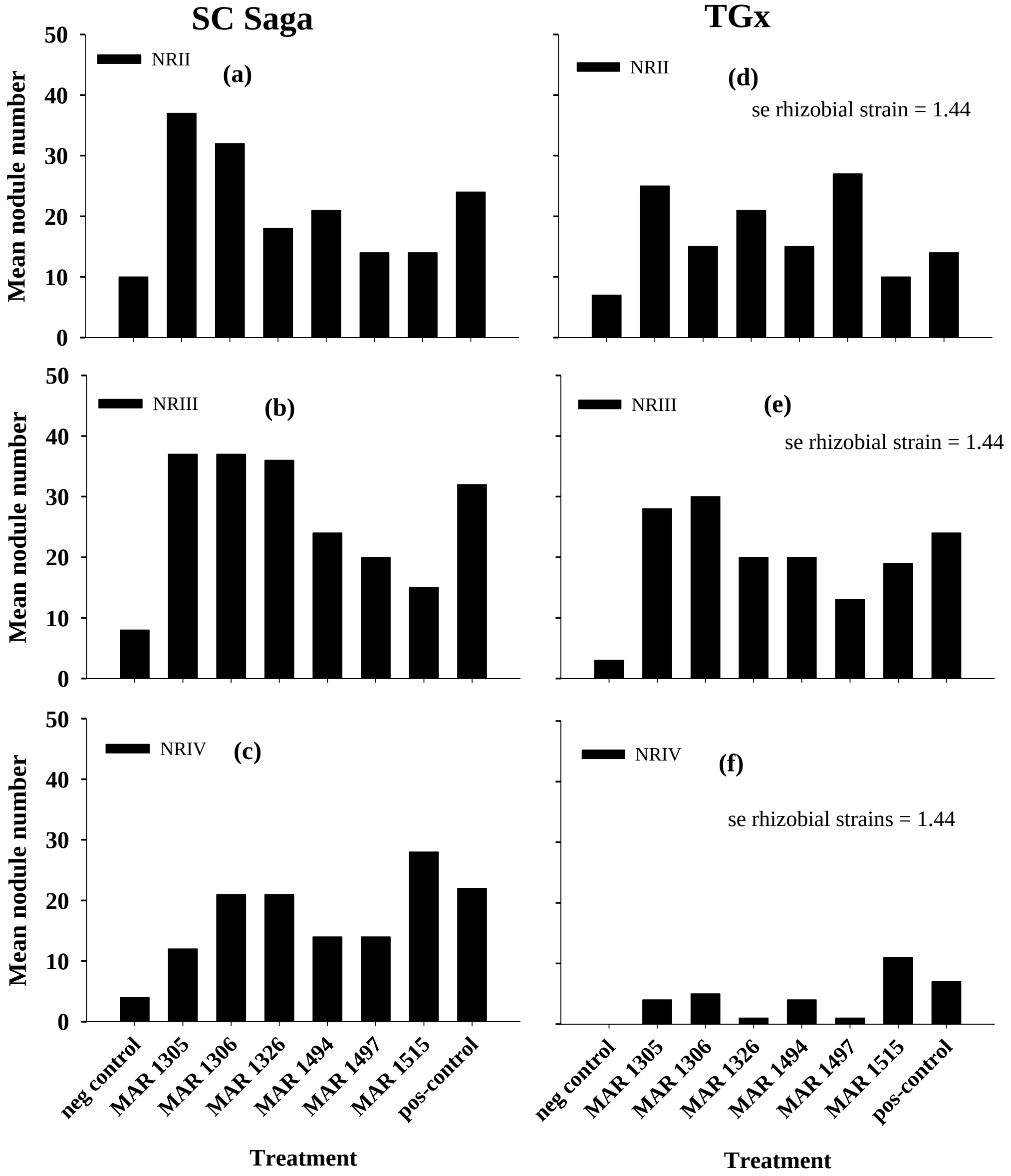


#Soyabean varieties- SC Saga and TGx

Figure 5: Soyabean total nitrogen yield response to rhizobial inoculation at three sites located in 3 NRs in smallholder farms in Zimbabwe (2012/2013).

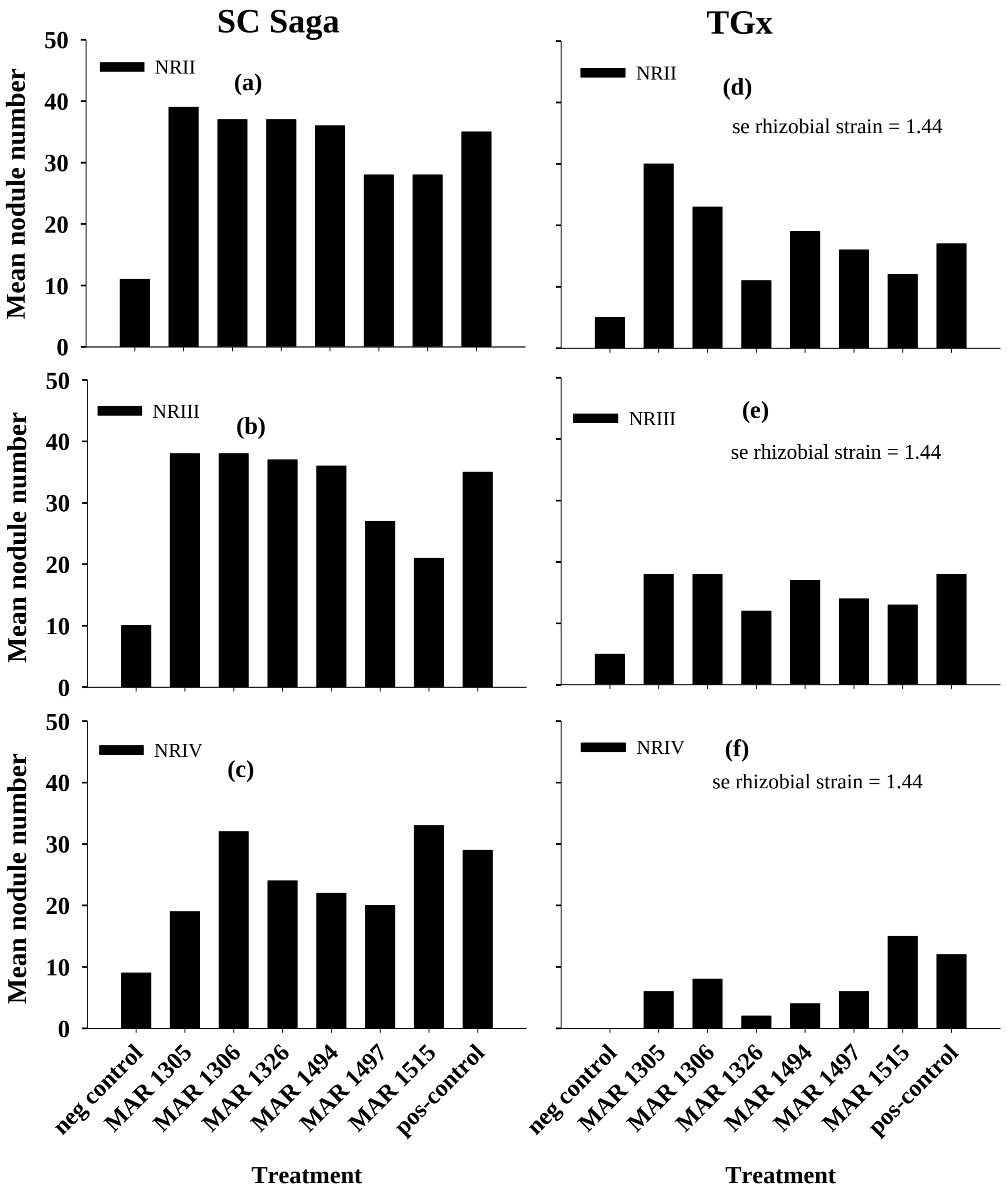
Nodulation response to inoculation with six rhizobial strains

Soyabean nodulation responded significantly to rhizobial inoculation with the test strains ($p<0.0001$). Rhizobial strains MAR 1305, 1306, 1326, 1494 and 1515 produced mean nodule numbers ranging between 28-37 nodules per plant that were significantly higher than those of the positive control (Figure 6 and 7). At sites in NRII and NRIII rhizobial strains MAR 1305, 1306, 1326 and 1494 had high mean nodule numbers while at sites in NRIV rhizobial strain inoculation with MAR 1306 and 1515 resulted in high mean nodule numbers (27-38 nodules per plant). The cropping season significantly influenced the nodule numbers ($p<.0001$), with 2011/2012 season having higher nodule numbers than 2012/2013 season.



#Soyabean varieties- SC Saga and TGx

Figure 6:   Soyabean nodulation responses to rhizobial inoculation at three sites located in 3 NRs in smallholder farms in Zimbabwe (2011/2012).

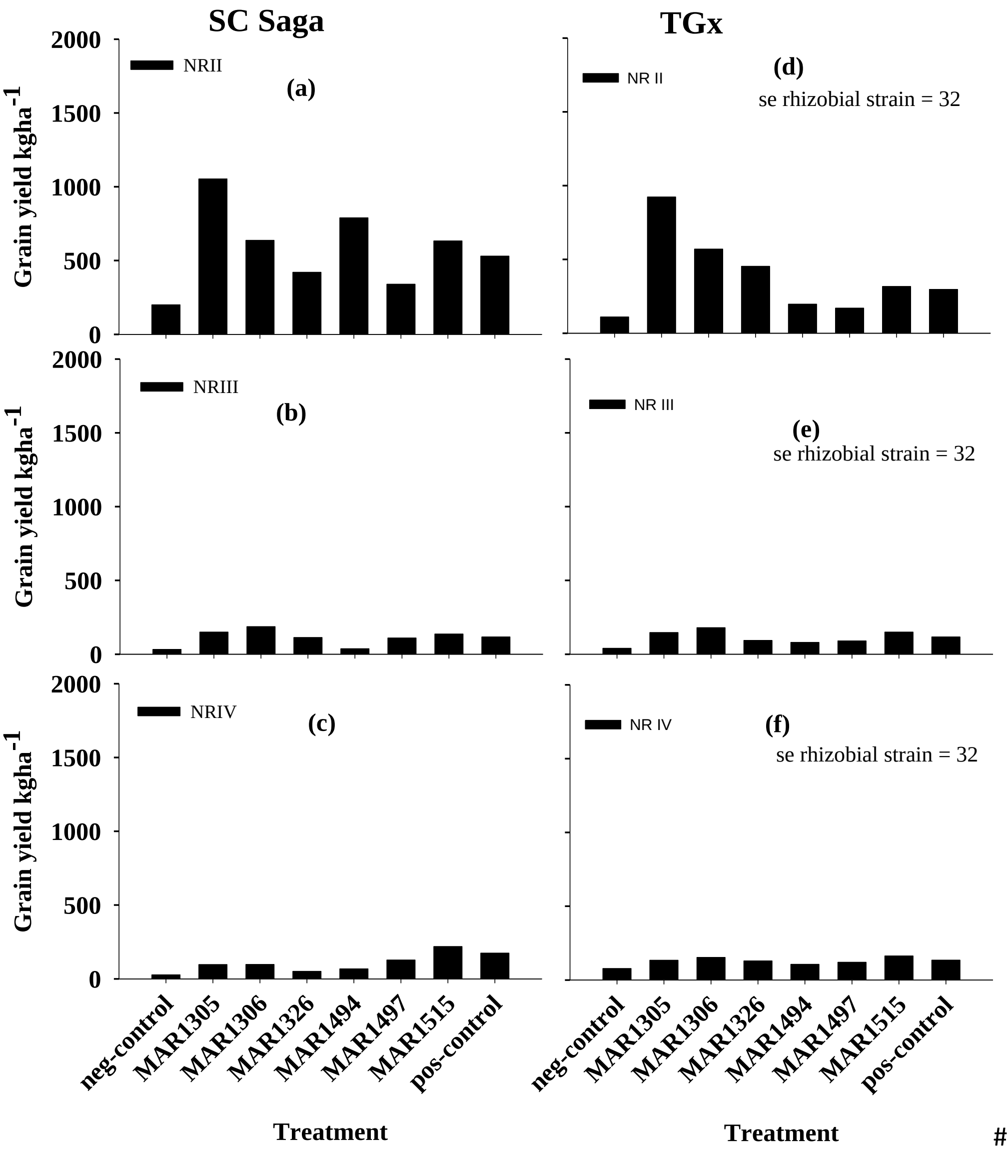


#Soyabean varieties- SC Saga and TGx

Figure 7: Soyabean nodulation responses to rhizobial inoculation at three sites located in 3 NRs in smallholder farms in Zimbabwe (2012/2013).

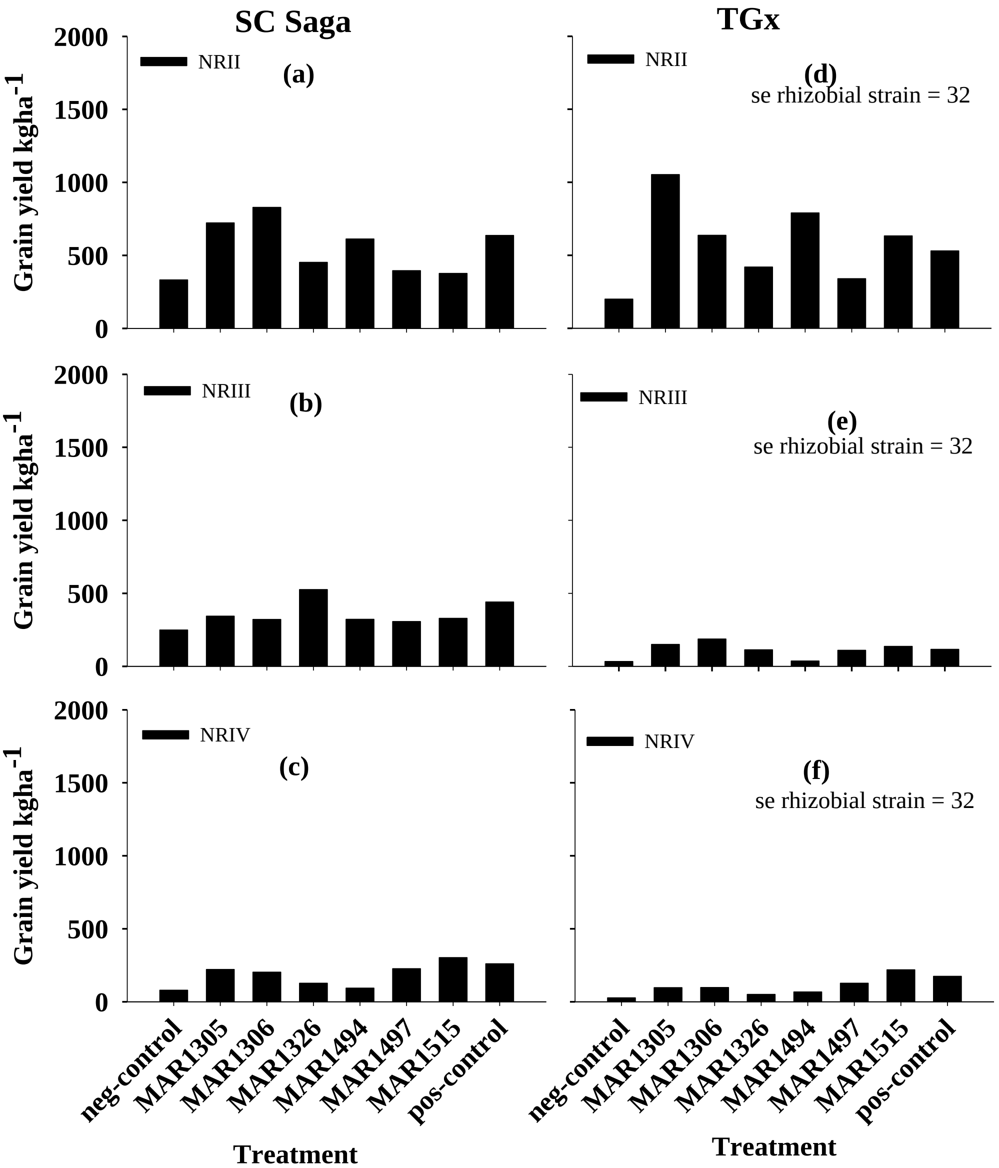
Soyabean grain yield

Grain yields were significantly influenced by the rhizobial strain ($p<0.0378$) with the highest yields attained after inoculation with rhizobial strains MAR 1305, 1306, 1494 and 1515 at sites in NRII and NRIII while at sites in NRIV, after inoculation with MAR 1515 and 1306 (Figures 8 and 9). Grain yields ranged from 150 – 1050 kg ha $^{-1}$ across the sites with an average of 600kg ha $^{-1}$ over the two seasons compared to an average of 400 kg ha^{-1} by the reference strain MAR 1491.The soybean variety also significantly influenced the grain yields achieved ($p<0.0009$) with SC saga (average 600 kg ha^{-1}) yielding higher than TGx varieties (480 kg ha^{-1}). Soyabean grain yields were significantly influenced by the agro-ecological region ($p<0.0001$) with yields in the order NRII > NRIII > NRIV.



#Soyabean varieties- SC Saga and TGx

Figure 8: Soyabean grain yield response to rhizobial inoculation at three sites located in 3 NRs in smallholder farms in Zimbabwe (2011/2012).



#Soyabean varieties- SC Saga and TGx

Figure 9: Soyabean grain yield response to rhizobial inoculation at three sites located in 3 NRs in smallholder farms in Zimbabwe (2012/2013)

Discussion

Rhizobial strain performance

Results show that soyabean varieties inoculated with rhizobial strains MAR1305, 1306, 1494 and 1515 consistently yielded and nodulated better than the commercial inoculant strain MAR 1491used as the positive control. Prior characterization of the rhizobial strains showed that they had been highly effective after being inoculated on selected varieties (Table 5.3). However, the cultivars they were tested on where no longer available commercially and hence they proved they are still effective strains after being tested on the new available cultivars.

Nodulation and total nitrogen response to inoculation

Nodulation of soyabean varieties tested responded significantly to rhizobial inoculation at all the study sites (P<0.0001). Nodulation is understood to be a summation of the host plant, rhizobial population and soil and climatic conditions (Bordeleau and Prevost, 1994). One factor affecting nodulation is inoculation rate and the significant nodulation response may be attributed to the increased application rates. Rhizobia inoculum were applied at five times the manufacturers' recommended rate (Chirinda et al, 2003) after a prior study had concluded that increasing the application rate up to five times significantly increased nodulation resulting in increased yields. Increased inoculum results in increased cells in the soil. Results from our study support these findings as inoculation resulted in moderately abundant mean nodule numbers of 28-37 in soils where rhizobia had not been detected by most probable number estimations.

However, with such increased application rates expectations was that mean nodule numbers would be very abundant reaching mean nodule numbers of greater than 50. The moderately abundant mean nodule numbers of 28-37 may be attributed to several factors. The soils in which experimental trials were established had acidic soils with pH ranging from 4.17- 5.46 with the exception of one site which had a pH of 6.14. Nodulation is affected by soil acidity which can affect solubility of inorganic nutrients and induce nutrient deficiencies resulting in conditions of high and potentially toxic levels of Al and Mn and low deficient levels of Ca and P (Bell and Edwards, 1987; Bordeleau and Prevost, 1994; Dilworth et al., 2001). Calcium is critical in the permeability of cell membranes and attachment of rhizobia during the initiation and formation of nodules while P is a part of important enzyme systems responsible for nodule formation and other plant and soil process (Peoples et al., 1989; Bardin et al., 1996). Since pH was acidic this could have partially inhibited nodulation.

All the soils in which the experimental trials were conducted had low soil organic carbon percentages of lower than 2.5% with some soils having SOC % as low as 0.5 %. Soil organic carbon is important in the survival of rhizobia as they are saprophytic in the absence of their legume host. Organic matter provides rhizobia with nutrients for their multiplication in the rhizosphere before attachment to the root hairs of soyabean roots (O'Hara, 2001) particularly carbon. Low soil C content could have contributed to the relatively low mean nodule numbers observed.

The agro-ecological region in which the experimental trials were established significantly influenced nodulation response ($P<.0001$). Yields were in the order NRII> NRIII> NRIV as temperature increases and rainfall decreases in that order. Nodulation requires good soil moisture for nutrient and ion movement and this could have been affected by the sandy soils which had low water holding capacity and were susceptible to drying. Drying of soils also reduces the presence of normal root hairs resulting in short stubby root hairs which are inadequate for infection by rhizobia (Lie, 1981). This may explain why nodulation was very low in NR IV as rainfall distribution was poor with several dry spells during the season.

Nodulation decreased from NR II down to NR IV as the altitude decreased. At lower altitudes day time temperatures increase resulting in increased soil temperatures which lead to reduced photosynthesis and increased respiration as well as reduced amounts of N fixed (Waughman, 1977; Lie, 1981). Also, at high temperatures the amount of plant roots produced are usually thin and do not branch and hence have few lateral roots (Waughman, 1977) resulting in low root hair concentration in the rhizosphere. Nodulation occurs at the root hairs hence increased root hair concentration affords more rhizobia attachment sites for initiation of nodulation (Downie, 2010). Increased soil temperatures also result in reduced survival of soil bacteria (Dudeja and Khurana, 1989) and this may also have attributed to reduced nodulation in the soils particularly those in natural regions at low altitudes.

The cropping season also significantly influenced nodulation ($P<.0001$). The 2011/2012 season's crop had significantly higher mean nodule numbers than 2012/2013 season. Soyabeans have one rhizobial infectible period close to the beginning of the root hair growth and are also sensitive to weed growth. This period coincided with the heavy rains and waterlogged conditions during the 2012/2013 season making weeding difficult. Also, under waterlogged conditions carbon dioxide concentrations increase and can inhibit nodule formation (Wei et al., 2008).

The total nitrogen fixed ranged from 80 kg ha^{-1} to 150 kg ha^{-1} after rhizobial inoculation compared to the negative and positive controls after employing the N- difference method. This observation was in agreement with previous studies done in Hurungwe and Guruve districts of Zimbabwe that found total nitrogen ranging from 60-130 kg ha^{-1} (Kasasa, 1999).

Biomass and grain productivity in response to rhizobial inoculation

Biomass yields responded to rhizobial inoculation ($p<0.0239$) as well as the agro-ecological region in which the trials were established. The TGx varieties produced higher biomass compared to SC Saga at mid-flowering consistent with the findings of previous studies which concluded that promiscuous varieties had higher biomass yields compared to specific ones (Giller, 2008).

Soyabean grain yield responded significantly to rhizobial strain inoculation ($p<0.0378$) with all varieties yielding higher than the negative control. Also, in both seasons' inoculation with rhizobial strains MAR 1305, 1306, 1494 and 1515 out yielded the recommended commercial rhizobial strain. Total nitrogen content was significantly higher than the reference strain for all varieties after inoculation. This shows that there are other strains that have better adaptation to the smallholder cropping systems than the commercially available strain and further exploration of them is important in the success of soyabean production in these cropping systems.

Soyabean grain yields were significantly influenced by the agro-ecological region ($p<0.0001$) with yields decreasing in the order NRII> NRIII> NRIV and also by the cropping season ($p<0.0001$) with 2011/2012 cropping season having better yields than 2012/2013 cropping season. The highest grain yields achieved were 1053 kg ha^{-1} and these were lower than expected yields of 2-4 t ha^{-1} after inoculation. This can be attributed to the fact that grain yields are affected by several factors which include variety selection, planting date, plant populations, nutrient deficiency, weed, disease and insect pressure and rainfall distribution (Pederson, 2007). The promiscuous soyabean varieties were imported at the beginning of the 2010/2011 season hence the seed used in the seasons was first and second generations and seeds lose vigor and genetic potential with every successive generation (Pederson, 2007). Another cause of low grain yields could have been the dry spells during the season which could have affected critical reproductive stages such as pod formation, filling and setting especially during the 2012/2013 season when the rains stopped in early march (Carlson and Knapp, 1992; Gibson and Mullen, 1996; Hu and Wiatrack, 2012).

Plant populations of 250,000 plants per hectare were used at all experimental sites but however closed canopies were achieved in a few fields mainly those in NR II. This failure of the canopy to close could be attributed to low soil fertility in which soyabean plants were established. The soils had low N, soil organic carbon, P and low pH (Table 2 & Table 3). Canopy closure is a great advantage for reduction of soil moisture loss and weed competition (Paz, Batchelor and Seild, 2000; Pederson, 2007). Hence because canopy closure was not attained this could have resulted in increased soil moisture loss as well as competition for nutrients affecting the reproductive stages such as pod formation and seed fill.

All the soils investigated had pH below 6 except one with most of them ranging from 4.17-5.46 and acidic soil conditions are known to result in nutrient deficiencies. Although a basal fertilizer containing N, P, K and S was applied and it is probable that fixation could have occurred as the soils was acidic. Phosphorus, a limiting nutrient, is important in the development of roots and nodules as well as plant energy systems and seed development and maturity hence if its fixation occurred this could have attributed to these low grain yields (Sprent and Sprent, 1990; Marschner, 1995; Vladimir, 2012). If phosphorus were low even after significant nodulation, nodule functions such as seed development and seed maturity could have been affected.

The soyabean variety significantly influenced the grain yield ($p<0.0009$). The commercial variety SC Saga yielded significantly higher than the TGx varieties. This is supported by previous findings which noted that specific varieties produced higher grain yield but lower biomass compared to promiscuous varieties (Giller et al., 2011). However, these results did not offer further support to another study which concluded that promiscuous varieties had the potential to nodulate and produce high yields in low input cropping systems (Kasasa, 1999).

Conclusions

Achieving optimal soyabean yields in smallholder farming areas is a summation of the soyabean variety, rhizobial strain, climatic conditions and soil conditions. Good soil management practices such as the addition of organic manure which results in improved SOC and good rhizobia proliferation as well as improved soil water holding capacity are critical for sustainable soyabean production in smallholder areas. As most soils in these areas are acidic liming is important in order to improve soil health even after addition of basal inorganic fertilisers. For sustainable soyabean in NR IV supplementary irrigation is required as the incidence of dry spells in this region is increased. Inoculation is a prerequisite in these cropping systems and plans to find other carrier materials need to be explored to reduce application rates making soyabean production even more economical as well as further characterization of available strains to increase options.

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