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Response of soybean [*Glycine max* (L.) Merrill] varieties to Bradyrhizobium Japonicum inoculation, N and P supplementation in Northern Tanzania

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Abstract

Despite that soybean [Glycine max (L.) Merrill] is easily introduced in places where it is not native, its production, especially Africa is constrained by the lack of *Bradyrhizobium japonicum* which is needed for biological nitrogen fixation (BNF). Inoculating soybean with proper rhizobacteria is a key solution to enhance benefits of BNF. A study was done in Northern Tanzania in Arusha and Kilimanjaro regions to assess the effects of *B. japonicum*, nitrogen (N) and phosphorus (P) application on four selected soybean varieties namely Uyole 1, Uyole 2, Uyole 3 and Uyole 4. Inoculation with *B. japonicum* strongly ($P<0.001$) increased nodule number, nodule efficiency and nodule dry weight. Total chlorophyll accumulation in plant leaves was significantly ($P<0.05$) reduced in control (15.26mg L^{-1}) treatment compared with *B. japonicum* plus phosphorus (18.54mg L^{-1}) and sole *B. japonicum* (18.11mg L^{-1}) treatments. Leaf area was significantly lower when soybean was applied with phosphorus (33.46cm^2) compared with sole *B. japonicum* (44.23cm^2) inoculation and inoculation + P (42.18cm^2) supplementation. Soybean varieties had a significant effect ($P<0.05$) on nodule dry weight, plant shoot length and a stronger effect ($P<0.01$) on root dry weight.

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Introduction

Soybean [*Glycine max* (L.) Merrill] is an annual legume widely grown throughout the world (Herridge *et al*., 2008). Soybean is comprised of 36% protein, 19% oil, 35% carbohydrate, 5% minerals and other components including vitamins (Liu, 1997). Thus soybean is the source of the most consumed vegetable oil (El-Hamidi & Zaher, 2018) and the least expensive dietary protein to both livestock (Singh *et al*., 2008) and human beings (Derbyshire *et al*., 1976; Hassan, 2013). According to Salvagiotti *et al*. (2008) and Machido *et al*. (2011), soybean growth and yield production requires high (up to 400kg ha^{-1}) doses of nitrogen needed for high (5t ha^{-1}) yield. To meet requirements, soybean crop can convert nitrogen (N) from the atmosphere when the proper symbiotic relationship with specific rhizobacteria (mostly with *Bradyrhizobium japonicum*) is established in the roots (Albuquerque *et al*., 2017). In Africa, reaping benefits of the N-fixation is limited due to lack *B. japonicum* in most soils (Agoyi, 2018). Nevertheless, the African continent has also been facing an enormous challenge of increasing the agricultural production (Naseem & Kelly, 1999; Shiferaw *et al*., 2004) and the Sub-Saharan Africa region has always demanded use of fertilizer (Reardon *et al*., 1999). Giller *et al*. (1998) reported that poor N fixation is a factor for reduced common bean yield production in Northern Tanzania and, soil N, phosphorus (P) and potassium (K) are also the major constraints hindering enough production (Wortmann, 1998). These constraints may also hinder the growth of other legumes such as soybean.

However, one of the ways to increase N uptake is through biological nitrogen fixation (BNF) (Peoples *et al*., 1995; Graham & Vance, 2000). The BNF process also require P which is crucial for root development, energy transformation, biochemical reactions including BNF (Rotaru & Sinclair, 2009). Both N and P are also demanded for the manufacture of plant food via the process of photosynthesis (Walker *et al*., 2014). Plants use a conspicuous pigment (Bruuinsma, 1963) called chlorophyll which is mainly found in their leaves to harvest light (Mackinney, 1941; Thornber, 1975; Baker, 2008) and is used to combine carbon dioxide, water and tiny amount of minerals into sugars. The end product, carbohydrates are is used as a plant food for its growth and development (Rabinowitch & Govindjee, 1965). Efforts to gain further knowledge and enhance nutrient use efficiency and the biological contribution to crop nutrient uptake are now developing (Dungait *et al*., 2012). Inoculating soybean with specific *B. japonicum* has been reported to increase plant nodulation, growth and yield parameters via BNF (Giller & Cadisch, 1995; Han & Lee, 2005; Njira *et al*., 2013; Masciarelli *et al*., 2014; van Heerwaarden *et al*., 2018) and Chl content of the plant (Nyoki & Ndakidemi, 2014). Some studies in Tanzania have also looked at supplementing inoculation with fertiliser such as N and or P and its effect on soybean (Ndakidemi *et al*., 2006; Massawe *et al*., 2017). Inoculated and P supplemented soybean was also found to contain increased Chl and had higher growth over control plants as reported by Tairo & Ndakidemi (2013).

In crop development, there is one inevitable thing, changes in response of crop cultivars in varying environmental conditions (Kempton *et al*., 1996). With new soybean varieties being released there is need to research their response to inoculation and fertiliser supplementation. This study was done to evaluate nodulation, Chl accumulation and growth response of four selected soybean varieties to inoculation, phosphorus and nitrogen supplementation in Northern Tanzania.

Materials and methods

Experimental layout description

Four selected non-promiscuous soybean varieties were used in the study namely: Uyole 1, Uyole 2, Uyole 3 and Uyole 4, which were obtained from Uyole Research Station in Mbeya, Tanzania. The soybean inoculant used was *Bradyrhizobium japonicum* strain USDA 110 obtained from MEA Company Nairobi-Kenya. The treatments included: (1) un-inoculated soybean which was used as a negative control, (2) soybean inoculated with *B. japonicum*, (3) soybean inoculated with *B. japonicum* +

phosphorus fertiliser (P, 26kg ha^{-1} as triple super phosphate), (4) nitrogen applied at a rate of 30kg ha^{-1} as urea as a positive control and, (5) phosphorus fertiliser applied at a rate of 26kg ha^{-1} as triple super phosphate (TSP). Plots were laid out in a randomized complete block design with three replicates and plot size was 3m x 3m, with plant spacing of 0.5m x 0.2m. The experimental plots were kept weed free throughout the growing season.

Sites description: location, climate and soil characterization

The research was conducted in Northern regions of Tanzania, Kilimanjaro and Arusha, at Kibosho farm (at latitude of 3°17'02.4" S, longitude of 37°18'15.1" E and with an altitude of 1200m) and Nelson Mandela African Institution of Science and Technology farm (at latitude of 3°23'56.8" S, longitude of 36°47'48.3" E and with an altitude of 1100m) respectively. The 2018/2019 average monthly rainfall for the sites is provided in

Fig. **1** and its data compiled from World Weather Online (2020a) and World Weather Online (2020b). Soil was sampled from five points in each field using a zig zag method and it was tested for different chemical and physical properties at Tanzania Coffee Research Institute using specific methods: pH by potentiometric determination (Fullen & Catt, 2004), total N by micro Kjeldahl (Miller & Houghton, 1945), P determination by Bray and Kurtz P1 method Bray & Kurtz (1945) while for K, atomic absorption spectrophotometer method was used (David, 1960).

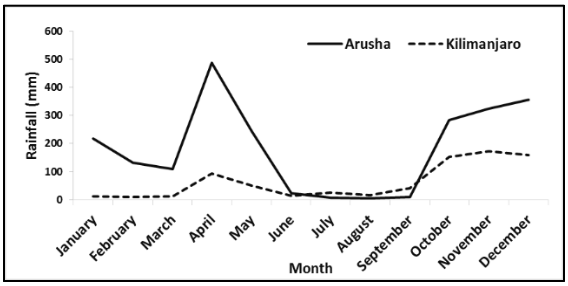

Fig. 1. The 2019 average monthly rainfall.

Estimation of Chlorophyll Content

During flowering stage, the third top leaf was plucked from each of the four randomly sampled soybean plants per plot and was taken for analysis of chlorophyll content. Dimethylsulphoxide (DMSO) was used as an extraction solvent and a Synergy HTX Multi-Mode Microplate Reader (BioTek Instruments, Winooski, VT, USA) was used to read wavelengths at 356nm and 645nm. All procedures were done according to Arnon (1949).

Evaluation of nodulation and plant growth parameters

Destructive nodule counting was used for the evaluation of nodulation. Four randomly selected plants were dug out from each plot at flowering stage and immediately washed in half full bucket of water which had a sieve to trap any detached nodules from the plant roots. Then each plant was cut at the cotyledonary node to separate roots from shoots. Right after cutting, nodules were detached from roots of each soybean plant and counted to compute average nodule number per plant (NN, n° $plant^{-1}$). Assessment of nodule effectiveness was done right after counting by dissecting the nodule into halves and observing the inside centre colour. Effective nodules were determined by red, pink and brown colours whereas yellow, green and other colours indicated ineffectiveness (Sylvia *et al.*, 2005). Thereafter, the nodules, roots and shoots were oven dried at 65°C for 72 hr to a constant weight for determination of nodule dry weight (NDW, g $plant^{-1}$), root dry weight (RDW g $plant^{-1}$) and shoot dry weight (SDW, g $plant^{-1}$). Plant shoot length (PSL, cm $plant^{-1}$) and leaf area (LA, cm^2 $plant^{-1}$) were recorded using a ruler and the latter was calculated using formula, LA=0.624+0.723 (leaf length*leaf width) by Wiersma & Bailey (1975).

Statistical analysis

Data for all parameters were subjected to analysis of variance (ANOVA) using Genstat software, 15^{th} edition (VSN International, Hemel Hempstead, United Kingdom). Separation of treatment means were done using Fischer least significant differences (LSD) at P=0.05.

Results

Soil properties

The soils at Kilimanjaro site were brown and at Arusha it was observed to be greyish in colour. At both sites, soils were clay loam in texture and were slightly acidic, whereby the soils at Arusha inclined much to the acidic condition (**Error! Reference source not found.**). Kilimanjaro site had higher N present in the soil compared with Arusha, similarly with P. However, at Arusha, the presence of K in the soil was slightly above the amount found at Kilimanjaro.

Table 1. Chemical and physical properties of soil.

Property	Units	Arusha	Kilimanjaro
pH (H_2O)	-	6.37	6.53
Total N	cmol(+)kg^{-1}	0.08	0.22
P	ppm	18.7	29.6
K^+	cmol(+)kg^{-1}	1.93	0.69
Texture	-	Clay loam	Clay loam

Effect of Bradyrhizobium inoculation and supplementation with N and P fertilisation on nodulation parameters (number of nodules, nodule effectiveness and nodule dry weight) of selected soybean varieties

The nodulation performance on nodule number (NN) and nodule effectiveness (NEF) at Arusha and Kilimanjaro sites did not significantly differ, however Arusha had significantly higher (0.12g plant^{-1}) nodule dry weight (NDW) compared with Kilimanjaro (0.09g plant^{-1}). Inoculation and application of fertiliser had a significant effect on nodulation parameters (**Error! Reference source not found.**).

There were no significant differences among the effects of N, P and control treatments on the nodulation parameters. The combination of Bradyrhizobium inoculation and P supplementation significantly increased NN (21.17), NEF (81.1%) and NDW (0.25) compared with other treatments but the effect on NEF was significantly at par with sole *B. japonicum* (76.3%).

There were significant ($P<0.01$) interactions between fertilisation and site on NDW which showed that the combination of inoculation and P was the highest at both sites but did not significantly differ from sole inoculation at Arusha site (Fig. 2).

Soybean varieties had no significant effect on NN but significantly ($P<0.05$) affected NEF and NDW as shown in **Error! Reference source not found.**. Variety Uyole 2, had the highest (41.1%) NEF which did not significantly differ from Uyole 1 (35.3%) and the NEF for latter was at par with the remaining two varieties. Among soybean varieties, Uyole 4 had the highest (012.g plant^{-1}) NDW and the lowest (0.08g plant^{-1}) was in Uyole 3. There were significant interactions between site and varieties on NEF and NDW. As shown in Fig. 2, Uyole 2 increased NEF compared with the remaining three varieties at Arusha, and there were significant variety variations at Kilimanjaro. The interactions between site, fertiliser and varieties on NDW showed no significant differences at Kilimanjaro, and between control, N and P treatments at Arusha site (Fig. 2).

Table 2. Nodule number (NN, n° plant^{-1}), nodule effectiveness (NEF,% plant^{-1}) and nodule dry weight (NDW, g plant^{-1}) of soybean varieties as affected by inoculation and supplementation with N and P fertilization.

Treatment		NN	NEF	NDW
Site	Arusha	8.02 ± 1.17^a	34.82 ± 4.85^a	0.12 ± 0.02^a
	Kilimanjaro	8.81 ± 1.60^a	35.56 ± 5.22^a	0.09 ± 0.01^b
Fertilisation				
Control		2.34 ± 1.34^c	7.1 ± 4.21^b	0.02 ± 0.01^c
Nitrogen		1.36 ± 0.55^c	6.79 ± 3.80^b	0.02 ± 0.01^c
Phosphorus		0.98 ± 0.46^c	4.63 ± 3.20^b	0.02 ± 0.01^c
Rhizobium		16.22 ± 1.37^b	76.32 ± 2.20^a	0.2 ± 0.02^b
Rhizobium + P		21.17 ± 2.27^a	81.11 ± 1.75^a	0.25 ± 0.02^a
Variety				
Uyole 1		9.61 ± 2.51^a	35.3 ± 7.56^{ab}	0.11 ± 0.02^{ab}
Uyole 2		8.75 ± 1.78^a	41.08 ± 6.58^a	0.1 ± 0.02^{ab}
Uyole 3		6.05 ± 1.45^a	30.97 ± 6.79^b	0.08 ± 0.02^b
Uyole 4		9.24 ± 2.04^a	33.41 ± 7.63^b	0.12 ± 0.03^a
3-Way ANOVA F-statistics				
Site		0.42^{ns}	0.08^{ns}	19.92^{***}
Fertilisation		49.31^{***}	182.53^{***}	138.76^{***}
Variety		1.75^{ns}	3.65^{*}	3.48^{*}
Fertilisation*Site		0.69^{ns}	1.67^{ns}	3.21^{*}
Fertilisation*Variety		1.02^{ns}	1.5^{ns}	1.79^{ns}
Variety*Site		1.75^{ns}	4.59^{**}	7^{***}
Fertilisation*Variety*Site		0.36^{ns}	1.3^{ns}	2.08^{*}

Values presented are means ± SE. *; **; *** is significant at $P<0.05$, $P<0.01$, $P<0.001$ respectively, ns = not significant, SE = standard error. Means followed by similar letter(s) in a column are not significantly different ($P=0.05$, Fischer LSD).

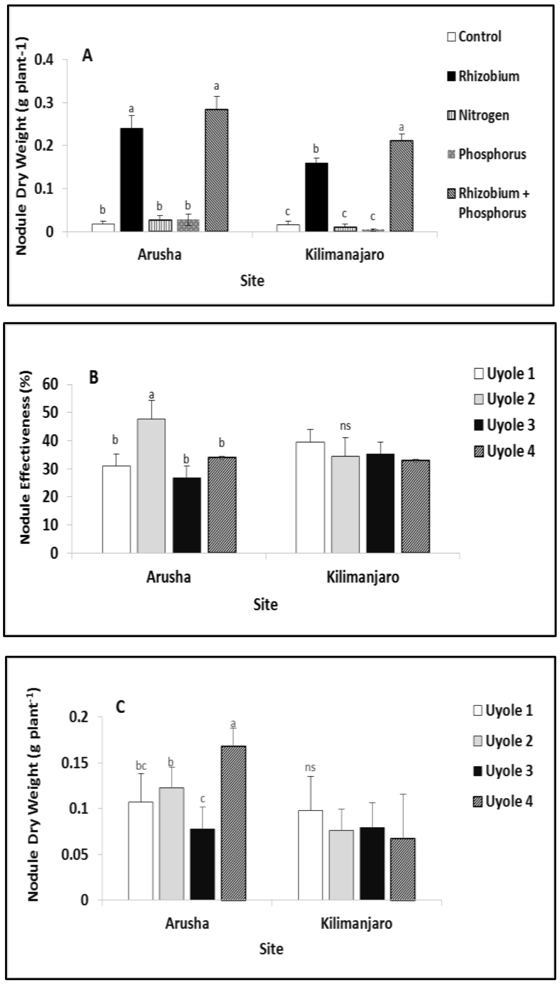

Fig. 2. Interactive effects of, variety and site on nodule effectiveness (A) and nodule dry weight (B); and inoculation (supplemented with N and P) and site on nodule dry weight (C). Error bars indicate standard errors of the means and when followed with similar letter are not significantly different (p=0.05, Fischer LSD).

Effect of Bradyrhizobium inoculation and supplementation with N and P fertilisation on Leaf chlorophyll content of selected soybean varieties

The amount of chlorophyll (Chl) that accumulated in soybean leaves grown at Arusha and Kilimanjaro sites did not significantly vary (

Table 3). Fertilisation significantly affected Chlorophyll a (Chl a) and total Chl (Chl a + b). On accumulation of Chl a, there were non-significant differences between P and control treatments, but the latter did not significantly vary with N and sole Bradyrhizobium treatments. Chl a levels reached at peak (13.66mg $^{L-1}$) when plants were inoculated with Bradyrhizobium and supplemented with P

Table 3. Total Chl content was significantly higher (18.54mg L^{-1}) in inoculated plus P supplemented and sole inoculated (18.11mg L^{-1}) plants compared with the control (15.26mg L^{-1}) treatment. However, total Chl in control plants did not significantly differ from N (17.46mg L^{-1}) and P (16.71mg L^{-1}) treated plants, although the latter two did also not differ from the inoculated treatments (

Table 3). Since total Chl was derived from the combination of Chl a and Chl b, the contents of both Chl a and Chl b found in leaves determined the final total Chl. Thus it was not coincidence that the performance rank of treatments for the total Chl resembled the one for Chl a (

Table 3). Soybean varieties had no effect on Chl content levels and there were no significant factor-interactions observed.

Table 3. Chlorophyll a (Chl a,mg L^{-1}), chlorophyll b (Chl b,mg L^{-1}) and total chlorophyll (Chl a + b,mg L^{-1}) content of soybean varieties as affected by inoculation and supplementation with N and P fertilization.

Treatment		Chl a	Chl b	Total Chl
Site	Arusha	12.25±0.47^{a}	4.83±0.20^{a}	17.08±0.55^{a}
	Kilimanjaro	12.46±0.31^{a}	4.75±0.14^{a}	17.22±0.37^{a}
Fertilisation				
Control		10.75±0.62^{c}	4.51±0.31^{a}	15.26±0.74^{b}
Rhizobium		13.27±0.64^{ab}	4.84±0.22^{a}	18.11±0.74^{a}
Nitrogen		12.94±0.62^{ab}	4.52±0.31^{a}	17.46±0.78^{ab}
Phosphorus		11.69±0.82^{bc}	5.02±0.35^{a}	16.71±0.99^{ab}
Rhizobium + Phosphorus		13.66±0.61^{a}	4.88±0.34^{a}	18.54±0.79^{a}
Variety				
Uyole 1		13.75±0.76^{a}	4.76±0.23^{a}	18.51±0.90^{a}
Uyole 2		11.78±0.48^{a}	4.38±0.26^{a}	16.15±0.58^{a}
Uyole 3		12.08±0.64^{a}	4.88±0.29^{a}	16.95±0.77^{a}
Uyole 4		12.25±0.53^{a}	5±0.31^{a}	17.25±0.66^{a}
3-Way ANOVA F-statistics				
Site		0.55^{ns}	0.29^{ns}	0.15^{ns}
Fertilisation		3.41*	0.52^{ns}	2.56*
Variety		2.25^{ns}	0.90^{ns}	1.83^{ns}
Site*Fertilisation		0.71^{ns}	0.61^{ns}	0.75^{ns}
Site*Variety		1.44^{ns}	0.58^{ns}	0.57^{ns}
Fertilisation*Variety		1.60^{ns}	1.04^{ns}	1.68^{ns}
Site*Fertilisation*Variety		0.50^{ns}	0.66^{ns}	0.49^{ns}

Values presented are means ± SE. * is significant at $P<0.05$, ns = not significant, SE = standard error. Means followed by a similar letter in a column are not significantly different (P=0.05, Fischer LSD).

Effect of Bradyrhizobium inoculation and supplementation with N and P fertilisation on plant growth parameters of selected soybean varieties

Stem Girth

Site had a strong effect on stem girth (SG) of plants whereby Kilimanjaro had higher (0.64g) SG relative to Arusha (0.45g) site (**Error! Reference source not found.**). Plant SG did not significantly vary in response to inoculation, fertilization and soybean varieties. The interaction between soybean variety and site had a significant effect on SG (**Error! Reference source not found.**). As shown in

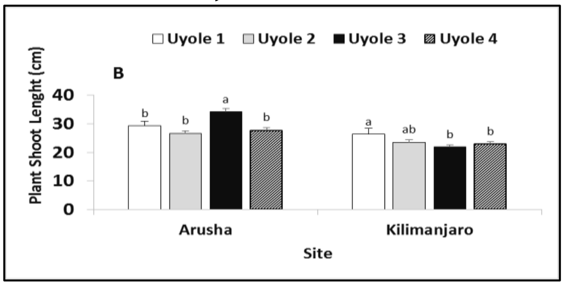

Fig. , the interactive effect between soybean varieties and site caused higher (0.54g) SG in Uyole 2 than other varieties at Kilimanjaro while at Arusha, site and variety interaction showed no significant differences.

Table 4. Stem girth (SG,cm plant^{-1}), shoot length (SL,cm plant^{-1}), leaf area (LA,cm^{2} plant^{-1}), root dry weight (RDW, g plant^{-1}) and shoot dry weight (SDW, g plant^{-1}) of soybean varieties as affected by inoculation and supplementation with N and P fertilization.

Treatment	SG	PSL	LA	RDW	SDW
Site Arusha	0.64±0.01^{a}	29.45±0.69^{a}	50.17±1.96^{a}	0.9±0.04^{a}	10±0.47^{a}
Kilimanjaro	0.45±0.02^{b}	23.63±0.66^{b}	27.73±0.80^{b}	0.4±0.02^{b}	2.25±0.13^{b}
Fertilisation					
Control	0.57±0.05^{a}	26.74±1.41^{a}	38.3±3.60^{abc}	0.67±0.08^{a}	5.76±0.98^{a}
Rhizobium	0.55±0.03^{a}	27.29±1.15^{a}	44.23±3.85^{a}	0.67±0.07^{a}	7.14±1.11^{a}
Nitrogen	0.54±0.03^{a}	26.48±1.24^{a}	36.56±3.15^{bc}	0.66±0.08^{a}	6.22±1.01^{a}
Phosphorus	0.51±0.02^{a}	24.72±1.07^{a}	33.46±2.67c	0.57±0.07^{a}	5±0.85^{a}
Rhizobium + phosphorus	0.55±0.02^{a}	27.46±1.21^{a}	42.18±2.85^{ab}	0.68±0.06^{a}	6.52±0.85^{a}
Variety					
Uyole 1	0.52±0.02^{a}	27.78±1.35^{a}	40.12±3.58^{a}	0.55±0.06^{b}	6.13±0.90^{a}
Uyole 2	0.58±0.04^{a}	25.08±0.66^{b}	40.89±2.76^{a}	0.62±0.05^{b}	5.67±0.74^{a}
Uyole 3	0.55±0.03^{a}	27.97±1.34^{a}	38.36±2.55^{a}	0.75±0.06^{a}	6.58±0.88^{a}
Uyole 4	0.53±0.02^{a}	25.33±0.76^{b}	36.43±2.88^{a}	0.67±0.06^{ab}	6.12±0.95^{a}
3-Way ANOVA F-statistics					
Site	68.80***	55.68***	113.87***	186.87***	259.43***
Fertilisation	0.69^{ns}	1.57^{ns}	3.38*	1.24ns	2.24^{ns}
Variety	1.21^{ns}	3.93*	0.89^{ns}	5.29**	0.59^{ns}
Site*Fertilisation	1.34^{ns}	2.26^{ns}	0.75^{ns}	1.17^{ns}	0.91^{ns}
Site*Variety	3.20*	8.06***	0.37^{ns}	1.34^{ns}	0.66^{ns}
Fertiliser*Variety	1.02^{ns}	1.69^{ns}	0.41^{ns}	1.24^{ns}	0.89^{ns}
Site*Fertilisation*Variety	1.33^{ns}	0.44^{ns}	0.60^{ns}	1.17^{ns}	0.66^{ns}

Values presented are means ± SE, n=12. *; **; *** is significant at P<0.05, P<0.01, P<0.001 respectively, ns = not significant, SE = standard error. Means of three replicates and when followed by a similar letter in a column are not significantly different (P=0.05, Fischer LSD).

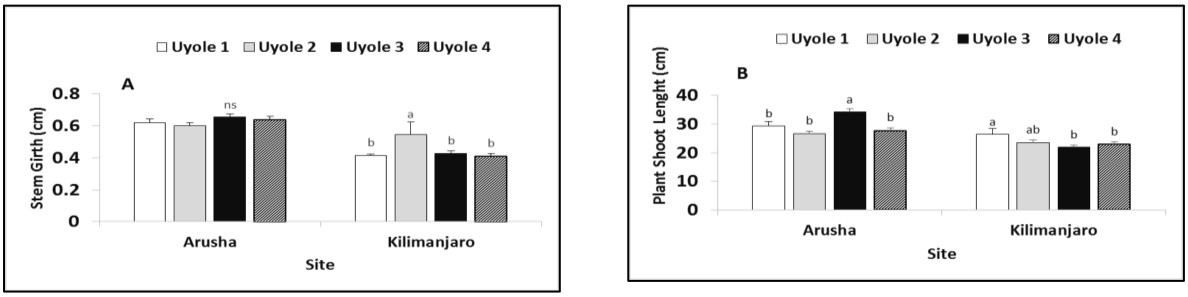

Fig. 3. Interactive effects of soybean variety and site on stem girth (A) and shoot length (B).

Plant shoot length

Soybean that was grown at Arusha site significantly grew taller (29.45cm) than the one grown at Kilimanjaro (23.63cm). Inoculated, N and P treated plants did not show any significant variations in their plant shoot length (PSL). Despite showing a non-significant variation between inoculated and fertilised treatments, inoculated plants had numerically higher PSL compared with un-inoculated treatments. Soybean varieties significantly (P<0.05) affected the length of shoots (**Error! Reference source not found.**). Shoot lengths of Uyole 3 (27.97cm) and Uyole 1 (27.78cm) were significantly at par as it was between Uyole 4 (25.33cm) and Uyole 2 (25.08cm) and vice versa. There was a significant interactions between soybean varieties and site. Uyole 3 interacted well with the agro-conditions at Arusha site and at Kilimanjaro it was Uyole 1 that had a better interaction with the site conditions than other varieties (

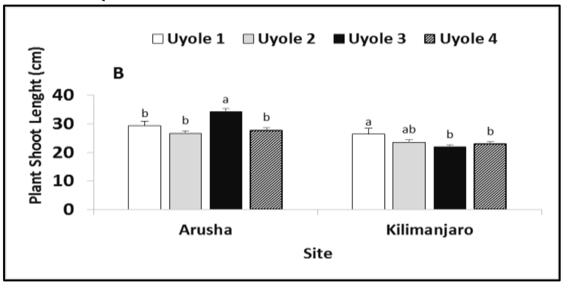

Fig.).

Leaf area

Leaf area (LA) at Arusha was significantly higher (50.17cm²) relative to Kilimanjaro (27.73cm²). Fertilisation had a significant effect on LA (**Error! Reference source not found.**). P treatment did not significantly differ from N and control treatments, and the latter was also at par with the sole Bradyrhizobium and Bradyrhizobium + P treatment. However, sole Bradyrhizobium increased LA with 15.48% over the control treatment (**Error! Reference source not found.**). On average, soybean treated with sole inoculation produced slightly higher (44.23cm²) compared with the combination of inoculation + P (42.18cm²) and control (38.3cm²) significantly performed better than P (33.46cm²) treatment.

Root dry weight

Root dry weight (RDW) per plant significantly (P<0.001) differed between sites, in which higher (0.94 g) RDW values were recorded at Arusha compared with Kilimanjaro (0.4) site (**Error! Reference source not found.**). The response of plants to inoculation and application of fertilisers showed no significant effect on RDW. Soybean varieties significantly (P<0.01) affected RDW. There were no significant variations in RDW between Uyole 1, Uyole 2 and Uyole 4. However, the RDW of Uyole 4 (0.67g) did not significantly differ from the highest (0.75g) RDW of Uyole 3. The interactions between all factors on RDW showed no significant differences.

Shoot Dry Weight

Shoot dry weight (SDW) of soybean grown at Arusha (10g) increased more than four times to that of Kilimanjaro (2.25 g) site. This was the biggest increase in the studied growth parameters compared with the ones already presented. Soybean varieties and factor interaction had no significant effect on SDW.

Discussion

Effect of Bradyrhizobium inoculation and supplementation with N and P fertilisation on nodulation parameters of selected soybean varieties

Symbiotic nitrogen fixation require large amounts of phosphorus (Schulze *et al.*, 2006). Bradyrhizobium inoculation + P supplementation increased all nodulation parameters, nodule number (NN), nodule dry weight (NDW) and nodule effectiveness (NEF) as shown in Fig. 2 and **Error! Reference source not found.**. A similar study conducted in Benin on inoculation and P supplementation by Zoundji *et al.* (2015) found similar results as ours. Sole Bradyrhizobium inoculation also increased nodulation parameters as similarly reported by Njira *et al.* (2013) and Chibeba *et al.* (2018). However, in this study it was found that inoculation supplemented with phosphorus enhanced nodulation parameters results which are similar to *Fatima et al.* (2007) and Tarekegn & Kibret, (2017) findings. In very few cases, nodules were observed in control plots (**Error! Reference source not found.**) and this could be as a result of presence of native rhizobia species as also reported by Abaidoo *et al.* (2007) and Mathu *et al.* (2012) and they can cause nodules to develop in non-promiscuous soybean (Zoundji *et al.*, 2015). Soybean varieties significantly contributed to nodule effectiveness and nodule dry weight. Several authors, Salvucci *et al.* (2012), Alam *et al.* (2015) and N'Zi *et al.* (2016) reported that soybean genotypes affected nodulation parameters which concurs with the findings in this study. The interactions between inoculation (supplemented with N and P), soybean variety and site indicated dependency between the factors on increasing nodule dry weight. As shown in **Error! Reference source not found.**, sole inoculation and inoculation plus P supplementation resulted in more nodule dry weight in variety of Uyole 4 at the Arusha site than in other treatments.

Effect of Bradyrhizobium inoculation and supplementation with N and P fertilisation on chlorophyll accumulation in selected soybean varieties

Generally, plants treated with rhizobia accumulated higher Chl in their leaves relative to control treatment. Total Chl levels (mg L^{-1}) were slightly elevated when sole inoculation (18.11) was augmented by P supplementation (18.54). Since Chl is a vital mechanism of plant physiology as it aids in photosynthesis (Handa *et al.*, 2016), then supplementing soybean inoculation with P can enhance the crop's physiology. The slight Increase in Chl content in inoculated plus P supplemented plants could be also as a result of increased N uptake via biological N uptake. In a study where inoculation was supplemented with P, Tairo & Ndakidemi (2013) found that both inoculation and P increased total Chl in soybean plants, and Shen *et al.* (2019) reported that inoculation and N application increased Chl content, root length and shoot biomass in leguminous *M. sativa*. Legumes require both P and N for successful symbiotic N fixation (Saxena & Rewari, 1991; Graham & Vance, 2000) and similarly, the nutrients are crucial to the photosynthesis process (Marschner, 2002; Taiz & Zeiger, 2010; Croft *et al.*, 2017). The content of photosynthetic pigments, can vary depending on (i) species (Sumanta *et al.*, 2014); (ii) internal factors and; (iii) environment conditions (Wolf, 1958; Jolliffe & Tregunna, 1968; Porra, 1991; Vicaş *et al.*, 2010). In this study, variations in total Chl across treatments was significant ($P<0.05$) probably, due to environmental conditions, inoculation and supplementation with N and P which highly increased the photosynthetic pigments in such treatments compared with control treatment.

Effect of Bradyrhizobium inoculation and supplementation with N and P fertilisation on growth characteristics of selected soybean varieties

In this study, all evaluated growth parameters changed between sites which can be attributed to slight variation in rainfall. Despite that Kilimanjaro had higher total N, P and pH (**Error! Reference source not found.**), the rainfall pattern from sowing date to mid flowering was not reliable which was the opposite at Arusha site. Hamisi (2013) also reported that high rainfall variability in Kilimanjaro has made rains in the region least reliable. As shown in

Fig. **1**, Arusha received higher rains compared with Kilimanjaro. Moisture content affects mobility of nutrients such as potassium and root growth (Kuchenbuch *et al.*, 1986). Lack of enough soil moisture during early soybean development at Kilimanjaro site could be factor for lower growth characters compared with Arusha site.

Lower root lengths and dry biomass accumulation have been reported in many soybean accessions under water stress conditions (Thu *et al.*, 2014). Roots with great total surface area enhance maximal moisture and nutrient extraction which maintains photosynthesis (Blum, 2011; Lopes *et al.*, 2011; Comas *et al.*, 2013) and water stress in soybean reduces photosynthesis (Ribas-Carbo *et al.*, 2005) and yield (Sionit & Kramer, 1977).

There were minimal leaf area (LA) variations between inoculated, N and P supplemented treatments (**Error! Reference source not found.**). Plant leaf area response to inoculation, and supplementation with N and P was ideal under sole rhizobium inoculation but such performance did not differ from the combination of inoculation and P supplementation and control treatment results. A study on soybean response to inoculation and P supplementation by Tairo & Ndakidemi, 2013 found an increase in LA values. Similarly, Nyoki & Ndakidemi (2014) found that P supplementation increased LA values of field grown cowpea which is in coherent with Zhang *et al.* (2017) finding, that P can facilitate in plant growth. P deficiency also caused lower leaf area in wheat (Rodríguez *et al.*, 1998). However, leaf expansion has also been assumed to be affected by water-related conditions (Hsiao, 1973), which could also be the reason for lower LA values at Kilimanjaro study site relative to Arusha as the latter received considerably enough rainfall to support soybean growth.

Soybean varieties differed in plant shoot length (PSL) and root dry weight (RDW). Uyole 1 and Uyole 3 grew taller than Uyole 2 and Uyole 4. PSL response of the varieties also indicated a significance interaction with the environment (

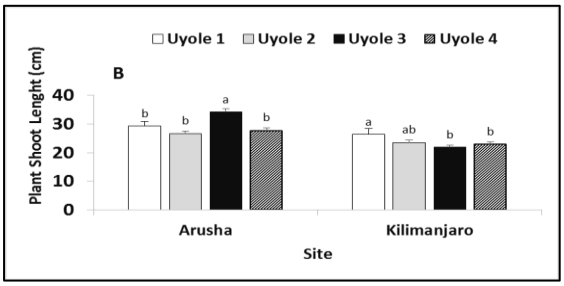

Fig.), and Uyole 3 grew than other varieties at Arusha site. Among the grown varieties, Uyole 3 also produced the highest shoot dry weight (SDW). This shows that nutrient uptake, assimilation, translocation, and remobilization (when ageing) in Uyole 3 was efficient than other varieties. Better nutrient acquisition and utilisation improves plant productivity and selecting genotypes with improved nutrient uptake and use capacity is important (Clark, 1990). Soybean varieties show different characteristics and responses to treatments. Solomon *et al.* (2012) found variations in performance of soybean varieties treated with different strains of bacteria and similar observations have also been reported in varieties of cereal crops (Abera *et al.*, 2017).

Conclusion

The response of soybean when inoculated with *B. japonicum* and supplemented with N and P of suggests that the dependency on synthetic fertiliser can be reduced, especially for those that cause environmental damage. It is rational to utilise *Bradyrhizobium japonicum* inoculation in soybean which can eliminate the costs associated with use of synthetic fertilisers.

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