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Growth response of maize (*Zea mays*) intercropped with *Rhizobium* inoculated soybean (*Glycine max* (L.) Merr.) and P and K fertilization

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Abstract

The research was carried out for the two consecutive cropping seasons in northern Tanzania to evaluate the effects of cropping systems and P and K fertilization on maize growth. A split-split plot design experiment with 2x4x7 factorial arrangements and replicated thrice was conducted. The main plots comprised of two rhizobia inoculation treatments on Soybean, the sub plots comprising sole maize (SM) at a spacing of 75x60cm; sole soybean at a spacing of 75x40cm; maize-soybean intercropped at 75x60 and 75x20cm, maize and soybean respectively; and the last cropping system was maize-soybean intercropped at 75x60 and 75x40cm, maize and soybean respectively. The fertilizer levels: control; 20K; 40K; 26P; 52P; 26P+20K; 52P+40K (kg ha^{-1}) were assigned to sub-subplots. The results indicated that both cropping systems and P and K fertilization improved maize growth for the two cropping seasons. The plant height was significantly higher in M+B(A)+R and M+B(B)+R. The stem girth, plant vigor and greenness were statistically similar in sole maize (SM), M+B(A)+R and M+B(B)+R for both seasons. Any level of P and K significantly increased all growth attributes measured. The 40K+52P (kg ha^{-1}) performed better than all other fertilizers treatments. Therefore, intercropping maize with *Rhizobium* inoculated soybean at wider spacing and supplemented with P and K applied at higher rate of 40K+52P (kg ha^{-1}) will result in improved plant growth and hence final yield.

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Introduction

Maize (*Zea mays* L.) is the most important grain crop being grown in different parts of world and is produced under diverse environments. In sub-Saharan Africa (SSA), maize is the most important cereal crop and staple food for about 1.2 billion people (IITA, 2009) and occupy a third of the cultivated area (Blackie, 1990). Tanzania is one of the major maize producers in the world, ranked 1, 5, and 18 top maize producing countries in East Africa (EA), Africa and in the world respectively (FAOSTAT, 2015; United States Department of Agriculture, 2016). Successful maize production depends on the correct application of production inputs and correct agronomic practices that will sustain the environment as well as agricultural production (du Plessis, 2003). These includes adapted and improved cultivars; plant population; soil tillage; fertilisation; weed, insect and disease control and harvesting (du Plessis, 2003). Management of these inputs results in a better growth performance of crops which eventually results in higher crop yield performance. Among other factors, soil fertility is the most critical input for crop growth, development and production. In many regions of East Africa, soils have negative balances of nutrients such as NPK affecting crop production (Bekunda *et al.*, 2004). Growing crops by intercropping cereals and legumes may also influence the growth of respective crop and improve yields (Carr *et al.*, 2004; Dusa and Stan, 2013). The main parameter of plant growth is its height which can be measured at different growth stages of plant or at the end (at physiological growth). Several studies have shown different growth responses as a result of different treatments applied to crop plants. The varied crop growth following intercropping or cropping patterns have been reported by several researchers (Lemlem 2013; Hirpa 2013; Hirpa 2014; Nyoki and Ndakidemi 2017). Availability or deficiency of soil nutrients such as N, P and K is another factor affecting plant growth. Availability of these nutrients for plants will improve plant growth and finally increase yield, while their deficiency will result in poorly developed crops there by reducing the final yields.

Each of these elements has important function(s) in plant growth and development. For example, in plant cell, phosphorus plays role in a variety of plant functions such as energy transfer, photosynthesis, translocation of sugars and starches as well as movement of nutrients within the plant (Brady, 2002; Shahid *et al.*, 2009). Plants physiological processes such as activation of several enzymes, synthesis and degradation of carbohydrates, production of proteins and regulation of stomata pores for gas exchange and photosynthesis are regulated in presence of potassium (Lissbrant *et al.*, 2009). Currently, there is little information on the growth response of maize intercropped with *Rhizobium* inoculated soybean, supplemented with P and K fertilizers. Therefore, the current study was carried out to assess the growth response of maize intercropped with *Rhizobium* inoculated soybean, and fertilized with different levels of P and K applied singly and combined.

Materials and methods

Experimental Design and Treatments

The field experiment was carried out at Tanzania Coffee Research Institute (TaCRI) farm for two consecutive years (2015 and 2016). The experiment was laid out in split-split plot design with 2x4x7 factorial arrangements and replicated thrice. The plot size was 3x3m, with main plots having two rhizobia inoculation treatments, while the sub plots comprised: Maize pure stand at a spacing of 75x60cm; Soybean pure stand at a spacing of 75x40cm; Maize-soybean intercropped at a spacing of 75x60cm and 75x20cm, maize and soybean respectively; and the last cropping system was Maize-soybean intercropped at a spacing of 75x60cm and 75x40cm, Maize and soybean respectively. The sub-subplots were assigned the following fertilizer levels (kg ha^{-1}): control; 20K; 40K; 26P; 52P; 26P + 20K; 52P + 40K.

Data collection

Growth and development parameters of maize that were measured includes: plant eight (H), number of leaves per plant, stem girth (SG). The plant height was measured at different growth stages using a meter ruler, while stem girth (cm) was measured with veneer caliper. This was done three times at 2 weeks interval during the growing period of the crops.

Statistical analysis

The collected data was analyzed using statistical software called STATISTICA. The statistical analysis was performed using the 3-way analysis of variance (ANOVA) in factorial arrangement. The fisher's least significance difference (L.S.D.) was used to compare treatment means at p = 0.05 level of significance (Steel and Torrie, 1980).

Results

Plant height

The results of the current study indicated that the cropping systems had significant effect on the plant height where the intercropped maize appeared taller than pure stand maize.

For the two cropping season and across the growth stages, pure stand maize were significantly shorter than the intercropped ones. The data recorded from maize that were intercropped with *Rhizobium* inoculated soybean showed that maize were significantly taller than those intercropped with un-inoculated soybean and the pure stand maize for the two cropping seasons. Although there were slight differences of plant height with regard to the spacing, the differences were not significant (Table 1). The results of this study also showed that fertilizers had significant effects on plant height. When compared with the control (unfertilized plots), all other fertilizer treated plots significantly improved the maize height for both cropping season. The data recorded at different growth stages showed that whether applied singly or combined, at lower rates or doubled rates, P and K fertilizers improved plant height over the control for the two cropping seasons (Table 1).

Table 1. Plant height measured at different growth stages of maize as affected by cropping systems and P and K fertilizers for the two cropping seasons.

	Mean plant height (cm) 2015 cropping season			Mean plant height (cm) 2016 cropping season		
	2 WAP	4 WAP	6 WAP	2 WAP	4 WAP	6 WAP
SM	16.24±0.51d	28.78±1.09a	67.32±2.33b	20.70±0.64b	38.06±0.94c	145.89±5.73c
M+B(A)-R	17.92±0.37c	28.94±0.64a	66.13±2.14b	21.75±0.91b	40.62±1.21b	155.95±4.54ab
M+B(B)-R	18.40±0.50bc	29.49±0.79a	68.17±1.93b	21.83±0.76b	39.22±1.42bc	149.86±3.06bc
M+B(A)+R	19.19±0.54ab	29.05±0.83a	69.30±2.60ab	23.48±0.62a	43.11±1.27a	154.05±4.17ab
M+B(B)+R	19.32±0.50a	31.11±1.12a	72.27±2.20a	24.21±0.80a	42.98±1.15a	157.24±5.16a
Fertilizer (kg ha⁻¹)						
0	15.38±0.40f	25.09±0.82e	54.80±1.38e	18.24±0.70e	33.49±1.02f	121.02±4.69f
20K	16.51±0.40e	27.22±0.80de	60.73±1.20d	20.07±0.56d	36.22±0.91e	137.76±3.13e
40K	17.73±0.32d	28.29±0.78cd	65.18±1.49c	21.47±0.59cd	38.82±0.75d	147.24±2.68d
26P	18.11±0.43cd	29.31±0.92cd	68.53±1.43bc	21.89±0.58bc	40.53±0.62d	155.93±2.38c
52P	19.07±0.39bc	30.29±0.85bc	72.00±1.33b	23.33±0.74b	42.64±0.54c	160.87±2.04bc
20K+26P	19.87±0.60ab	32.18±0.79ab	78.40±1.31a	25.18±0.70a	45.13±0.89b	166.47±2.29b
40K+52P	20.82±0.59a	33.93±0.87a	80.82±2.12a	26.56±0.65a	48.76±1.02a	178.89±3.28a
F-Statistics						
CropSyst	15.43***	1.51 ns	3.45**	7.40***	16.25***	3.52*
Fert	25.44***	10.63***	39.07***	21.69***	63.03***	42.33***
CropSyst*Fert	0.59ns	0.21 ns	0.60 ns	0.329ns	1.01 ns	0.84 ns

CroSyt: Cropping Systems; Fert: Fertilizers; SM: Sole maize; WAP: Weeks after Planting; M+B(A): Maize/soybean intercropped at a spacing of 75x60cm and 75x20cm, maize and soybean respectively; M+B (B): Maize/soybean intercropped at a spacing of 75x60cm and 75x40cm, maize and soybean respectively; –R and +R un-inoculated and inoculated soybean respectively; Values presented are means ± SE; *,**, ***: significant at $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$ respectively, ns = not significant, SE = standard error. Means followed by dissimilar letter(s) in a column are significantly different from each other at p=0.05 according to Fischer least significance difference (LSD).

Number of leaves

The results presented in (Table 2) showed that for the first cropping season, both cropping systems and fertilizers had no significant effect on the number of leaves. In the second cropping season, data recorded from 4 Weeks after Plant (4 WAP) and 6 Weeks after Plant (6 WAP) indicated that P and K significantly increased the number of leaves over the control. All levels of fertilizers significantly increased the number of leave compared with unfertilized plots. However, the highest number of leaves was recorded in plots treated with (kg ha^{-1}) 52P, 20K +26P, and 40K + 52P (Table 2)

Table 2. Mean number of leaves counted at different growth stages of maize as affected by cropping systems and P and K fertilizers for the two cropping seasons.

	Mean number of Leaves 2015 cropping season			Mean number of Leaves 2016 cropping season		
	2 WAP	4 WAP	6 WAP	2 WAP	4 WAP	6 WAP
SM	5.56±0.08a	7.30±0.14a	11.05±0.19a	6.48±0.15a	9.97±0.23a	13.11±0.13a
M+B(A)-R	5.75±0.09a	7.43±0.07a	11.46±0.21a	6.76±0.09a	10.27±0.20a	12.83±0.17a
M+B(B)-R	5.56±0.07a	7.47±0.11a	11.19±0.16a	6.43±0.09a	9.75±0.13a	12.98±0.17a
M+B(A)+R	5.63±0.08a	7.56±0.11a	11.22±0.17a	6.51±0.10a	10.09±0.17a	12.97±0.22a
M+B(B)+R	5.65±0.07a	7.70±0.13a	11.40±0.19a	6.56±0.08a	10.27±0.18a	13.06±0.19a
Fertilizer (kg ha^{-1})						
0	5.51±0.10a	7.38±0.14a	11.02±0.32a	6.27±0.12a	9.27±0.19d	12.18±0.22c
20K	5.69±0.09a	7.51±0.10a	10.93±0.19a	6.47±0.09a	9.84±0.17c	12.73±0.23b
40K	5.56±0.06a	7.44±0.10a	11.24±0.17a	6.44±0.09a	9.96±0.19bc	13.22±0.16ab
26P	5.71±0.11a	7.44±0.14a	11.49±0.24a	6.56±0.11a	10.43±0.25ab	12.73±0.24b
52P	5.76±0.07a	7.67±0.10a	11.67±0.18a	6.73±0.15a	10.16±0.23abc	13.38±0.13a
20K+26P	5.64±0.12a	7.43±0.18a	11.24±0.20a	6.64±0.13a	10.27±0.16abc	13.29±0.14a
40K+52P	5.53±0.10a	7.56±0.18a	11.24±0.18a	6.71±0.16a	10.56±0.19a	13.40±0.13a
F-Statistics						
CropSyst	0.93 ns	1.76 ns	0.69 ns	1.51 ns	1.78 ns	0.50 ns
Fert	0.98 ns	0.53 ns	1.14 ns	1.79 ns	4.87***	6.29***
CropSyst*Fert	0.78 ns	1.34 ns	0.39 ns	0.80	0.99 ns	1.18ns

CroSyt: Cropping Systems; Fert: Fertilizers; SM: Sole maize; WAP: Weeks after Planting; M+B(A): Maize/soybean intercropped at a spacing of 75x60cm and 75x20cm, maize and soybean respectively; M+B (B): Maize/soybean intercropped at a spacing of 75x60cm and 75x40cm, maize and soybean respectively; –R and +R un-inoculated and inoculated soybean respectively; Values presented are means ± SE; ***: significant at p ≤ 0.001 respectively, ns = not significant, SE = standard error. Means followed by dissimilar letter(s) in a column are significantly different from each other at p=0.05 according to Fischer least significance difference (LSD).

Stem Girth

The results of the current study showed that the cropping systems had significant effects on the maize stem girth. The maize pure stand and maize intercropped with inoculated soybean planted at wider spacing had significantly higher stem girth compared with other cropping systems for the two cropping seasons (Table 3). Phosphorus and potassium also significantly increased the maize stem girth over the control (Table 3).

The stem girths were increasing with the increasing fertilizer levels and this trend was observed in the two cropping seasons. It was generally observed that the combined doubled P and K resulted in greater stem girth relative to other treatments.

Plant vigor and Greenness

The cropping systems and P and K fertilization had significant effects on plant vigor and greenness for the two cropping seasons. For the two cropping seasons, maize pure stand (SM) and maize intercropped with inoculated soybean planted at wider spacing (M+B(B)+R) significantly improved the plant vigor over the rest of the cropping systems used in this study (Table 3).

In the 2015 cropping season, maize intercropped with inoculated soybean planted at wider spacing (M+B(B)+R) significantly improved the greenness of the plants. However, this was statistically the same with maize intercropped with inoculated soybean planted at narrower spacing (M+B(A)+R) and the maize grown as pure stand (SM).

Table 3. Stem girth, vigor and greenness of plant as affected by cropping systems and P and K fertilizers for the two cropping seasons.

Cropping Systems	2015 cropping season			2016 cropping season		
	Stem Girth (mm)	Plant Vigor	Greenness	Stem Girth (mm)	Plant Vigor	Greenness
SM	13.51±0.25a	2.00±0.21b	2.02±0.21b	14.76±0.32a	1.79±0.19c	1.76±0.19c
M+B(A)-R	12.89±0.31bc	2.55±0.23a	2.48±0.25a	13.98±0.41b	2.25±0.28a	2.37±0.29a
M+B(B)-R	12.74±0.35c	2.12±0.24b	2.12±0.22ab	13.57±0.39b	2.14±0.25ab	2.14±0.22ab
M+B(A)+R	13.35±0.27ab	2.07±0.20b	2.00±0.21b	13.81±0.27b	2.11±0.26ab	2.11±0.26ab
M+B(B)+R	13.42±0.30ab	2.00±0.21b	1.91±0.21b	14.62±0.37a	1.98±0.21bc	2.03±0.23bc
Fertilizer (kg ha⁻¹)						
0	11.55±0.21d	3.53±0.13a	3.63±0.12a	11.84±0.34e	3.71±0.14a	3.81±0.16a
20K	12.19±0.23cd	3.00±0.13b	2.87±0.12b	13.19±0.28d	3.22±0.10b	3.12±0.13b
40K	12.86±0.21bc	2.40±0.16c	2.23±0.19c	14.01±0.27c	2.30±0.15c	2.27±0.17c
26P	13.13±0.25b	2.07±0.17c	1.97±0.19cd	14.03±0.22c	1.77±0.17d	1.77±0.22d
52P	13.45±0.27b	1.57±0.16d	1.53±0.17de	14.54±0.29bc	1.30±0.11e	1.37±0.11e
20K+26P	14.23±0.21a	1.27±0.15d	1.20±0.15e	15.24±0.23b	1.07±0.05ef	1.13±0.08e
40K+52P	14.87±0.26a	1.20±0.14d	1.30±0.15e	16.19±0.30a	1.00±0.00f	1.11±0.07e
F-Statistics						
CropSyst	2.86*	3.11*	2.48*	5.19***	4.22**	3.60**
Fert	22.40**	33.32***	28.64**	27.17***	109.74***	59.12***
CropSyst*Fert	0.45	0.50 ns	0.36ns	0.63 ns	1.64 ns	0.97 ns

CroSyt: Cropping Systems; Fert: Fertilizers; SM: Sole maize; WAP: Weeks after Planting; M+B(A): Maize/soybean intercropped at a spacing of75x60cm and 75x20cm, maize and soybean respectively; M+B (B): Maize/soybean intercropped at a spacing of 75x60cm and 75x40cm, maize and soybean respectively; –R and +R un-inoculated and inoculated soybean respectively; Values presented are means ± SE; *,**, ***: significant at $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$ respectively, ns = not significant, SE = standard error. Means followed by dissimilar letter(s) in a column are significantly different from each other at p=0.05 according to Fischer least significance difference (LSD). Note: plant vigor and Greenness was assessed in a scale of 1-5. 1=Good; 5= Bad.

In the second cropping season (2016), maize grown as pure stand (SM) and maize intercropped with inoculated soybean planted at wider spacing (M+B(B)+R) had significantly greener maize plants over all other cropping systems (Table 3). On the other hand, plant vigor and greenness were strongly and significantly improved in the plots treated with both P and K over the control. Of all the treatment, 52P, 20K+26P, 40K+52P (kg ha^{-1}) had excellent plant vigor and the greenness for the two cropping seasons (Table 3).

Discussion

In the current study we examined the effects of cropping systems and P and K fertilization on growth parameters of maize intercropped with inoculated and un-inoculated soybean. The results showed that both cropping systems and fertilizers had significant effects on almost all growth parameters such plant height; number of leaves; stem girth; plant vigor and greenness of plant leaves. Intercropping maize with inoculated soybean resulted in taller maize that those intercropped with un-inoculated soybean and those under the pure stand.

The reason for that might be due to the effects of competition for light because inoculated soybean grew quicker and pose enough competition to maize in early weeks of plant growth leading to increased plant height. When soybean stopped growing the other factor that may have contributed to increased plant height in maize is the effects nitrogen fixed from legume component of intercrop. This argument is supported by many researchers who reported the presence of direct nitrogen transfer from legumes to cereals in intercropping leading to improved growth of both crop components (Giller and Wilson, 1991; Giller *et al.*, 1991; Shen and Chu, 2004). Maize intercropped with un-inoculated soybean were relatively shorter than those under *Rhizobium* inoculated soybean because there was no strong competition as un-inoculated soybean could not strongly compete for light with maize, and there was no additional nitrogen from legumes. Maize grown as pure stand were relatively of the same height with those intercropped with un-inoculated soybean except those recorded 2 WAP in the first cropping season and 4 and 6 WAP in the second cropping season which were shorter than those under intercropping. Our findings do not agree with those found by Ndiso *et al.* (2017) who reported that the height of both cowpea and maize were shorter in intercropping than those under respective pure stand crops. They argued that their results could have been attributed by competition for resources among the component crops. In our study cropping systems did not have significant effect on the number of leaves of maize plant for the two cropping seasons. However, there were significant effects of the cropping systems on the maize stem girth, plant vigor and greenness. For the two cropping seasons, the maize pure stand and maize intercropped with inoculated soybean at wider spacing resulted in improved and greater stem girth over the maize intercropped with inoculated soybean at narrower spacing and un-inoculated soybean. These results could have been attributed by lack of completion in maize sole crop. The improved maize stem girth in maize intercropped with inoculated soybean at wider spacing could have been attributed by less competition due to enough spacing and also the fixed nitrogen (data not presented in this paper) from soybean (Karim *et al.*, 1993 Oliveira *et al.*, 2016). Plant vigor and greenness was significantly influenced by cropping systems where maize plants were greener and grew vigorously in plots that had maize intercropped with soybean at wider spacing compared with other treatment. This could have been contributed by the fixed nitrogen which improved the plant vigor and greenness over other treatments. Although intercropping at narrower spacing (M+B(A)+R) also had fixed nitrogen, but the was strong completion which led to thinner maize stem girth.

On the other hand, phosphorus and potassium fertilization also showed a significant effect on plant growth parameters measured in this study. The plant height of P and K fertilized maize was significantly higher compared with the unfertilized maize, indicating the needs of these elements in plant growth. The number of leaves was also increased in fertilized plots relative to the control plots. Maize stem girth, plant vigor and greenness were also improved following P and K fertilization.

Generally, any level of P and K has significantly contributed to the growth performance of the crop. Similar to our findings Yilmaz (2008) reported that there was a significant increase in plant height following fertilization with higher (75kg ha^{-1}) rate of phosphorus on Narbon Vetch. Phosphorus is an essential element required in large quantities in young cells, such as shoots and root tips, where metabolism is high and cell division is rapid making its availability to improve growth of plants (Uchida, 2000). Other function of phosphorus is root development. Well-developed roots can explore enough growth resources leading to the improved plant growth than in P deficient Plants (Brady, 2002; Shahid *et al.*, 2009). Apart from phosphorus, potassium also has contributed much in the improved growth of maize in this study because K is known to be an enzyme activator that promotes metabolism (Lissbrant *et al.*, 2009; Barragán *et al.*, 2012). Other functions of K is to promote the translocation of photosythates (sugars) for plant growth, K also assists in regulating the plant's use of water by controlling the opening and closing of leaf stomata (Talbott and Zeiger, 1996; Uchida, 2000; Lissbrant *et al.*, 2009; White and Karley, 2010; Andrés *et al.*, 2014).

It was observed in this study that fertilization with P and K at higher rates whether singly or combined significantly increased plant growth parameters than when they were applied at lower rates. The combined doubled fertilizers (40K+52P kg ha^{-1}) showed super performance in improving plant growth over all other fertilizers treatments for the two cropping seasons. Similar to our study, Mallarino *et al.* (1999) have reported the increased in early growth of corn following fertilization with P and K. In another study conducted by Zafar *et al.* (2011) it was reported that different sources of P applied along with plant Growth Promoting Rhizobacteria (PGPR) significantly increased morphological parameters of the plants in *P. vulgaris.* In this study, there were no significant interactions of cropping systems and fertilizer application

Conclusion

The results of this study have showed that both cropping systems and P and K fertilization has contributed much to the improved maize growth over the period of two cropping seasons. Plant height was significantly higher in plots that were intercropped maize with inoculated soybean compared with other treatments. It was generally observed that stem girth, plant vigor and greenness were found to be statistically similar in sole maize (SM) and maize intercropped with *Rhizobium* inoculated soybean at both narrower and wider spacing for the two cropping seasons. P and K fertilization significantly increased plant growth parameters such as plant height, number of leaves, stem girth, plant vigor and greenness over the control. The higher rates of these fertilizers significantly increased the plant growth parameters over the lower rates. Interestingly, their combined application significantly increased growth traits than when they were singly applied. When the combination of these fertilizers was doubled (40K+52P (kg ha^{-1})) they showed a super performance in improving plant growth over all other fertilizers treatments for the two cropping seasons. Therefore, based on these findings we recommend that maize should be intercropped with *Rhizobium* inoculated soybean at the recommended spacing and supplemented with combined P and K applied at higher rate of 40K+52P (kg ha^{-1}).

Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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