Original Research Article

Determination of the effects of aluminium application and *Rhizobia* inoculation on growth, yield and nutrients uptake of three Kenyan soy bean genotypes

Comment [AS1]: Aluminium and *Rhizobia* inoculation effects on growth, yield and nutrients uptake of Kenyan soy bean genotypes

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ABSTRACT

Soy bean (*Glycine max* L.) grains are important legume crops commonly grown in Kenya. Soy bean genotypes are grown in western Kenyan soils that are highly saturated with Aluminium ions. Aluminium toxicity majorly limits crop production. Many soy bean genotypes do not tolerate high acidity in soils. Aluminium stress have effects on root growth which limits plant growth and development. Inoculation of plants with *Rhizobia* can restore nitrogen under acid affected soils to produce competitive crop yields. There is little information on response of soy bean genotypes grown in Western Kenya to aluminium toxicity under rhizobium inoculation. This study was designed to determine the effect of aluminium application and *Rhizobia* inoculation on growth, yield and nutrients uptake of soy bean genotypes. Eight treatments of Al ($AlCl_3.6H_2O$) levels and *Rhizobia* were imposed. Randomized Complete Block Design with three replicates was used. Parameters determined included; number of branches, days to 50% flowering, pod clearance, number of pods, days to harvest maturity and nitrogen, phosphorus and potassium contents. Tukey's HSD test at 5% level was used to separate the means. Significant differences in growth and nutrients were observed in all the genotypes. These findings show that *Bradyrhizobium japonicum* inoculation alleviates Al effects to a level that is significant to improve soy bean yield. Therefore, genotypes GAZELLE and NAMSOI under *Rhizobia* inoculation were identified to be more tolerant to Al-stress, hence are recommended for growing in Al prone soils. It provides the best conditions in improving soy bean production under Al stress prone soils.

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Keywords: *Aluminium, Growth parameters, NPK concentration, Rhizobium inoculation, Secondary metabolites a*
beans,

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Comment [AS6]: Growth and yield

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1. INTRODUCTION

Soy bean (*Glycine max* L.) grains are the world's most important among legumes crops and commonly grown in (Ambe, 2019). Soy bean products have low levels of saturated fat and cholesterol-free (Lamptey *et al*., 2014) them highly nutritious. Between 1990 and 2000, Food and Agriculture Organization (FAO), German Agent for Technical cooperation (GTZ) and Kenya Agriculture and Livestock Research Organization (KALRO) promoted soy bean farming in western Kenya (Edna *et al*., 2019). However this was not successful, as the soy bean seeds used were farm sav poor quality (Edna *et al*. 2019).

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Aluminium ions have been considered as a major limiting factor in acidic soils. It affects about 40-70% of the worlds cultivated land (Dechessa *et al*., 2010), which has potential for soy bean crop production. Soy bean genotypes are grown in these areas hence may face a problem of Al stress. Edna *et al*. (2019) notes that compared to other countries such as USA, Al has led to low soy bean production in Kenya.

The challenge of the increasing production of soy bean is that, in Africa, soils are known to become exhausted due to over cultivation (Aliyu *et al*., 2013). This means, mineral fertilizers are needed to be applied, yet they are too expensive for the generally resource-poor farmers to afford quantities sufficient for sustainable agricultural intensification. *Rhizobia* inoculation may therefore, partly address the problem of soil fertility in acidic soils caused by Al. Inorganic fertilizers are expensive and out of reach for the smallholder farmers in western Kenya sub-counties. Therefore, cheaper biological means like the use of microorganisms such as *Rhizobia* should be used as a means to replenish soil fertility (Naomi, 2009).

Soil acidity disturbs and limits nitrogen fixing symbiosis (Edna *et al*., 2019). *Rhizobia* species have metabolic abilities to mitigate Al stress, hence inoculation with *Rhizobia* can restore nitrogen under acid affected soils to produce competitive crop yield (Hussain *et al*., 2002). Biological nitrogen fixations (BNF) contribute approximately 70 million tons of fixed N annually to agricultural lands (Sanjay *et al*., 2018). However, the amount of N fixed can vary between species, locations due to differences in soil factors, soy bean genotype and *Rhizobia* strain.

Aluminium toxicity is a hindrance to soy bean production. One of the approaches to reduce the effect of Al toxicity is inoculation of the seeds with *Rhizobia* before planting (Lamptey *et al*., 2014). Soy bean seeds are rich in proteins; therefore the plants require a large amount of nitrogen (N). Nitrogen accumulation in the soy bean shoots correlate positively to the soy bean seed yield as found by Aftab *et al*. (2010). Soy beans might suffer from nitrogen deficiency under field conditions (Ambe, 2019). For instance, at flowering when the nodules start to senescence or when seeds are either planted in soils without inoculation with proper symbiotic bacteria. In particular areas where soy bean has not been grown before (Aftab *et al*., 2010), there is no success in nodulation therefore low seed yield especially if not inoculated. Inoculation of soy beans seeds with appropriate strain of *Rhizobia* before planting therefore helped reduce the problem of low soil fertility. The bacteria is able to fix up to 300kg/ha atmospheric nitrogen that lead to increased grain and biomass yield (Stanislava *et al*., 2015). It can therefore alleviate low biomass and low grain production of soy bean plants in acidic soils caused by Al. Consequently, the problem of soil pollution, which arises from excessive use of nitrogen fertilizers may be solved (Naomi, 2009) as well as food insecurity.

Aluminium ions (Al^{3+}) in acidic soils bind to minerals and reduce their uptake (Miguel *et al*., 2013). This may cause infertility in acid soils, therefore, mineral element deficiency of N, phosphorous (P) and potassium (K). For instance, Al can lower P availability and block the normal uptake of Ca^{2+} and Mg^{2+} causing an imbalance in plant mineral nutrition (Igual *et al*., 1997). These effects are manifested in soy bean plants as non-efficient use of nutrients of the subsoil, because the plants have difficulties in root system formation (Dias *&* Bruggemann, 2010). According to Dias *&* Bruggemann (2010), the symptoms that are similar to those of water stressed plants are exhibited due to the same effects.

Plants that are not infected with *Rhizobia* might have had lower rates of nitogenese activity thus less photosynthetic activities. Enzyme activities may be lowered as nitrogen is rechanneled into tissues such as leaves, flowers and pods (Pedrol & Pilar, 2016). Therefore, inoculation meant amino acids and proteins remained available to decrease senescence as photosynthetic activity was maintained at this stage of remobilization (Duchessa *et al*., 2010). Inoculation done to soy beans, might have to some level reduced the effect of Al in reducing nitrate reductase enzyme and therefore delayed senescence (Emel *et al*., 2018).

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Compared to inorganic fertilizer use, there is low cost for this process, although it is under-utilized due to it`s poor understanding (Onyango *et al*., 2015). According to Dechassa *et al*. (2010) cellular response to Al toxicity involves synchronization of events ranging from induction of proteins and secondary metabolites that ultimately detoxify Al. Therefore, determining different levels of metabolites accumulation in soy bean genotypes under *Rhizobia* infections helped in understanding this phenomenon.

Mechanisms of Al toxicity and resistance are complex and have not yet been fully explained. Furthermore, there is a dath of information on tolerant and high yielding soy bean genotypes to Al stress under *Rhizobia* inoculation. Al ions in acidic soils binds to minerals and reduce their uptake that leads to NPK mineral deficiency in plants. Soy bean strains that can improve nodulation under Al are much yet to be identified. Adding *Rhizobia* bacteria to the soil can ameliorate the effects of aluminium stress. Poor yield in soy beans production due to Al toxicity due to reduction on growth parameters has a negative impact to food security. Therefore, there is need to ameliorate the effect of Al toxicity by *Rhizobia* inoculation. Little is documented on the effects of *Rhizobia* inoculation and Al stress on growth, Al and NPK concentrations and biochemistry of soy beans grown in Kenya. This will help meet food requirement for the ever increased population through improvement of grain yield, other desirable agronomic traits and phonological characters. The objective of our study was to determine the effect of aluminium application and *Rhizobia* inoculation on growth, aluminium concentration, NPK nutrients and secondary metabolites concentration of soy bean genotypes.

2. MATERIAL AND METHODS

2.0 Experimental site

Research was carried out within greenhouse at Maseno University Research Farm between August 2021 and December 2022. The site is located approximately 1504m above sea level on Latitude and Longitude extents of 0^0 $1^/$ $0^{//}$S and 34^0 $36^/0^{//}$E respectively with a UTM position XE79 and a joint operation graphic reference SA36-04.

2.2 Planting procedure

The genotypes (SB 17 (TGx 1871-12E), SB 19 (NAMSOI) and SB 72 (GAZELLE)) were obtained from farmers linked to the Consortium of International Agricultural Centers (CGIAR) station at Maseno. Land was sampled and soils collected from Maseno University farm in consideration to Keino (2015). Soils were characterized according to Kiflu *et al*. (2016) and the results were recorded in Table 1 below.

2.3 Inoculation of seeds

Soy bean seeds were sterilized and spread in water-agar plates then incubated in the darkness for germination as done by Gicharu *et al*. (2013). Sterile disposable pipette tip was used to inoculate 1ml (approximately contained 10^9 cells mL^{-1}) of pure isolate *Bradyrhizobium japonicum* (USDA-Rhizobia) culture suspension directly around seedling hypocotyl at a recommended rate of 10g per kg of seed (Ajeigbe *et al*., 2010). Thereafter, the inoculated surface was covered with steam sterilized sand to inhibit conformation.

2.4 Planting and experimental design

Twenty litre PVC pots were filled with soil then ten seeds were planted per pot. Fertilization was done as recommended in soy bean legume plants by Gicharu *et al*. (2013). Randomized Complete Block Design with three replicates was used. Interactive treatments comprised of Control (Water)*Inoculated, 480µM Al*Inoculated, 750µM Al*Inoculated and 960µM Al*Inoculated, Control (Water), 480µM Al, 750µM Al and 960µM Al which are T1, T2, T3, T4, T5, T6, T7 and T8 respectively.

Table 1

Comment [AS12]: Experimental soil characteristics

Soil characters	Units	Result	Range low	Range high	L.	A.	H
pH (KCl)	pH	4.9	4.9	6.4		√	
Organic carbon	g.kg^{-1}	19	20	50	√		
Total nitrogen	g.kg^{-1}	1.9	1.0	2		√	
Total phosphorous	g.kg^{-1}	1.1	0.2	0.6			√
Total sulfur	g.kg^{-1}	0.3	0.3	0.5		√	
Potassium (exch.)	mmol.kg^{-1}	6.7	1.5	3			√
Calcium (exch.)	mmol.kg^{-1}	59.1	15	25			√
Magnesium (exch.)	mmol.kg^{-1}	15.5	4.5	10			√
Zinc (M3)	mg.kg^{-1}	3.4	2.5	4	√		
Copper (M3)	mg.kg^{-1}	4.5	1	2			√
Cation exchange capacity	mmol.kg^{-1}	91.9	75	200	√		
Clay	%	53.8	25	50			√
Sand	%	20.4	35	55	√		
Total Aluminium	g.kg^{-1}	100	56	91			√
Total potassium	g.kg^{-1}	4.7	9.8	22	√		
Total silicon	g.kg^{-1}	270.6	250	330		√	
Total iron	g.kg^{-1}	84	27	72			√
Phosphorous (M3)	mg.Pkg^{-1}	24.6	20	40	√		
Total manganese	g.kg^{-1}	4541	610	2300			√

Soil characteristics of Maseno. L., A. and H. indicate low, adequate and high content of parameter cited in the table 1.

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2.5 Determination of growth

Comment [AS14]: No number

2.5.1 Number of leaves

Leaves were counted and recorded.

2.5.2 Number of branches

Branches were counted for the three plants and the number recorded on average.

2.5.3 Days to 50% flowering

These were determined at a stage when 50% of the plants (5) were with at least one fully open flower.

2.5.4 Pod clearance

Pod clearance was the average number of three randomly selected plant heights (cm) from ground level to the lowest pods in a PVC pot.

2.5.5 Number of pods

Pods were counted for the three plants and the number recorded on average.

2.5.6 Days to harvest maturity

This was determined by recording number of days from planting to a stage when 95% of the pods had changed from yellow to brown.

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2.6 Determination mineral nutrients

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2.6.1 Determination nitrogen

Comment [AS18]: Nitrogen

Determined according to the procedure of Motsara & Roy (2008). Both mixed reagents were made considering the procedure of Revati *et al*. (2021). Standard solution of nitrogen of 300mg/L was then formed by dissolving 1.4159g of $(NH_4)_2$ SO_4 in 50ml of 0.7M sulphuric acid solutions, then used to make standard series: 0, 1, 2, 3, 4, 5ml of standard solution. Plant sample of 0.5g at harvest was wet-digested in di-acid and then made up to 100 ml volume. Sample diluted solutions (i.e, digest or standard series) of 0.2ml was added to 3 ml of mixed reagent I and 5ml of reagent II. Mixed after each addition, then measured the absorbance after 90 minutes by flame photometer (model 410, Sherwood Scientific LTD, Cambridge UK) at 630nm (Ye-Jin *et al*., 2017). The absorbance reading was used to determine the P concentration from the standard curve and calculation done as to Revati *et al*. (2021).

2.6.2 Determination phosphorous

Comment [AS19]: Phosphorus

Reagents for colour development were made using the procedure of Motsara & Roy (2008) where, ammonium molybdate-antimony potassium tartrate solution and antimony potassium tartrate were dissolved in water and diluted to 1L. Standard solution was made by procedure of Ye-jin *et al*. (2017). The absorbance was then read using flame photometer and used to make the standard curve of absorbance against N concentrations. Plant sample of 0.5g at harvest for each PVC pot was wet-digested in di-acid and then made up to 100 ml volume (Motsara & Roy 2008). Absorbance was measured by flame photometer (Model 410, Sherwood Scientific LTD, Cambridge UK) at 650nm (Ye-Jin et al., 2017). The absorbance reading was then used to determine the P concentration from the standard curves and calculations done using procedure of (Revati *et al*., 2021).

2.6.3 Determination potassium

Comment [AS20]: Potassium

Standard potassium stock solution was prepared by procedure of Motsara & Roy (2008), where 0.1253 of reagent grade Kcl was dissolved. Stock solution was diluted to a mark by distilled water and mixed thoroughly to give concentrations of 0, 0.2, 0.4, 0.6, 0.8 and 1 g.L^{-1} K respectively. Plant sample of 0.5g was made up to 100 ml volume after it was digested in di-acid (Motsara & Roy, 2008). Potassium content was measured at plant harvest for each pot using flame photometer (Model 410, Sherwood Scientific LTD, Cambridge UK). The absorbance of plant sample was then used to determine K content from the standard curve. K concentration was then calculated using the equation of Revati *et al*. (2021).

2.7 Statistical data analysis

Comment [AS21]: No number

The effect of genotypes and treatments was tested using the general linear model (Steel *et al*., 2006) in a factorial way by statistical analysis software (SAS) 9.1. Tukey's HSD test at 5% level was used to separate the means.

3. RESULTS

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3.1 Plant growth

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3.1.1 Number of leaves

Number of leaves on day 45 after treatment showed that there was a statistically significant difference (p=.05) amongst genotypes as determined by ANOVA. Tukey's studentized range (HSD) showed that there were no significant differences whenever each of the means for aluminium treatments {480 µM Al (2.89), 750 µM Al (2.89), Control (2.89), and 960 µM Al (2.78)} was compared to the other. Number of leaves for TGX (2.96) and NAMSOI (2.96) soy bean genotypes treated with *Rhizobia* and Al were significantly higher than that of TGX. Number of leaves on 49 DAT showed that there were a statistically significant differences (p=.05) amongst eight treatments and genotypes as determined by ANOVA. Mean number of leaves for NAMSOI (3.96) and TGX (3.92) soy bean genotypes treated with *Rhizobia* and Al were significantly higher than that of GAZZELE (3.58). Number of leaves on 56 DAT showed that there were a statistically significant differences (p=.05) amongst aluminium treatments and genotypes as determined by ANOVA. There was a statistically significant interaction between the effects of aluminium treatments and genotypes on day 56 after treatments. Mean number of leaves for USDA-inoculated (4.97) did not show significant differences whenever it was compared to mean at non-inoculated (4.89). Mean number of leaves for NAMSOI (5.00) soy bean genotype treated with *Rhizobia* and Al was significantly higher than those of TGX (4.96) and GAZZELE (4.83), respectively.

Number of leaves on 63 DAT showed that there were a statistically significant differences (p=.05) amongst eight treatments and genotypes as determined by ANOVA. Tukey's HSD showed that mean at control (7.00) and 750 µM Al (6.89) significantly higher compared to means at 480 µM Al (6.83) and 960 µM Al (6.61) for number of leaves on 63 DAT, respectively. Mean number of leaves for NAMSOI (7.00) soy bean genotype treated with *Rhizobia* and Al was significantly higher than those of TGX GAZZELE (6.75) and TGX (6.75), respectively. Fig. 1 shows number of leaves in the three soy bean genotypes. No significant differences were observed whenever each mean of GAZZELLE, NAMSOI and TGX was compared at any of the eight treatments levels and combined treatments on 45 DAT, 49 DAT, and 56 DAT and on 63 DAT.

3.1.2 Number of branches

Number of branches on day 61 after treatments showed that there were a statistically significant differences amongst eight treatments (p=.05) and genotypes (p<.01) as determined by ANOVA. Tukey's HSD showed that there were significant differences for number of branches on 61 DAT whenever mean at either control (3.83) or at 480 µM Al (3.33) was compared to mean at either 750 µM Al (3.11) or 960 µM Al (2.94). Mean number of branches for USDA-inoculated (6.92) was significantly higher whenever it was compared to mean at non-inoculated (6.75). Mean number of branches for TGX (3.96) soy bean genotype treated with *Rhizobia* and Al was significantly higher than those of NAMSOI (3.25) and GAZZELE (2.71), respectively.

Number of branches on day 72 after treatments showed that there were a statistically significant differences amongst eight treatments and genotypes (p=.05) as determined by ANOVA. Tukey's HSD showed that mean at control (5.28) was significantly higher whenever it was compared to mean at either 480 µM Al (4.83), 750 µM Al (4.33), or at 960 µM Al (4.17) for number of branches on 72 DAT. Mean number of branches for USDA-inoculated (3.50) was significantly higher whenever it was compared to mean at non-inoculated (3.11). Mean number of branches for TGX (4.92) soy bean genotype treated with *Rhizobia* and Al was significantly higher than that of NAMSOI (4.67) while mean for GAZZELE (4.38) was significantly lower whenever compared to mean at NAMSOI.

Number of branches on day 78 after treatment showed that there was a statistically significant difference amongst eight treatments (p<.01) as determined by ANOVA. Tukey's HSD showed that means at control (6.56) and 480 µM Al (6.05), were significantly higher compared to means at 750 µM Al (5.50) and 960 µM Al (5.17) for number of branches on 78 DAT, respectively. Number of branches on day 96 after treatment showed that there was a statistically significant differences amongst eight treatments (p<.01) as determined by ANOVA. Tukey's HSD showed that means at control (7.33) or at 480 µM Al (6.56) were significantly higher compared to mean at 750 µM Al (5.89) and 960 µM Al (5.00) for number of branches on 96 DAT, respectively. Mean at USDA-inoculated (6.00) was significantly higher whenever it was compared to mean at non-inoculated (5.63).

Fig1 shows number of branches in the three soy bean genotypes measured on 61, 72, 78 and 96 days after treatment. Mean of branches for NAMSOI was significantly higher compared to mean branches for GAZZELLE and TGX at treatment 4 (T4) on 61 DAT, respectively (Fig. 2). Mean of branches for TGX was significantly higher (p<0.05) compared to mean for GAZZELLE and NAMSOI at T5, respectively.

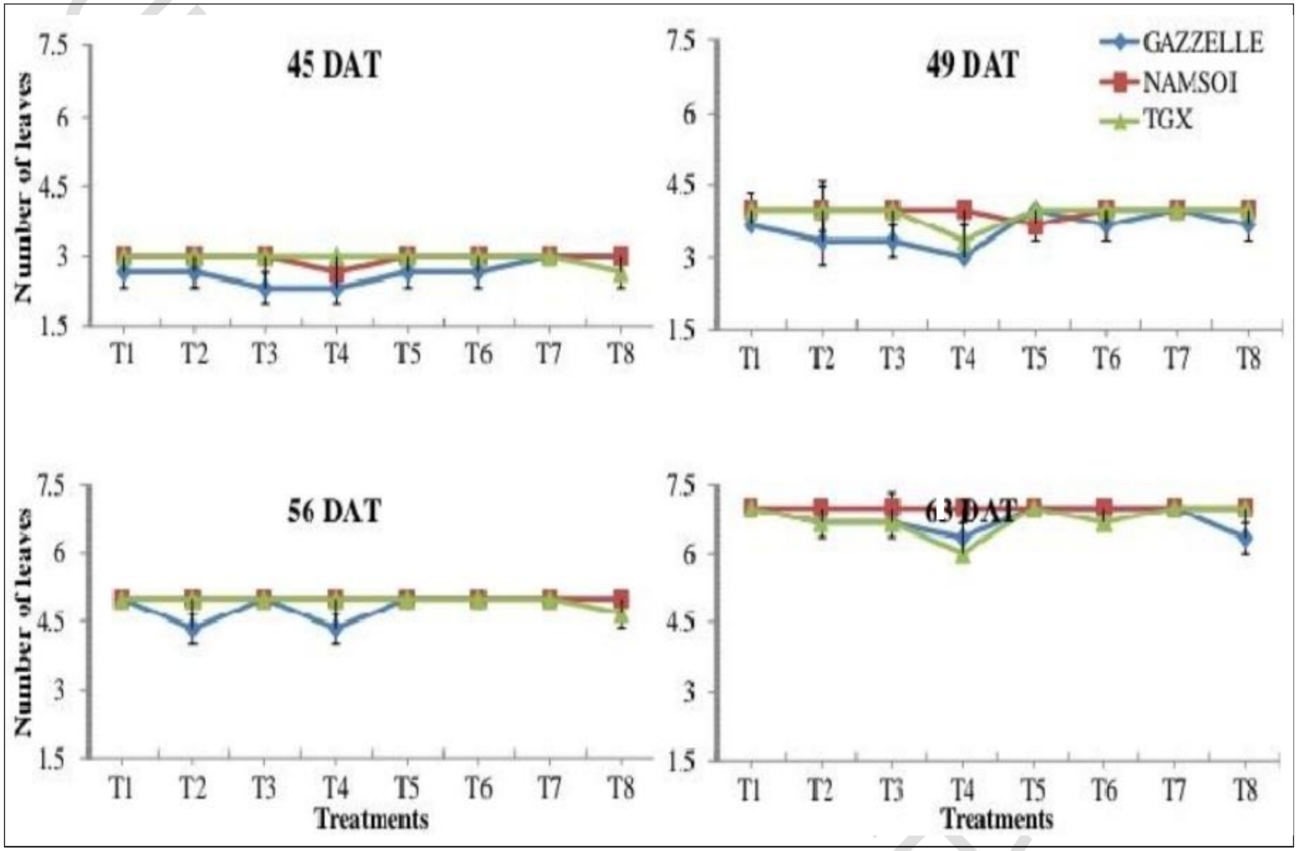

Fig. 1 Number of leaves per plant of three soy bean genotypes at 45 DAT, 49 DAT, 56 DAT and 63 DAT subjected to various treatments. Values are means of three replicates±SEs. Control (Water)*Inoculated (T1), 480µM Al*Inoculated (T2), 750µM Al*Inoculated (T3) and 960µM Al*Inoculated (T4), Control (T5), 480µM Al (T6), 750µM Al (T7) and 960µM Al (T8).

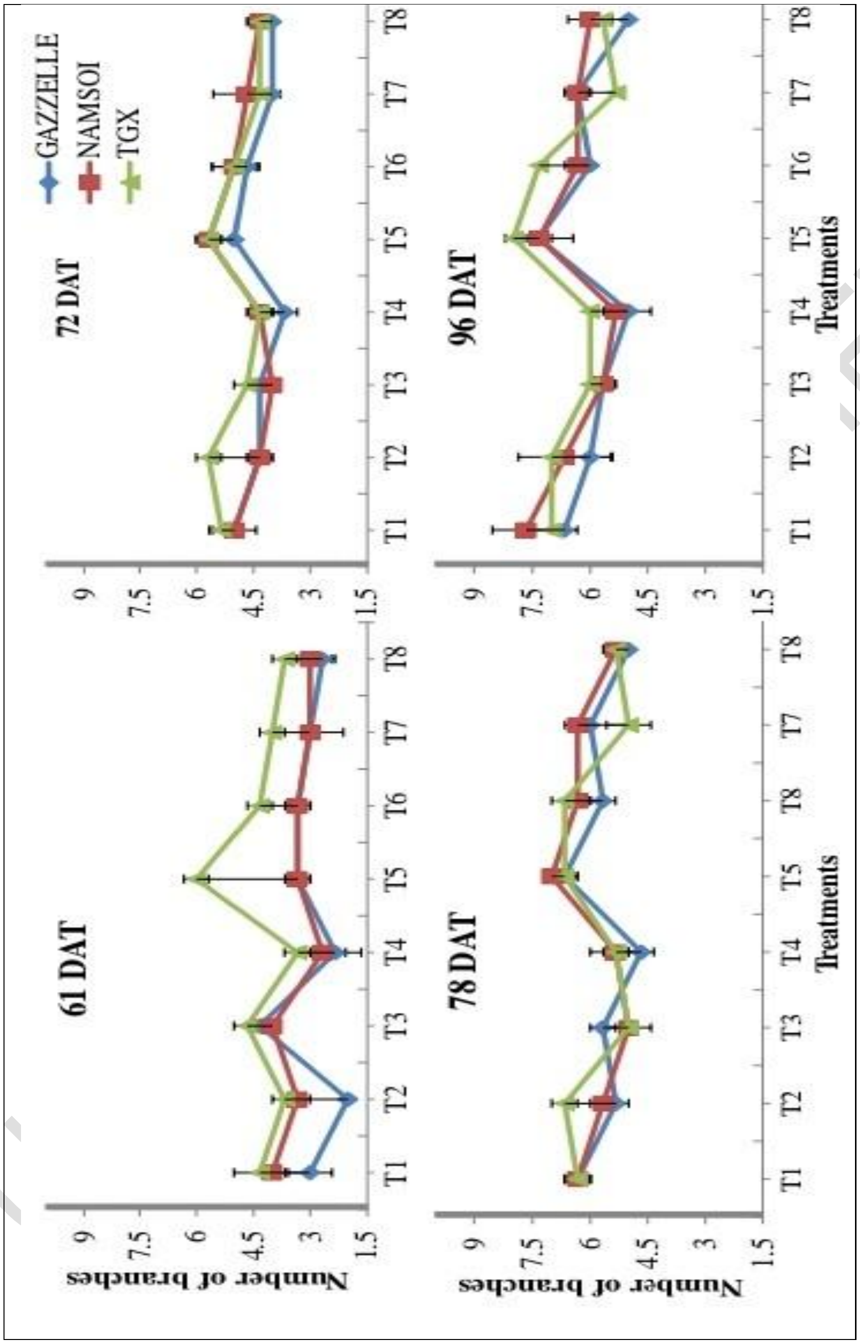

Fig.2 Number of branches per plant of three soy bean genotypes at 61 DAT, 72 DAT, 78 DAT and 96 DAT subjected to various treatments. Values are means of three replicates±SEs. Control (Water)*Inoculated (T1), 480µM Al*Inoculated (T2), 750µM Al*Inoculated (T3) and 960µM Al*Inoculated (T4), Control (T5), 480µM Al (T6), 750µM Al (T7) and 960µM Al (T8).

3.1.3 Days to 50% flowering

Days to 50% flowering in plants showed that there was a statistically significant difference amongst genotypes ($p<.01$) as determined by ANOVA. Tukey's HSD showed significant differences whenever each of the means for aluminium treatment {480 µM Al (42.78), Control (42.50), 750 µM Al (42.39), and 960 µM Al (42.11)} were compared for days to 50% flowering. Mean number of days to 50% flowering for NAMSOI (43.50) soy bean genotype treated with *Rhizobia* and Al was significantly higher than those of TGX (42.08) and GAZZELE (41.75), respectively. Table 2 shows mean number of days to 50% flowering determined in the three soy bean genotypes. Mean number of days to 50% flowering of NAMSOI was significantly higher than that of GAZZELLE and TGX genotypes, respectively at T3.

3.1.4 Pod clearance

Pod clearance in plants showed that there was a statistically significant difference amongst genotypes ($p=.05$) as determined by ANOVA. Tukey's HSD showed that mean pod clearance for eight treatments concentrations {control (20.46 cm), 960 µM Al (20.43 cm), 750 µM Al (19.44 cm) and 480 µM Al (18.16 cm)} were not significantly different when measured. Mean number of pods for USDA-inoculated (19.93cm) was highly not significantly different from that of non-inoculated (19.32cm). Mean Pod clearance for NAMSOI (21.53cm) soy bean genotype treated with *Rhizobia* and Al was significantly higher than those of TGX (19.15 cm) and GAZZELE (18.19 cm), respectively.

Table 2 shows pod clearance of the three soy bean genotypes. Mean of pod clearance of NAMSOI was significantly higher than that of GAZZELLE and TGX genotypes, respectively, at T2 and T3 (Table 2). Meanwhile, mean of pod clearance for TGX was significantly higher than that of GAZZELLE and NAMSOI at T7, respectively.

3.1.5 Number of pods

Number of pods on day 55 after treatment showed that there were statistically significant differences among the three genotypes ($p=.05$) and amongst eight treatments ($p<.01$) as determined by ANOVA. Mean number of pods at control (7.67) was significantly higher than mean at 480 µM Al (6.67), 750 µM Al (6.56) and 960 µM Al (6.06) respectively, for number of pods. Mean number of pods of NAMSOI (7.04) soy bean treated with *Rhizobia* and Aluminium was significantly higher than those of GAZZELE (6.63) and TGX (6.54), respectively.

Number of pods on day 62 after treatment showed that there was a statistically significant difference ($p=.05$) amongst eight treatments as determined by ANOVA. Tukey's HSD showed that mean at control (7.83) was significantly higher than mean at 480 µM Al (7.00), 750 µM Al (6.94) and 960 µM Al (6.39), respectively for number of pods. Mean number of pods of USDA-inoculated (7.08) was highly significantly different than the mean at non-inoculated (7.00).

Fig 3shows mean number of pods in the three soy bean genotypes on 55 DAT, 62 DAT, 68 DAT and 116 DAT. Mean numbers of pods of NAMSOI was significantly higher than mean of either TGX or GAZZELLE (Fig. 3.) on 62 DAT at T2 and on 116 DAT at T6.

Table 2. Days to 50% flowering and Pod clearance of three soy bean genotypes subjected to various treatments. Values are means of three replicates±SEs. Means with the same latter in the row are not significantly different.

Days to 50% flowering for genotypes				Pod clearance (cm) for genotypes			
GZZL	NMSI	TGX	Tukey`s grouping for Treatments	GZZL	NMSI	TGX	Tukey`s grouping for Treatments
42.33±0.33a	43.00±0.00a	43.00±0.00a	**42.78±0.15a**	18.73±0.89a	23.6±3.22a	21.5±1a	**21.28±1.23a**
41.67±0.33a	45.00±2.00a	42.33±0.33a	**42.56±0.78a**	13.77±1.13b	23.23±2.87a	19.13±2.94ab	**18.71±1.84a**
41.33±0.33b	44.00±0.00a	41.67±0.33b	**42.33±0.44a**	17.83±2.13a	20.83±1.2a	20.53±1.35a	**19.73±0.94a**
41.33±0.67a	43.00±0.00a	42.33±0.58a	**42.22±0.32a**	19.5±0.76ab	23.33±2.8a	17.17±0.83b	**20±1.25a**
40.67±0.33a	45.00±2.00a	41.00±0.58a	**42.22±0.92a**	19.17±3.07a	19.93±6.38a	19.83±1.59b	**19.64±2.09a**
42.33±0.88a	43.00±2..00a	42.33±0.58a	**43.00±0.29a**	18.5±1.32a	15.83±0.44a	18.5±2.36a	**17.61±0.91a**
42.33±0.88a	42.67±0.33a	42.33±0.58a	**42.44±0.29a**	18.77±1.79a	21.5±0.5a	17.17±0.83b	**19.14±0.86a**
42.00.± 0.0a	42.33±0.67a	41.67±0.58a	**42.00±0.24a**	19.27±3.6a	24.±3.06a	19.33±1.09a	**19.64±1.6a**
41.75±0.20b	**43.50±0.37a**	**42.08±0.16b**		**18.19±0.71b**	**21.53±1.07a**	**19.15±0.57ab**	

Control (Water)*Inoculated (T1), 480µM Al*Inoculated (T2), 750µM Al*Inoculated (T3) and 960µM Al*Inoculated (T4), Control (T5), 480µM Al (T6), 750µM Al (T7) and 960µM Al (T8).

TREATMENTS
Control*USDA - Inoculated
480 µM Al*USDA- Inoculated
750 µM Al*USDA- Inoculated
960 µM Al*USDA- Inoculated
Control (Water)*No inoculation
480 µM Al*No inoculation
750 µM Al*No inoculation
960 µM Al*No inoculation
Tukey`s grouping for genotypes

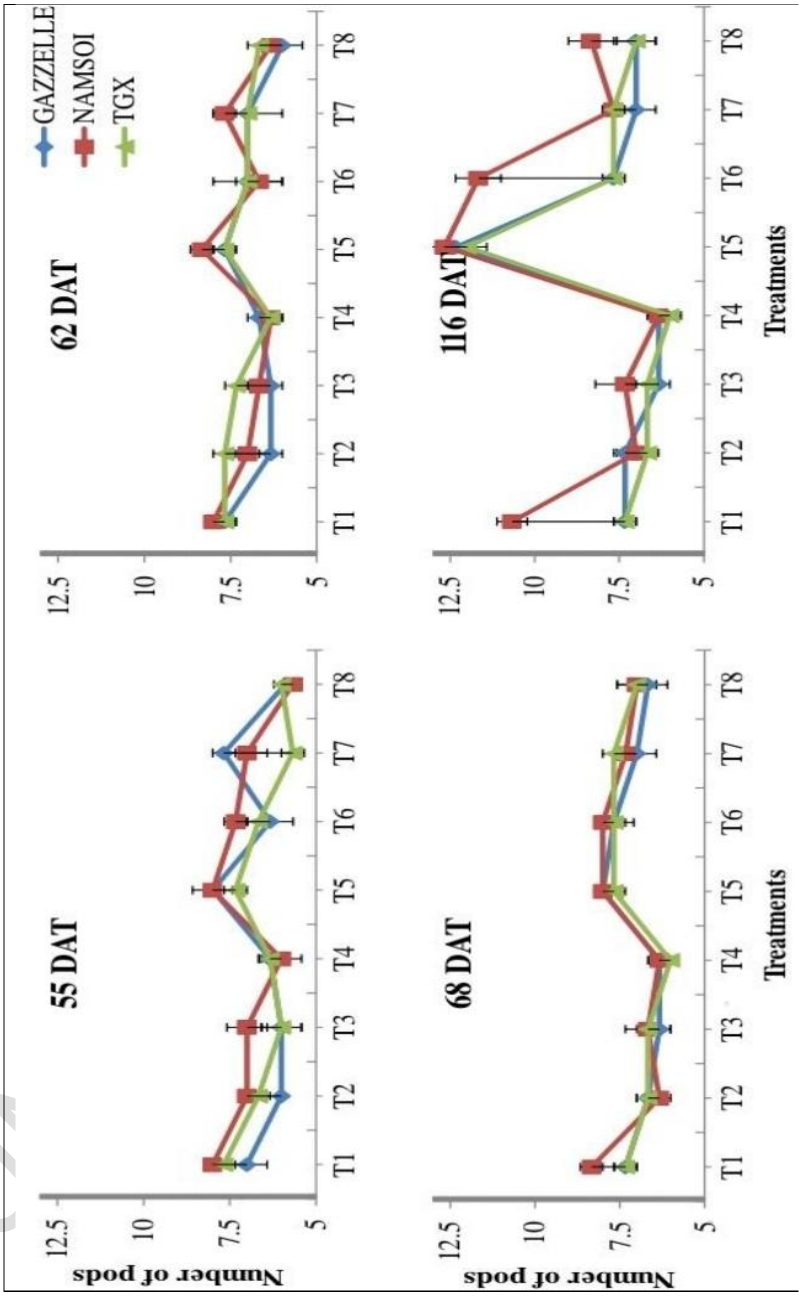

Fig. 3. Number of pods per plant of three soy bean genotypes at 55 DAT, 62 DAT, 68 DAT and 116 DAT subjected to various treatments. Values are means of three replicates±SEs.. Control (Water)*Inoculated (T1), 480µM Al*Inoculated (T2), 750µM Al*Inoculated (T3) and 960µM Al*Inoculated (T4), Control (T5), 480µM Al (T6), 750µM Al (T7) and 960µM Al (T8).

3.1.6 Days to harvest maturity

Days to harvest maturity showed that there was a statistically significant difference among the three genotypes (p<.01) as determined by ANOVA. Mean days to harvest maturity for GAZZELE genotype (97.17) was significantly higher compared to mean of either NAMSOI (93.83) or TGX (90.00). Noteworthy, mean day to maturity for NAMSOI was higher than that of TGX, respectively.

Table 3 shows the mean number of days to harvest maturity. The mean of genotype GAZZELLE was significantly different than those of NAMSOI and TGX at T4. The mean of NAMSOI was significantly higher than those of GAZZELLE and TGX in T6.

Table 3. Days to harvest maturity of three soy bean genotypes subjected to various treatments. Values are means of three replicates ±SEs. Means with the same latter in the row are not significantly different.

Comment [AS24]: Give title of table separate and results

TREATMENTS	**Days to harvest maturity for genotypes**			**Tukey`s grouping for Treatments**
	GZZL	**NMSOI**	**TGX**	
Control*USDA- Inoculated	96.67±3.92a	93.00±1.00a	90.67±2.19a	**93.44±1.59a**
480 µM Al*USDA- Inoculated	97.33±2.33a	90.67±1.33a	93.33±2.96a	**93.78±1.51a**
750 µM Al*USDA- Inoculated	99.00±0.00a	96.33±4.26a	96.33±4.26a	**95.00±1.89a**
960 µM Al*USDA- Inoculated	95.33±1.00a	95.33±3.67ab	89.67±2.08b	**94.44±2.06a**
Control (Water*No inoculation	94.00±1.00a	92.67±2.33a	90.67±2.19a	**92.44±0.96a**
480 µM Al*No inoculation	96.33±4.26ab	100.00±1.00a	88.00±0.00b	**94.78±2.18a**
750 µM Al*No inoculation	98.67±2.03a	92.33±3.33a	90.00±1.73a	**93.67±1.74a**
960 µM Al*No inoculation	99.00±2.00a	90.33±2.33a	89.67±2.08a	**91.78±1.31a**
Tukey`s grouping for genotypes	**97.17±0.83a**	**93.83±1.01b**	**90.00±0.83c**	

Control (Water)*Inoculated (T1), 480µM Al*Inoculated (T2), 750µM Al*Inoculated (T3) and 960µM Al*Inoculated (T4), Control (T5), 480µM Al (T6), 750µM Al (T7) and 960µM Al (T8).

3.2 Mineral nutrient concentrations:

3.2.1 Nitrogen concentrations

Nitrogen concentration in plants showed that there were statistically significant differences ($p<.01$) amongst eight treatments and genotypes as determined by ANOVA. There was a statistically significant interaction between the effects of treatments and genotypes on N concentration in plants. The mean of plant nitrogen concentrations for each of aluminium treatments {Control (47.56 µg.L^{-1}), 480 µM Al (46.86 µg.L^{-1}), 960 µM Al (45.03 µg.L^{-1}), and 750 µM Al (42.46 µg.L^{-1})} were significantly different. Similarly, mean of USDA-inoculated (46.87 µg.L^{-1}) was significantly higher than the one in non-inoculated (44.10 µg.L^{-1}) plants. The means of nitrogen concentrations for genotype NAMSOI (47.31 µg.L^{-1}), GAZZELE (46.77 µg.L^{-1}) and TGX (42.36 µg.L^{-1}) showed significant differences.

Fig. 4 shows N concentration in the three soy bean genotypes. The mean nitrogen concentration of GAZZELLE was significantly higher compared to that of NAMSOI and TGX at treatment 1 (T1), respectively. The means of GAZZELLE and NAMSOI genotypes was significantly higher to TGX for treatments T3, T5, T6 and T7 (Fig. 4), respectively.

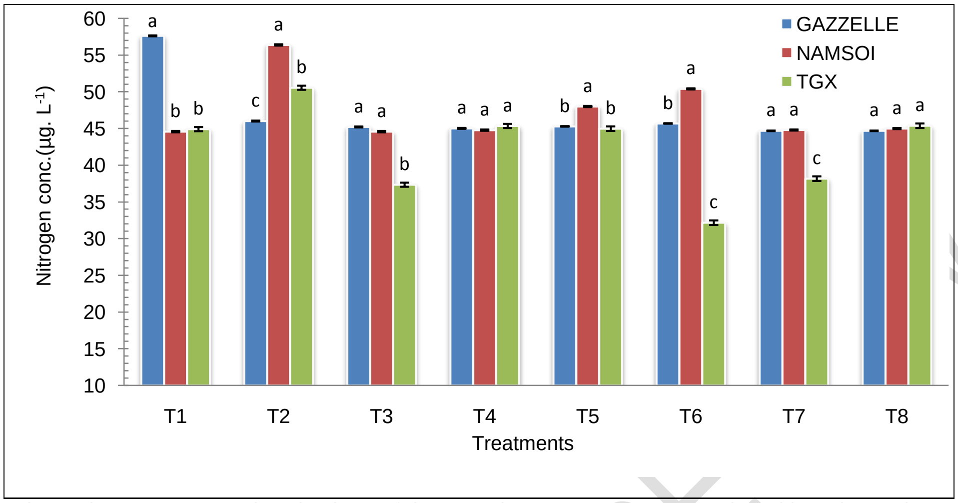

Fig. 4 Nitrogen concentrations in plants of three soy bean genotypes at maturity subjected to various treatments. Values are means of three replicates±SEs. Means with the same latter are not significantly different. Control (Water)*Inoculated (T1), 480µM Al*Inoculated (T2), 750µM Al*Inoculated (T3) and 960µM Al*Inoculated (T4), Control (T5), 480µM Al (T6), 750µM Al (T7) and 960µM Al (T8).

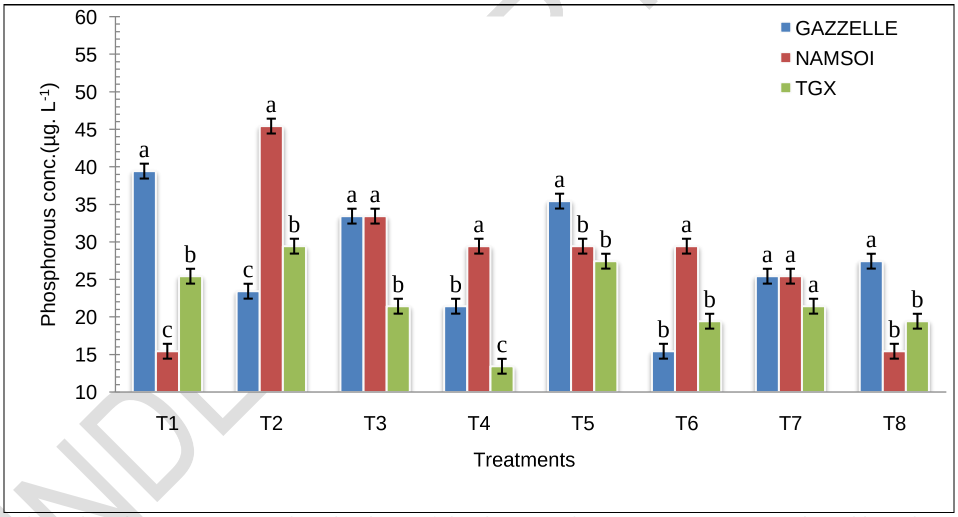

Fig.5 Phosphorous concentrations in plants of the three soy bean genotypes at maturity subjected to various treatments. Values are means of three replicates±SEs. Means with the same latter are not significantly different. Control (Water)*Inoculated (T1), 480µM Al*Inoculated (T2), 750µM Al*Inoculated (T3) and 960µM Al*Inoculated (T4), Control (T5), 480µM Al (T6), 750µM Al (T7) and 960µM Al (T8).

Give treatments detail at below the Figure or table 1, others below TREATMENTS AS FIGURE 1 OR TABLE 1

3.2.2 Phosphorous concentrations

Phosphorous concentration in plants showed that there were statistically significant differences (p<.01) amongst eight treatments and genotypes as determined by ANOVA. There was a statistically significant interaction between the effects of treatments and genotypes on P concentration in plants. The mean of phosphorous concentration at control (28.78 µg.L^{-1})

was significantly higher than those at 480 µM Al (27.11 µg.L^{-1}), 750 µM Al (26.78µg.L^{-1}) and 960 µM Al (21.11 µg.L^{-1}) treatments, respectively. The mean of phosphorous concentration at treatments 480 µM Al and 750 µM Al were significantly higher different than 960 µM Al. Mean phosphorous concentration for USDA-inoculated (27.61 µg.L^{-1}) was also significantly higher than the mean for non-inoculated (24.28 µg.L^{-1}). The mean P concentration of NAMSOI (27.94 µg.L^{-1}) and GAZZELE (27.69 µg.L^{-1}) soy bean genotypes treated with *Rhizobia* and aluminium were significantly than that of genotype TGX (22.19 µg.L^{-1})**.**
Fig. 6 shows P concentration in the three soy bean genotypes. The mean of phosphorous concentration of GAZZELLE genotypes was significantly higher than that of NAMSOI and TGX at treatments T1, T5 and T8, respectively.

3.2.3 Potassium concentrations

Potassium concentration in plants showed that there were statistically significant differences (p<.01) amongst eight treatments and genotypes as determined by ANOVA. There was a statistically significant interaction between the effects of treatments and genotypes on K concentration in plants. The mean of control treatment (169.67 µg.L^{-1}) was significantly higher than at treatments 750 µM Al (124.00 µg.L^{-1}), 960 µM Al (116.33µg.L^{-1}) and 480 µM Al (112.33 µg.L^{-1}), respectively. Similarly, mean of potassium concentration at either 480 µM Al and at 960 µM Al was significantly higher than at 750 µM Al. The mean of USDA-inoculated (147.00 µg.L^{-1}) soy bean plants was also significantly higher than non-inoculated (114.17 µg.L^{-1}). The mean K concentration for NAMSOI (149.33 µg.L^{-1}) and TGX (136.58 µg.L^{-1}) soy bean genotypes treated with *Rhizobia* and aluminium were significantly higher than that of genotype GAZZELE (105.83 µg.L^{-1})**.**
Fig.6 shows K concentration in the three soy bean genotypes. The mean of potassium concentrations for NAMSOI and TGX were significantly higher than that of GAZZELLE at treatments T4, T5, T6 and T7, respectively.

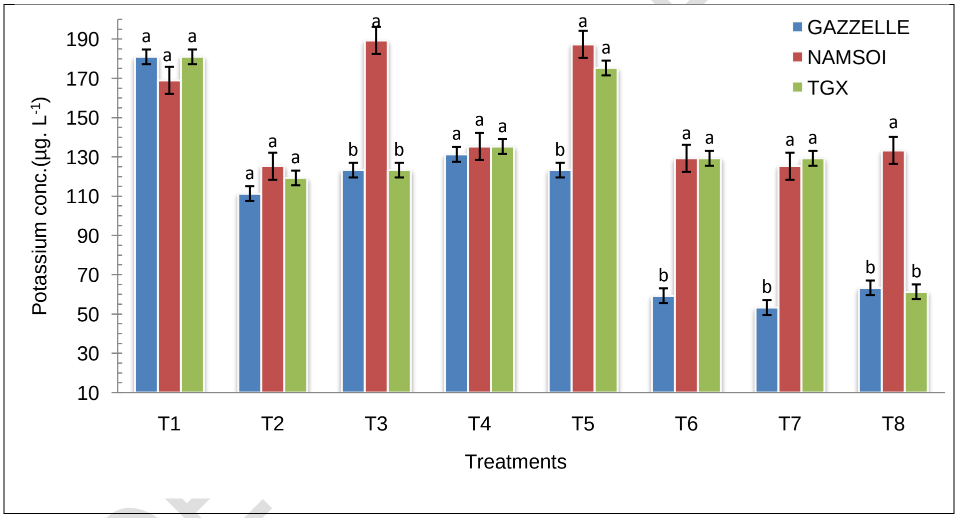

Fig.6 Potassium concentrations in plants of the three soy bean genotypes at maturity subjected to various treatments. Values are means of three replicates±SEs. Means with the same latter are not significantly different. Control (Water)*Inoculated (T1), 480µM Al*Inoculated (T2), 750µM Al*Inoculated (T3) and 960µM Al*Inoculated (T4), Control (T5), 480µM Al (T6), 750µM Al (T7) and 960µM Al (T8).

4. DISCUSSION

4.1 Effects aluminium application and *Rhizobia* inoculation on growth

The growth of plant shoots organs is affected in different ways when exposed to Al stress. Results show a decrease in leaf number for the three soy bean genotypes under Al-toxicity. Aluminum toxicity, therefore, may have affected the leaf development of soy bean genotypes inhibiting leaf growth among the genotypes (Trebelsi *et al*., 2011). Leaf development might have been disrupted hence affected this soy bean plant growth. Number of leaves for soy bean genotypes significantly increased when inoculated with USDA *Rhizobi*a strain. This increase was also found by Mfilinge & Ndakidemi (2014) when studying common beans, according to them this can be explained that, *Bradyrhizobium spp*. inoculums availed nitrogen to soy bean genotypes leave after fixation. This then offered much chlorophyll formation hence multiplication of leaves. Results of this present study are also congruent with Kumawat *et al.* (2017) findings which showed that soy bean developed optimum leaves number when inoculated with *Bradyrhizobium liaoningense*. Plants that are expose to Al and at the same time not inoculated with *Bradyrhizobium*, have been found to accumulate high concentration of Al in leaves (Mossi *et al.*, 2011), this phenomenon may have had greater impact on growth leading to reduced number of leaves found for such genotypes. For instance, soy bean genotype GAZELLE exhibited this effect due to Al toxicity with leave number. The results indicated that soy bean sensitivity to Al toxicity is determined by the days of exposure. It appears like leaves are altered in the early days, indicating Al toxicity to plants in their early stage of growth contrary to earlier findings by Edna (2019) where effects were more serious at old age. Previous studies (Naomi 2009; Miguel *et al*., 2023) linked reduced leaf number to low nutrient uptake.

Number of branches in soy bean was among the vegetative parameter used to determine plant growth and development. It varied for genotypes, Al treatments and for *Rhizobia* treatments. In this study, means at control on both DATs for number of branches was significantly higher compared those of Al treated plants. It is noteworthy that, the effect of Al-stress eventually decreased branching (Mossi *et al*., 2011). This might have adversely limited branching in GAZZELLE. Aluminium stress therefore might have also severely inhibited plant-water status and cell elongation in this genotype leading to much reduced branching. The study revealed reduced branching in the three genotypes at early stages of growth, for instance on 61 days after treatment. It is likely that, the apical meristem activity may have reduced under Al. It is also possible that, nitrogen availability in the soil significantly reduced branching, as earlier found by Gwata *et al*. (2004). However, Miguel *et al*. (2021) noted that high nitrogen content in the soil affects the normal BNF, hence in some occasion nitrogen application may have caused declined branch formation in the soy bean as a morphological traits. Therefore, critical nitrogen levels below or above is an impediment to attaining maximum productivity in legumes. For instance, in Miguel *et al*. (2013), a critical nitrogen range under inoculation may have been vital to inform the optimal conditions for increased branches. According to their research, high nitrogen levels above required optimum may have suppressed nodulation resulting in reduced branching and ultimately low shoot weight and shoot biomass in these soy beans under inoculation.

Mebrahtu & Teklay (2021) showed that *Bradyrhizobium spp*. lead to increased nitrogen fixation which caused increased branching. Therefore, increased number of branches in soy bean realised in this study depended on the inoculation with *Rhizobia*. This is inferred from the fact that non-inoculated plants had fewer branches. The differences in branching were noted among the soy bean genotypes. TGX genotype had more branches hence which may have influenced enzymatic reactions in these plants. For instance, it might have improved photosynthesis thus led to large number of branches as suggested by Miguel *et al*. (2023), as shown in cacao plants. It has been further explained that *Rhizobia* increases biological nitrogen fixation (BNF), but when coupled with phosphorus supplementation it increases branching. It therefore may have worked synergistically with phosphorous in NAMSOI to convert nitrogen to ammonia. This made nitrogen much readily available to increase branches for the two genotypes, NAMSOI and TGX.

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Days to 50 % flowering showed no significant differences ($p<0.05$) for both *Rhizobia* treatments and aluminium. According to Ntambo *et al*. (2017), this can be validated that nitrogen deficiency due to Al stress at flowering caused nodule senescence in GAZZELLE and TGX. *Bradyrhizobium japonicum*-USDA inoculum increases soy bean flowering days. NAMSOI had more days that were counted to be significantly higher compared to those of TGX and GAZZELLE, respectively. Therefore inoculation especially in NAMSOI led to high flowering ability. Flowering was triggered by survival of right proportion and type *Rhizobia* strain inoculum (Ulzen *et al.,* 2016). In this regard, nitrogen fixations and improved nutrient uptake, activated and increased cell elongation and division.

Some genotypes like the promiscuous nodulating genotype; GAZZELLE types respond adequately to *Rhizobia* inoculation hence was found to have less days to 50 % flowering compared to TGX which responded poorly to USDA *Rhizobia* as also found by Adenkambi *et al*. (2019) within soy bean genotypes they studied, which therefore, suggests it is a common phenomenon. For instance, in their study, it was established that there was a variation in number of days to 50% flowering. Precisely, in their study TGx1955-4F and TGx1951-3F genotypes flowered significantly earlier compared to others. There was no significant interaction between soy bean genotypes and inoculum treatments with regard to days to 50 % flowering, which is which was also found by other studies Mossi *et al.* (2011) when studying chemotypes.

Rhizobia treatments and Al applications and furthermore, interactions did not show significance level differences ($p<0.05$) for pod clearance. Ibie *et al*. (2021) whose results agree satisfactorily with the present study suggested reasons to be maximum environmental effects and high gene contribution to phenotypic expression of genes. The promiscuous NAMSOI genotype possesses a significantly different and very interesting pod clearance value compared to other genotypes indicating its adaptability to the prevailing conditions. Further, the only importance that is sound for pod clearance is to facilitate a good and mechanized harvesting condition (Edna *et al*., 2019). Low pod clearance height as for GAZZELLE and at 480 µM Al expose the soy beans to rainfall soil splash that attribute to susceptibility to white mould.

In this study significant differences found for pod number imply that *Rhizobia* inoculation reduce the adverse effects of Al toxicity. High number of pods found in NAMSOI and GAZZELLE would eventually suggest that they are likely to reap maximum yields according to Mebrahtu & Tekley (2021). Generally, USDA inoculated seeds led to plants with highly significant number of pods than non-inoculated plants. Therefore, using an appropriate *Rhizobium* species before planting enhance pod production and thus grain yield in legumes regardless of Al availability in soils (Thilakarathna & Raizada, 2017).

Mean for TGX was significantly lower ($p>0.05$) to those of GAZZELLE and TGX genotypes at T4 and T6, respectively when days to harvest maturity was analyzed. However GAZZELLE is an early maturing genotype (Jonas *et al*., 2008). The above-mentioned findings imply that TGX, a late maturing genotype might be affected much by aluminium in acid soils even if inoculation is done, a phenomenon that might have caused premature browning of pods in these genotype.

4.2 Effects of aluminium application and *Rhizobia* inoculation on NPK concentration

According to the finding of this study, the effects of Al application and *Rhizobia* inoculation on NPK concentration in different soy bean genotypes vary (Fig..1, 2 and.3). Al application led to significant differences in the accumulation of NPK in leaves of soy beans. Soy bean plants grown in soils with Al exhibited high negative effect of Al stress. For instance, nutrient became deficient in Al applied plants than in controls. Al ions may have accumulated to a large extent in root system but less was transported to the shoots of plants applied with Al as suggested by Trebelsi *et al*. (2011). Therefore such roots became inefficient in absorption of water and both nutrients due to their drastic reduction in cell elongation as they become stubby (Shi *et al*., 2022).

This study revealed reduced concentration of N in plants, interactions amongst eight treatments and genotypes were all significantly different. This strongly suggests that, Al in the soil lowers the uptake of N and N-use efficiency (Dogan & Goksel, 2014). However, the various soy bean genotypes responded variedly to Al application and *Rhizobia* inoculation. For instance, mean N for GAZZELLE was significantly higher than for NAMSOI and TGX at treatment 1(T1). Similarly, other differences were where TGX at T3, T5 T6, and T7 (Fig..1) was significantly lower than in the other two. In TGX, Al may affect the nitrification and the bacteria involved than in the other genotypes. On the other hand, the *Rhizobia* may have had a high potential to colonize the roots nodules of GAZZELLE plants. Therefore, much N fixed in GAZZELLE soy bean plants. Active uptake of N that probably led to loss of chlorophyll in TGX may be due to significant reduction of K uptake and therefore reduced respiration (Shi *et al*., 2022).

In genotypes like GAZZELLE and NAMSOI, less Al-phosphate complexes might form due to precipitation of P in roots leading to limited reduction of P in soy bean leaves as found by Dogan & Goksel (2014) in roman nettle (*U. Pilulifera*) plants. Formation of Complex ligands may have destructed the uptake of K^+ and NH^{4+} cations, mostly in TGX. Al affects N synthesis and inter-conversion within plants. In one case, while studying sorghum plants Zhao & Shen (2018) found that Al reduced NO-N but increased amino acid-N concentrations in xylem sap. Similarly, in soy bean plants, Al may have initiated more glutamine synthetase/glutamate synthase cycle, where glutamine synthetase catalyses NH-glutamate process when forming glutamine much less at control. Therefore, NH^{4+} may have been assimilated in large quantities within GAZZELLE even when control was inoculated (T1). Similarly, in maize, it was found that Al stimulates N assimilation in the roots of an Al-tolerant maize genotype (Mihailovic *et al*., 2015).

According to Mmayi *et al*. (2015), when studying how Al affect soy bean without inoculation, they suggested that, Al toxicity inhibited NO_3 uptake by plant roots by binding to the NO3 transporter and the deliberately cause NO_3 efflux. Therefore, in the current study, these may have also decreased internal NO_3 accumulation in Al treated plants regardless of *Rhizobia* inoculation.

Plants at control significantly accumulated much of P in leaves, a similar difference was found for USDA-inoculated plants when compared to non-inoculated plants. Al may have mostly affected H^+-ATPase actions as it disrupt H^+ gradient. H^+ gradient is mostly utilized in ion transport processes as a trans-membrane proton (Edna, 2019). In this case, P is very important in ATP-energy synthesis that is vital for nutrient uptake by active transport process by plants. Consequently in soy beans this might have highly altered ionic homeostasis of root cells of non-inoculated plants especially when served with Al. Al might have also reduced potassium utilizing rate (Mendoza-Sota *et al*., 2015) as less P was found to concentrate in leaves of Al treated plants.

Calcium may have been underutilized in Al treated plants. Therefore, leading to decreased respiration in Al applied and non-inoculated plants, which increase in polysaccharides deposition (Mendoza-Sota *et al*., 2015). The carbohydrates make conditions even more worse when they trap Al in the apoplast a phenomenon that reduce elongation of cells (Mfilinge & Ndakidemi, 2014) when there was inoculation at T4 while at T5, T6 and T7 when the genotypes GAZZELLE plants were not inoculated. According to Dogan & Goksel (2014), Al reduces P and K concentration by blocking their conducting channel which causes K^+ influx into guard cells. These then concomitantly reduces cell elongation. This decrease in K was found to be severe in roman nettle applied with 100 $AlCl_3$ by Dogan & Goksel (2014). GAZZELLE genotype may have undergone such K^+ influx effects considering T5, T6 and T7.

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4. CONCLUSION

Inoculation of legume seeds prior to planting is the best alternative to increase number of leaves and leaf area. Number of leaves increased by USDA inoculation that had a boost on nitrogen uptake. Poor soils that were applied with 960 µM Al affect the survival of the *Rhizobia* whose effects are detected by less days to 50 % flowering, branching, pod clearance, and days to harvest maturity. Out of the three genotypes studied GAZZELLE showed good performance when inoculated with USDA and applied to with Al.

Significant differences were observed for growth in genotypes under study at some treatments. This indicates that Al stress was down scaled on inoculation. Nodule number, root hair number per unit can be studied in future to determine their relationship between N_2-fixation capacity and root size and weight. It is very vital in determining effects of Al under inoculation since Al affects cell division at root.

USDA-*Rhizobia* improved symbiotic system in soy bean plants owing to increased nodulation that placed greater demand for phosphorous. Increased *Rhizobia* population even when Al was applied led to increased N levels that increased ability of such plants to absorb phosphorous. *Rhizobia* inoculation and Al application enhanced some significant differences within genotypes as GAZZELLE had high mean for N and P, while NAMSOI had high mean for K.

Aluminium is abundant in soils used in the study to levels that are detrimental as it was concentrated in both controls (T1 and T5); USDA inoculum is therefore recommended for use to ameliorate the effects of Al in soy bean plants. NPK mineral nutrients were strongly affected due to significant differences found in genotypes at various treatments. They are recommended for use as a routine check on effects of *Rhizobium* under other stress. Genotypes NAMSOI and GAZZELLE showed higher concentration of NPK. Therefore the two genotypes are recommended to be grown in areas with Al prone soils as they experienced reduced effect of Al stress under inoculation. Genotypes that are both Al-tolerant and NH4 -preferring should be bred for future use in Al prone soils; such genotypes have increased N-use efficiency and reduce NO3 loss. This will imply that reduced use of N fertilizers.
IN CONCLUSION NO NEED TO DISCUSS THE IMPORTANCE OF TREATMENTS SO ONLY GIVE WHAT TO BE USED FOR CULTIVATION GET HIGHER SEED YIELD OR WHICH CULTIVAR SHOULD BE FOLLOWED

DEFINITIONS, ACRONYMS, ABBREVIATIONS

HSD;- Tukey`s studentized range test (honestly significant difference)
IITA;-International Institute of Tropical Agriculture
K;- Potassium
N;- Nitrogen
P;- Phosphorous
TGx;-Tropical Glycine crosses series.
Ts;- Treatments.
TSBF;- Tropical soil Biology and Fertility.
YMA;- Yeast Mannitol Agar.
YMB;- Yeast Mannitol Broth.

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