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Gas Exchange, Photosynthetic Pigment and Secondary Metabolites Concentration of Soy bean (*Glycine max* L.) Plants Grown in Western Kenya under *Rhizobia*l Inoculation and Aluminium Application

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Authors' contributions

This work was carried out in collaboration among all authors. Author MMP designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors NGW and MDM managed the analysis of the study, and literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Soy bean production continues to be very low, there is potential for increased production of the crop. Acidic soils negatively affect plant nutrition and productivity. *Rhizobia* inoculation has previously been shown to improve legume production. There is need to understand how inoculation of *Bradyrhizobium japonicum* increase N content for leaf activity and enable pod filling that increase yield under Al application. The objective of the study was to investigate gas exchange, photosynthetic pigment concentration and secondary metabolites response of GAZZELLE,

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NAMSOI and TGX soy bean genotypes grown in western Kenya to aluminium application and *Rhizobium* inoculation. The experiment was carried out at Maseno University under greenhouse conditions. Three replicates, three genotypes and eight treatments {water (control), 480µM Al, 750µM Al, 960µM Al, Control & inoculation, 480µM Al & inoculation, 750µM Al & inoculation and 960µM Al & inoculation} were used in RCBD. Gas exchange parameters were collected. Absorbance were read at 480, 645 and 645nm to determine chlorophyll *a*, *b* and carotenoids. Plant secondary metabolites were also determined. Tukey`s HSD tests at 5% was used to separate treatment means. Significant differences were observed when GAZZELLE was compared to NAMSOI and TGX at T1, T3, T5, T6 and T7. Therefore USDA-*Rhizobia* may have high potential to colonize roots of GAZZELE. Significant differences found for C_i, *A* and g_i showed GAZZELLE and TGX to be photosynthesizing best under USDA-inoculation. Therefore they have potential production in acidic soils. Anthocyanin and phenolic compounds were found highly concentrated in GAZZELLE and NAMSOI, they then may have highly formed Al-complexes that limited Al stress. Inoculation ameliorated significantly the effects of Al to Chl *a*, Chl *b* and carotenoids. A reduction of Chl *a* was lowly in GAZZELLE indicating that it was less affected when Al was treated. These findings show that *Bradyrhizobium japonicum* inoculation alleviates Al effects and improve soy bean yield.

Keywords: Acidic soils; USDA-Rhizobia; photosynthesis; secondary metabolites; photosynthetic pigments.

ABBREVIATIONS

A	*: Net CO_2 assimilation rate;*
Chl. a	*: Chlorophyll a;*
Chl. b	*: Chlorophyll b;*
C_i/Ca	*: Internal to ambient CO_2 concentration rate;*
C_i	*: Intercellular carbon concentration;*
E	*: Transpiration rate;*
g_s	*: Stomatal conductance;*
HSD	*: Tukey`s studentized range test (honestly significant difference);*
NPQ	*: Non-photochemical quenching;*
PhiPSII or ϕPSI	*: Effective quantum yield;*
Pn	*: Net photosynthesis;*
qN	*: Coefficient of Non-photochemical quenching;*
Qp	*: PSII reaction centers;*
TGx	*: Tropical Glycine crosses series;*
Ts	*: Treatments.*

1. INTRODUCTION

Soy bean (*Glycine max* L.) is used majorly for medicinal purposes, human food, and as a source of bio-energy [1]. Kenya`s production level of soy bean is estimated at 450 kg/ha which is quite low [1] compared to countries like Brazil and United States of America (USA) at a proximate rate of 1301 to 2033 kg/ha [2]. One of the reasons for low production levels of soy beans in Kenya is acidic soils [2].

Air pollution by acids of nitrogen and sulphur lead to soil acidification through acid rain [3]. Such acid soil conditions are suitable for minerals dissolution that releases Al into the soil solution. A third of land area in Kenya that is approximately 7.5 million hectare is acidic [4]. Therefore, Al toxicity remains a major hurdle for increasing world`s food production especially in developing tropical and subtropical regions where Kenya belongs.

Symbiotic nitrogen fixation between soy bean plants and *Rhizobia* reduces free atmospheric nitrogen, which is converted to nitrate then absorbed to satisfy nitrogen demand for the host [5]. Despite the much more benefits of BNF to agricultural production, it`s exploitation has been limited by abiotic factors such as Al stress that affect the legume host, the micro-symbiont or both. *Rhizobia* bacteria fix atmospheric nitrogen thereby availing it for protein synthesis by the plant [6]. However, Al has been shown to adversely affect the process of nodulation through inhibition of root hair formation and nodule initiation [7].

Aluminium ions cause major effects on photosynthetic pigments associated with photosystem I and II. For instance, under high irradiance, the PSII reaction centers absorb excessive light energy which eventually results in the impairment or inactivation of the chlorophyll-containing reaction centers [8]. Carbon dioxide assimilation is significantly decreased under Al stress. Aluminium stressed leaves of inoculated

soy beans might interfere with the normal CO_2 assimilation and water use efficiency among other gas exchange processes. Under Al conditions rubisco, mitochondrial proteins and other chloroplast proteins are hydrolyzed and exported via phloem thus expected to reduce leave proteins of soy beans [9].

Aluminium ions in photosynthetic cells will majorly affect pigments associated to both photosystem I and II [10]. Therefore, Al stress may have decreased total electron carriers per reaction center and damaged all photochemical and non-photochemical parameters [10]. According to [11], electron transport capacities accompanied by lack of this reducing equivalent will eventually contribute to decreased carbon dioxide (CO_2) assimilation in stressed leaves. Furthermore, there was increased rate of photosynthesis in soy bean plants that had increased nodulation and symbiosis. According to [9], this was so regardless of the N effect, due to changes in the sink: source ratio.

Reduced photosynthesis therefore was the real cause of low soy bean crop production under Al due to poor performance of these physiological parameters. Inoculation of soy bean with *Rhizobium Japonicum* has been known to increase yield in soy bean [1]. Therefore, is possibility that this increase is linked to the effect on gas exchange parameters. A study on this is therefore warranted. Light absorption reduce as there is damage to photosystems under Al stress [12] and therefore soy bean plants under Al highly change their chlorophyll antennae size as an adaptive strategy. Inoculating of soy beans might gave an internal stop gap measure to this strategy [1], which needed to be explored in soy bean genotypes. Photosynthetic apparatus are affected by harmful effects of light and oxygen under Al [13]. Vital information is hereby established on how this makes excess light to be dissipated by carotenoids as heat in the antenna pigment complexes of inoculated soy beans. It is also now known how *Rhizobia* inoculation remove negative effect of light dissipation that drains off more production energy of the plants.

According to [14], secondary metabolites that are known to be increased under aluminium stress include phenolics, flavonoids, tannins, lignin, and certain organic acids. Synthesis and accumulation of phytochemical compounds due to Al have great impact to pharmacological properties of the plant [15]. This therefore called for a thorough investigation on their accumulation level in soy bean plants under *Rhizobia* inoculation.

Soy bean shows a large intraspecific variation in Al resistance but establishing the genetic basis for this variation has proved controversial. It is also not clear how *Rhizobia* inoculation may reduce or minimize Al stress effects. The objective to the study was to determine the effect of aluminium application and *Rhizobia* inoculation on gas exchange, photosynthetic pigment and plant secondary metabolites concentration of soy bean genotypes.

There is need to determine if *Rhizobia* can enable soy bean plants to have higher nodulation under Al application. Little is documented on the effects of *Rhizobia* inoculation and Al stress on yield, physiology and biochemistry of soy beans grown in Kenya. Therefore, yield determination, soy bean plant gas exchange and photosynthetic pigment determination were very important.

2. MATERIALS AND METHODS

Experimental Site: The research was carried out within greenhouse at Maseno university research farm between August 2021 to 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.

Seeds of Soy Bean: Three soy bean genotypes were selected based on their high quality and productivity. The genotypes were obtained from consortium of international agricultural centers (CGIAR) station at Maseno. Inoculation of seeds was done according to [16]. Seeds were inoculated with *Bradyrhizobia japonicum* soy bean inoculant using recommended rate of 10g per kg of seed [16].

Planting and Experimental Design: Twenty litre PVC pots were filled with soil from Maseno University Research farm. Calcium superphosphate, Diammonium phosphate and Potassium sulphate fertilizers were applied as recommended [1].

Randomized Complete Block Design (RCBD) with three replicates was used. Al-treatment concentrations ($AlCl_3.6H_2O$) were dissolved in water and applied according to [17]. Interactive treatments in the study 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 were then presented as T1, T2, T3, T4, T5, T6, T7 and T8 respectively.

Determination of Gas Exchange: Net photosynthesis rate (P*n*), transpiration rate (*E*), intercellular CO_2 concentration (C_i), net CO_2 assimilation rate (*A*) and total stomatal conductance (g_i) of single leaves were measured on the middle of first young fully mature healthy leaf on the elongating stage, with a portable photosynthesis system (LI-6400, LI-COR Inc., Lincoln, NE, USA) from 0900hrs to 1500 hours on a clear, cloudless day.

Determination of Photosynthetic Pigments: Chlorophyll content was determined as described in [18] where the third youngest leaf was sampled then 10 ml of 80% acetone was used to extract chlorophyll from 0.5g of the fresh leaf tissue. Absorbance was then measured using a spectrophotometer (Nova spec II, Pharmacia Biotech, Cambridge, England) at 480, 645 and 663nm to determine the carotenoids, chlorophyll *a* and *b* content. The respective chlorophyll content was calculated using the formula of [18].

2.1 Determination of Secondary Metabolites Concentration

Flavonoids Concentration: Flavonoids concentration was measured by the method of [19]. Acidified ethanol was used for extraction then followed by centrifugation [6]. Flavonoid was determined using a double bean scanning spectrophotometer (UV 190-1100, MRC). The formulae in [20] was used to calculate total flavonoids.

Anthocyanin Concentration: Acidified methanol was used for extraction according to [21]. Absorbance was measured using a spectrophotometer (Nova spec II, Pharmacia Biotech, Cambridge, England) at 550nm [22]. Anthocyanin concentration in $\mu g.l^{-1}$ was calculated using the formulae of [21].

Phenolic Compounds Concentration: Three (3) ml of 95% ethanol was used in extraction according to [21]. The absorbance of the supernatant was read on a spectrophotometer (Nova spec II, Pharmacia Biotech, Cambridge, England) at 725nm. Stock solution was prepared according to [21]. The formulae in was used to calculate phenolic compound concentration.

Statistical Data Analysis: The effect of genotypes and treatments was tested using the general linear model in a three way factorial using statistical analysis software (SAS) 9.1. Tukey's HSD test at 5% level will be used to separate the means.

3. RESULTS

3.1 Gas Exchange

Intercellular Carbon Concentration (C_i): Fig. 1 shows intercellular CO_2 concentration in the three soy bean genotypes measured on 35 DAT, 65 DAT and 95 DAT. The figure shows that there were no significant differences for mean C_i among GAZZELLE, NAMSOI and TGX, respectively at the eight treatments.

Analysis of variance run to determine the effects of aluminium treatments and *Rhizobia* treatments on intercellular carbon concentrations showed that there were statistical significant (p=.05) differences. The mean for Al treatments 750 µM Al (369.74 µmol mol^{-1}), 960 µM Al (366.2 µmol mol^{-1}) and 480 µM Al (362.86 µmol mol^{-1}) were significantly higher than mean of control (353.65 µmol mol^{-1}) treatment, respectively. Mean C_i for USDA-inoculated (366.10 µmol mol^{-1}) soy bean was significantly higher than that of non-inoculated (360.13 µmol mol^{-1}) treatment plants.

Analysis of variance run to determine the effects of aluminium treatments and *Rhizobia* treatments on intercellular carbon concentrations on day 65 after treatments showed that there were statistical significant (p=.05) differences. The mean C_i treatment 750 µM Al (370.75 µmol mol^{-1}) was significantly higher than treatments 960 µM Al (363.28 µmol mol^{-1}), 480 µM Al (357.28 µmol mol^{-1}) and control (352.66 µmol mol^{-1}), respectively. Mean C_i at 480 µM Al was significantly higher compared to the control treatment. Mean C_i of USDA-inoculated (366.92 µmol mol^{-1}) treatment was similarly significantly higher than non-inoculated (355.39 µmol mol^{-1}) treatment.

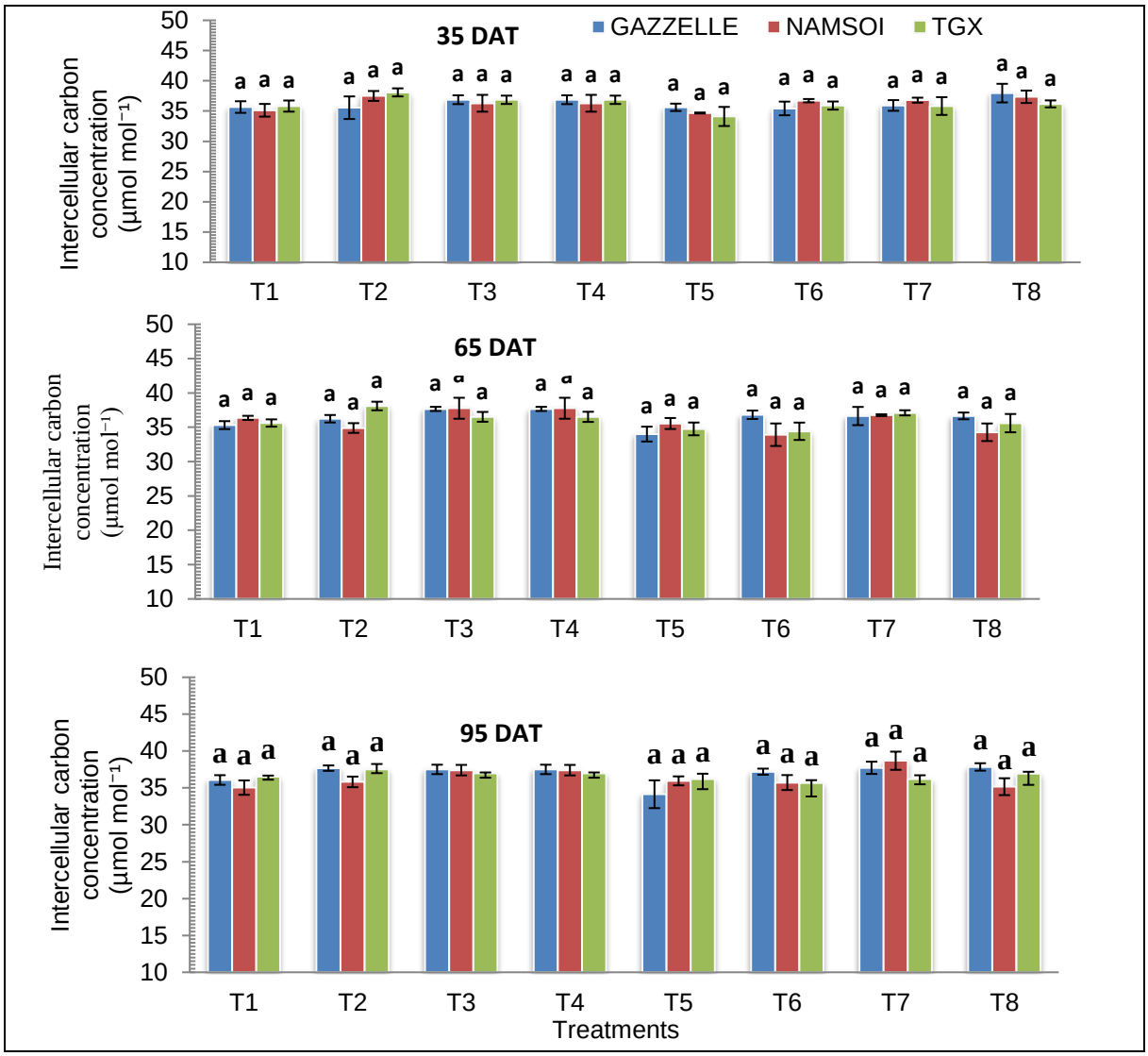

Fig. 1. Intercellular carbon concentration (C_i) in plants of the three soy bean genotypes on 35 DAT, 65 DAT and 95 DAT subjected to various treatments. Values are means of three replicates±SEs. Means with the same latter are not significantly different. Control (Water)*Inoculated (T1), 480µM Al*Inoculated (T2), 750µM Al*Inoculated (T3) and 960 µM Al*Inoculated (T4), Control (T5), 480 µM Al (T6), 750 µM Al (T7) and 960 µM Al (T8)

Analysis of variance run to determine the effects of aluminium treatments on intercellular carbon concentrations on day 95 after treatments showed that there were statistical significant (p=.05) differences. Mean C_i at 750 µM Al (374.18 µmol mol^{-1}) was significantly higher compared to mean at 480 µM Al (365.96 µmol mol^{-1}), 960 µM Al (365.63 µmol mol^{-1}) and Control (356.60 µmol mol^{-1}), respectively. Similarly, mean C_i at 480 µM Al and 960 µM Al were significantly higher than control treatment. Mean C_i for USDA-inoculated (366.62 µmol mol^{-1}) treatment did not show a significant difference compared to mean at non-inoculated (364.56 µmol mol^{-1}).

There were no statistical significant differences in C_i amongst the three genotypes for all the days of measurement.

Net CO_2 Assimilation Rate (A): Fig. 2 shows net CO_2 assimilation rate in the three soy bean genotypes measured on 35 DAT, 65 DAT and 95 DAT. The figure shows that there were no significant differences in net CO_2 assimilation rate between means for NAMSOI, GAZZELLE

and TGX, respectively for the treatments on 35 DAT, 65 DAT and 95 DAT, respectively.

Analysis of variance run to determine the effects of aluminium treatments and genotypes on *A* showed that there were statistical significant (p=.05) differences. Aluminium treatments significantly interacted with genotypes. Mean of *A* at control (7.73 mol.m-² s-¹) was significantly higher than mean at 960 µM Al (5.08 mol.m-² s-¹), 480 µM Al (4.75 mol.m-² s-¹) and 750 µM Al (3.53 mol.m-² s-¹), respectively. Mean for non-inoculated (5.32 mol.m-² s-¹) treatment was not significantly different from that of USDA-inoculated (5.22 mol.m-² s-¹) treatment. Mean of *A* for TGX (5.91 mol.m-² s-¹) was significantly higher than mean for NAMSOI (5.48 mol.m-² s-¹) and GAZZELE (4.43 mol.m-² s-¹), respectively.

Analysis of variance run to determine the effects of aluminium treatments on net CO_2 assimilation on day 35 after treatments showed that there was a statistical significant (p**=.**05) difference. Mean of *A* at control (7.83 mol.m-² s-¹) was significantly higher than mean at 480 µM Al (5.06 mol.m-² s-¹), 750 µM Al (4.53 mol.m-² s-¹) and 960 µM Al (4.05 mol.m-² s-¹), respectively. However, there was no significant difference among the latter three. Mean net CO_2 assimilation for non-inoculated (5.55 mol.m-² s-¹) was not significantly different from USDA-inoculated (5.19 mol.m-² s-¹) treatment. The mean net assimilation rate for TGX (6.02 mol.m-² s-¹) soy bean genotype treated with *Rhizobia* and aluminium were significantly higher than those of genotypes NAMSOI (5.49 mol.m-² s-¹) and GAZZELE (4.60 mol.m-² s-¹), respectively**.**

Analysis of variance run to determine the effects of aluminium treatments on net carbon assimilation on day 65 after treatments showed that there was a statistical significant (p=.05) difference. Mean CO_2 assimilation rate for control (8.12 mol.m-² s-¹) was not significantly from that of 960 µM Al (5.64 mol.m-² s-¹), 480 µM Al (4.47 mol.m-² s-¹) and 750 µM Al (3.60 mol.m-² s-¹), respectively. Mean at 960 µM Al was similarly, significantly higher compared to mean at 480 µM Al. Mean of assimilation at USDA-inoculated (5.62 mol.m-² s-¹), treatment was highly not significantly different from mean of non-inoculated (5.30 mol.m-² s-¹) plants. However, genotype mean for TGX (6.35 mol.m-² s-¹) was significantly higher than those of NAMSOI (5.46 mol.m-² s-¹) and GAZZELE (4.56 mol.m-² s-¹), respectively.

Analysis of variance run to determine the effects of aluminium treatments on net $C0_2$ assimilation on day 95 after treatments showed that there was a statistical significant (p**=**.05) difference. Mean CO_2 assimilation rate at control (7.24 mol.m-² s-¹) was significantly higher than that of treatments 960 µM Al (5.55 mol.m-² s-¹), 480 µM Al (4.72 mol.m-² s-¹) and 750 µM Al (2.47 mol.m-² s-¹), respectively. Similarly, mean at treatment 960 µM Al was significantly higher than 480 µM Al and 750 µM Al, respectively. Mean at 480 µM Al was significantly higher than that of mean at 750 µM Al treatment. Mean of net CO_2 assimilation rate of non-inoculated (5.13 mol.m-² s-¹) was not significantly different from that of USDA-inoculated (4.86 mol.m-² s-¹). Mean CO_2 assimilation rate of NAMSOI (5.50 mol.m-² s-¹) genotype was not significantly different than that of TGX (5.35 mol.m-² s-¹) and at GAZZELE (4.13 mol.m-² s-¹), respectively.

Net Photosynthesis (P*n*): Fig. 3 shows net photosynthesis in the three soy bean genotypes measured on 35 DAT, 65 DAT and 95 DAT. The figure shows that there were no significant differences between means of Pn for GAZZELLE, NAMSOI and TGX soy bean genotypes on 35 DAT, 65 DAT and 95 DAT.

Analysis of variance run to determine the effects of aluminium treatments on net photosynthesis showed that there was a statistical significant (p<.01) difference. The mean of net photosynthesis at control (406.82 µmol mol⁻¹) was significantly higher than those at 750 µM Al (396.64 µmol mol⁻¹), 750 µM Al (395.90 µmol mol⁻¹) and 960 µM Al (395.17 µmol mol⁻¹), respectively. Contrarily, mean P*n* for USDA-inoculated (399.68 µmol mol⁻¹) treatment was not significantly different from that of non-inoculated (397.59 µmol mol⁻¹). Means at TGX (399.6µmol mol⁻¹), NAMSOI (398.66 µmol mol⁻¹) and GAZZELE (397.55 µmol mol⁻¹) were not significantly different from each other.

Analysis of variance run to determine the effects of aluminium treatments on P*n* on day 35 after treatments showed that there was a statistical significant (p=**.**05) difference. The Pn mean at control (409.43 µmol mol⁻¹) was significantly higher than mean at 750 µM Al (401.57 µmol mol⁻¹), 480 µM Al (397.10 µmol mol⁻¹) and 960 µM Al (391.41 µmol mol⁻¹), respectively. Mean Pn at 750 µM Al was also significantly higher than 480 µM Al and 960 µM Al treatments, respectively. Contrarily, mean of P*n* for USDA-inoculated (401.23 µmol mol⁻¹) was not

significantly different NAMSOI (399.31 µmol mol^{-1}) and TGX (399.25 µmol mol^{-1}) were not significantly different from each other.

Analysis of variance run to determine the effects of aluminium treatments on P*n* on day 65 after treatments showed that there was a statistical significant (p=.05) difference. Mean of Pn for control (405.64 µmol mol^{-1}) was significantly higher than that of 960 µM Al (396.65 µmol mol^{-1}), 480 µM Al (395.67 µmol mol^{-1}) and 750 µM Al (391.80 µmol mol^{-1}) treatments, respectively. Mean at 960 µM Al was also significantly higher than those of 480 µM Al and 750 µM Al treatments, respectively. However, mean P*n* of USDA-inoculated (397.85 µmol mol^{-1}) was not significantly different than that of non-inoculated (397.03 µmol mol^{-1}). It was further observed that, means of genotypes GAZZELE (399.27 µmol mol^{-1}), NAMSOI (397.24 µmol mol^{-1}) and at TGX (395.81 µmol mol^{-1}) did not show significant differences among themselves.

Stomatal Conductance (g_i): Fig. 4 shows stomatal conductance of the three soy bean genotypes measured on 35 DAT, 65 DAT and 95 DAT. The figure shows that the mean of g_l for genotype NAMSOI was significantly higher than those of GAZZELLE and TGX, respectively at treatment T2 on 35 DAT. Similarly, mean g_i for GAZZELLE genotype was significantly higher than those of TGX and NAMSOI for treatment 8 (T8) on 65 DAT.

Analysis of variance run to determine the effects of aluminium treatments on g_l showed that there was a statistical significant (p=.05) difference. Mean of g_i for control (0.57 mol m^{-2} s^{-1}) was significantly higher than mean at 480 µM Al (0.51 mol m^{-2} s^{-1}), 960 µM Al (0.51 mol m^{-2} s^{-1}) and 750 µM Al (0.45 mol m^{-2} s^{-1}) treatments, respectively. Similarly, mean at 750 µM Al treatment was significantly higher than those at 960 µM Al and 480 µM Al. Mean g_l for USDA-inoculated (0.53 mol m^{-2} s^{-1}) plants was significantly higher than that of non -inoculated (0.48 mol m^{-2} s^{-1}). Contrarily, no significant differences in g_l were observed among GAZZELE (0.54 mol m^{-2} s^{-1}), TGX (0.49 mol m^{-2} s^{-1}) and NAMSOI (0.49 mol m^{-2} s^{-1}).

On 35 DAT, The mean g_l of USDA-inoculated (0.53 mol m^{-2} s^{-1}) plants was significantly higher than of non-inoculated (0.46 mol m^{-2} s^{-1}).

Analysis of variance run to determine the effects of aluminium treatments on g_l on day 65 after treatments showed that there was a statistical significant (p=.05) difference. Mean of g_i for control (0.64 mol m^{-2} s^{-1}) treatment was significantly higher than mean at 960 µM Al (0.52 mol m^{-2} s^{-1}), 480 µM Al (0.46 mol m^{-2} s^{-1}) and 750 µM Al (0.45 mol m^{-2} s^{-1}), respectively. Mean of g_l at 960 µM Al was significantly lower than those of 480 µM Al and 750 µM Al treatment, respectively. Similarly, g_i at 480 µM Al was significantly higher than that at 750 µM Al treatment. The g_l of USDA-inoculated (0.55 mol m^{-2} s^{-1}) plants was significantly higher than non-inoculated (0.48 mol m^{-2} s^{-1}).

Transpiration Rate (*E*): Fig. 5 shows transpiration rate in the three soy bean genotypes measured on day 35 after treatment (35DAT), 65 DAT and 95 DAT. The figure shows that there were significant differences in *E* among NAMSOI, TGX and GAZZELLE at treatments T2 on 35 DAT. Meanwhile, significant differences for mean of *E* were observed for TGX, GAZZELLE and NAMSOI at treatment 5 (T5).

Analysis of variance run to determine the effects of aluminium treatments on *E* showed that there was a statistical significant (p=.05) difference. Mean for control (0.013 mol.m^{-2} s^{-1}) treatment was significantly higher than those at treatments 480 µM Al (0.0127 mol.m^{-2} s^{-1}), 960 µM Al (0.0116 mol.m^{-2} s^{-1}) and 750 µM Al (0.0115 mol.m^{-2} s^{-1}), respectively. Treatment for *E* mean at 480 µM Al was significantly higher than those of 750 µM Al and 960 µM Al. There was no significant difference between USDA-inoculated (0.0126 mol.m^{-2} s^{-1}) soy bean plants and non-inoculated (0.0119 mol.m^{-2} s^{-1}). Similarly, there were no differences among the three genotypes TGX (0.0129 mol.m^{-2} s^{-1}), NAMSOI (0.012 mol.m^{-2} s^{-1}) and GAZZELE (0.12 mol.m^{-2} s^{-1}).

Analysis of variance run showed a statistically significant (p=.05) interaction between effects of aluminium treatments and the effects of three genotypes on *E* on day 35 after treatments. Means of *E* at control (0.0129 mol.m^{-2} s^{-1}), 480 µM Al (0.0125 mol.m^{-2} s^{-1}), 960 µM Al (0.011 mol.m^{-2} s^{-1}) and at 750 µM Al (0.0109 mol.m^{-2} s^{-1}) did not show significant differences. Similarly, *E* mean of USDA-inoculated (0.0126 mol.m^{-2} s^{-1}) plants was not significantly different than that of non-inoculated (0.0124 mol.m^{-2} s^{-1}). There were no significant differences among genotypes TGX (0.0127 mol.m^{-2} s^{-1}), GAZZELE (0.0125 mol.m^{-2}

s-1) and NAMSOI (0.0118 mol.m-2 s-1). On day 65 after treatment, mean *E* at treatments; control (0.0134 mol.m-2 s-1), 480 µM Al (0.0122 mol.m-2 s-1), 750 µM Al (0.0122 mol.m-2 s-1) and 960 µM Al (0.012 mol.m-2 s-1) did not show significant differences. Mean of USDA-inoculated (0.0125 mol.m-2 s-1) plants was not significantly different than that of non-inoculated (0.0124 mol.m-2 s-1). Similarly, means for genotypes TGX (0.013 mol.m-2 s-1), NAMSOI (0.0125 mol.m-2 s-1) and GAZZELE (0.118 mol.m-2 s-1) were not significantly different among themselves.

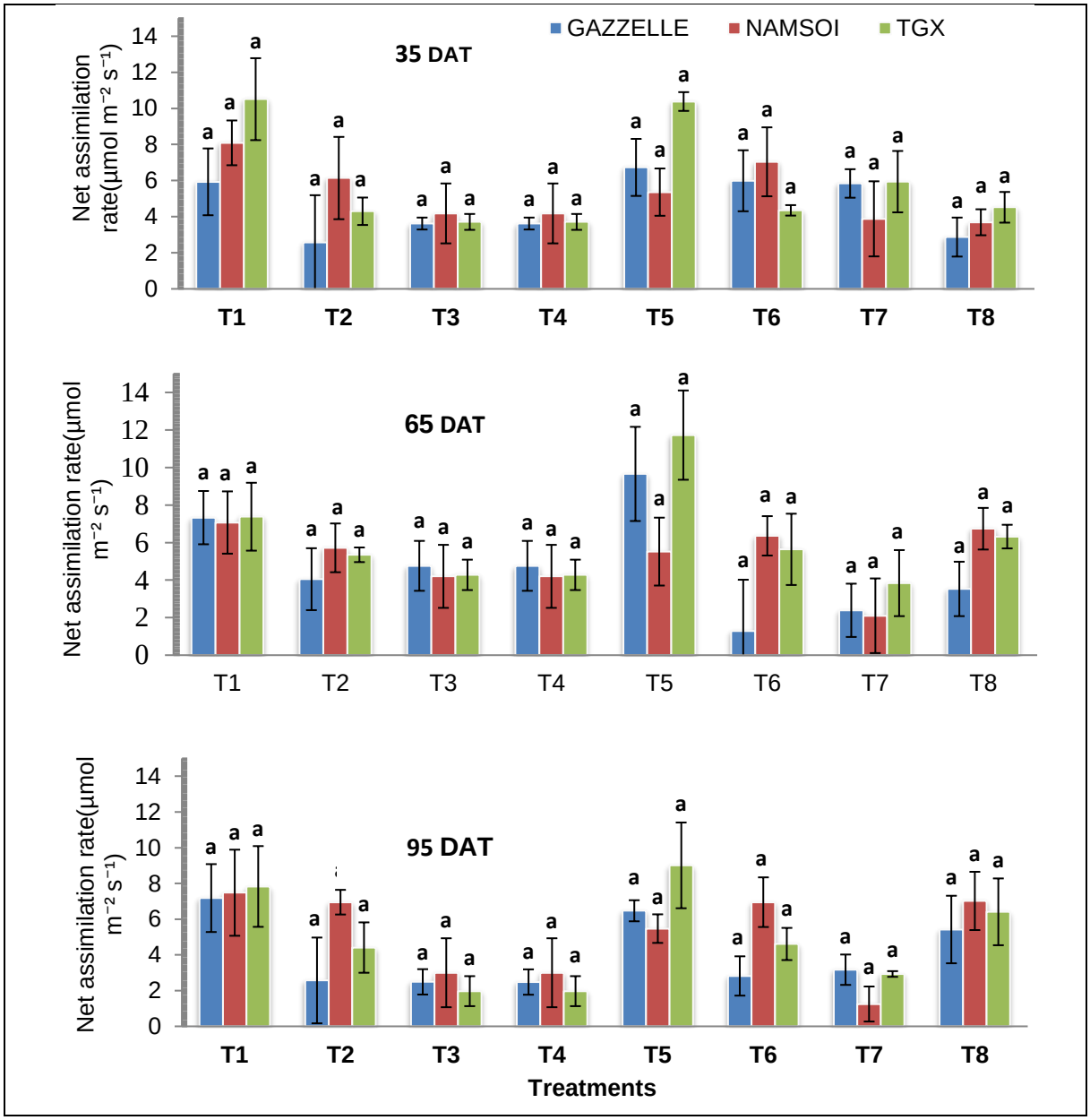

Fig. 2. Net photosynthetic CO_2 assimilation rate (*A*) in plants of the three soy bean genotypes on 35 DAT, 65 DAT and 95 DAT subjected to various treatments. Values are means of three replicates±SEs. Means with the same latter are not significantly different. Control (Water)*Inoculated (T1), 480 µM Al*Inoculated (T2), 750 µM Al*Inoculated (T3) and 960 µM Al*Inoculated (T4), Control (T5), 480 µM Al (T6), 750 µM Al (T7) and 960µM Al (T8)

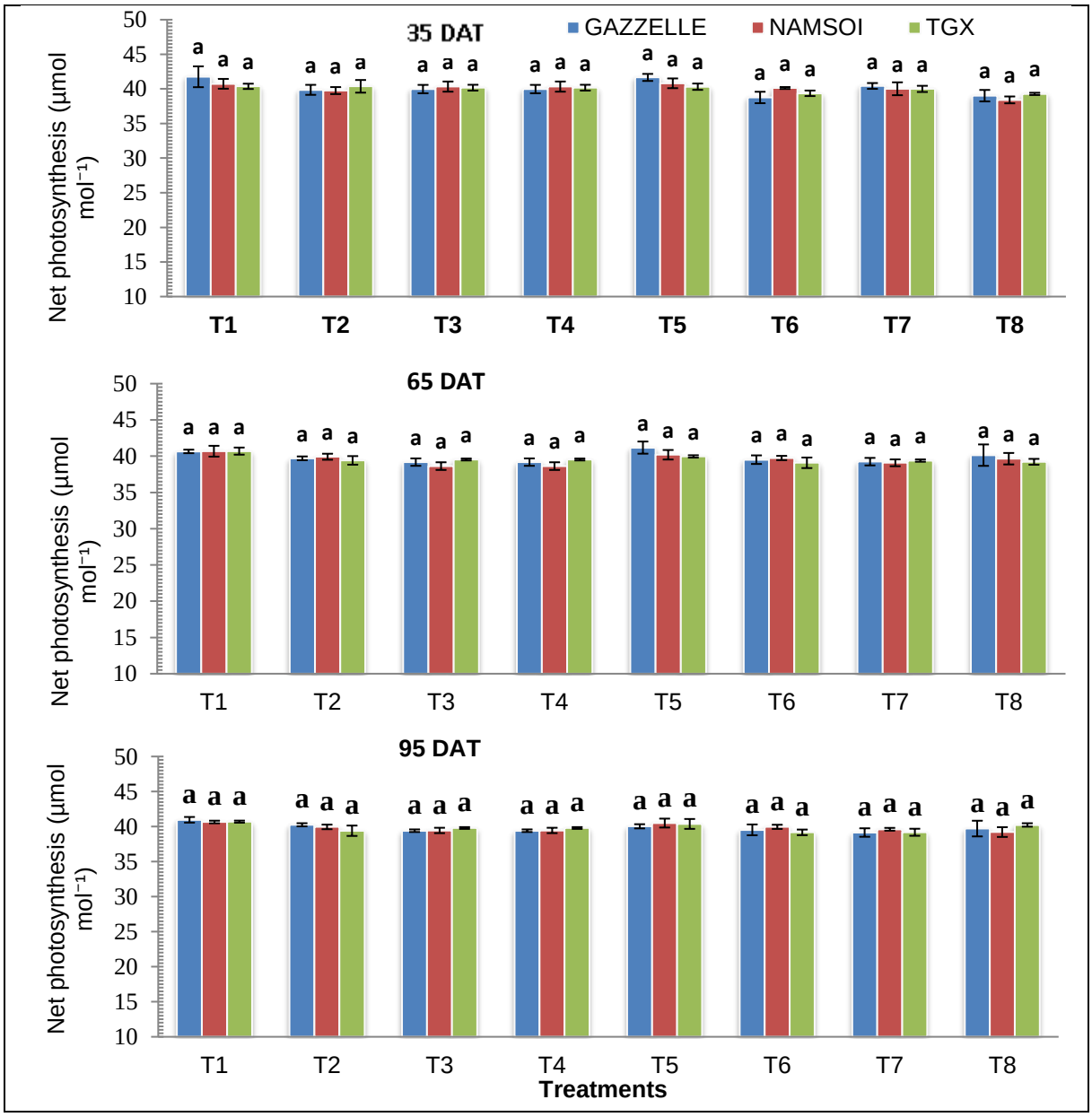

Fig. 3. Net photosynthesis (P*n*) in plants of the three soy bean genotypes on 35 DAT, 65 DAT and 95 DAT subjected to various treatments. Values are means of three replicates±SEs. Means with the same latter are not significantly different. Control (Water)*Inoculated (T1), 480 µM Al*Inoculated (T2), 750 µM Al*Inoculated (T3) and 960 µM Al*Inoculated (T4), Control (T5), 480µM Al (T6), 750 µM Al (T7) and 960 µM Al (T8)

3.2 Plant Photosynthetic Pigment Concentrations

Chlorophyll *a* Concentration: Fig. 6 shows chlorophyll *a* concentration in the three soy bean genotypes for 39 DAT, 73 DAT, 87 DAT and 101 DAT. The figure shows that mean of Chl. *a* for GAZZELLE was significantly higher than that of NAMSOI and TGX on 39 DAT at the following treatments T1, T3, T5 and T8; on 73 DAT at treatment T1, on 87 DAT at treatments T1, T3 and T5 and on 101 DAT at treatments T1, T3, T5 and T8, respectively. Mean for NAMSOI was significantly higher compared to mean for GAZZELLE and TGX on 73 DAT at treatment T5 and on 87 DAT at treatment T8.

A three way analysis of variance run to determine the effects of *Rhizobia* treatments, aluminium treatments and genotypes on Chl. *a* in plants

showed that there was a statistical significant (p=.01) three way interaction amongst them. Mean of Chl. *a* at control (3.80 mg.g^{-1}) treatment was significantly higher than those at 750 µM Al (3.61 mg.g^{-1}), 480 µM Al (3.49 mg.g^{-1}) and 960 µM Al (3.24 mg.g^{-1}), respectively. Means of Chl. *a* at 750 µM Al and 480 µM Al were also significantly higher than 960 µM Al. Meanwhile, mean for USDA-inoculated (3.62 mg.g^{-1}) was not significantly different from mean for non-inoculated (3.45 mg.g^{-1}). Mean of Chl. *a* for GAZZELE (4.52 mg.g^{-1}) soy bean genotype treated with *Rhizobia* and Al was significantly higher than those of NAMSOI (3.29 mg.g^{-1}) and TGX (2.80 mg.g^{-1}), respectively. Similarly, mean for NAMSOI was significantly higher than that of TGX.

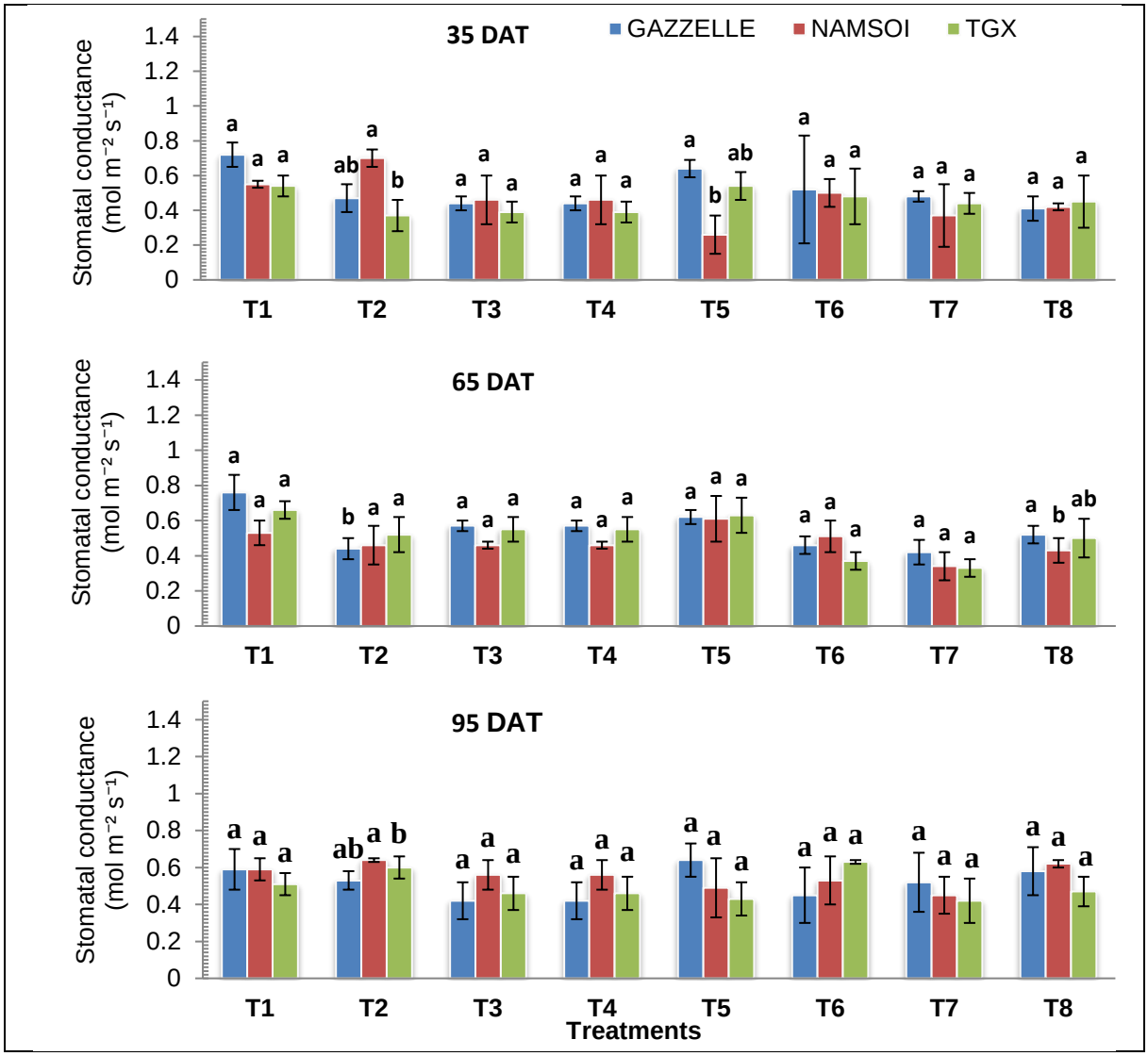

Fig. 4. Stomatal conductance (g_i) in plants of the three soy bean genotypes on 35 DAT, 65 DAT and 95 DAT subjected to various treatments. Values are means of three replicates±SEs. Means with the same latter are not significantly different. Control (Water)*Inoculated (T1), 480µM Al*Inoculated (T2), 750 µM Al*Inoculated (T3) and 960 µM Al*Inoculated (T4), Control (T5), 480 µM Al (T6), 750 µM Al (T7) and 960 µM Al (T8)

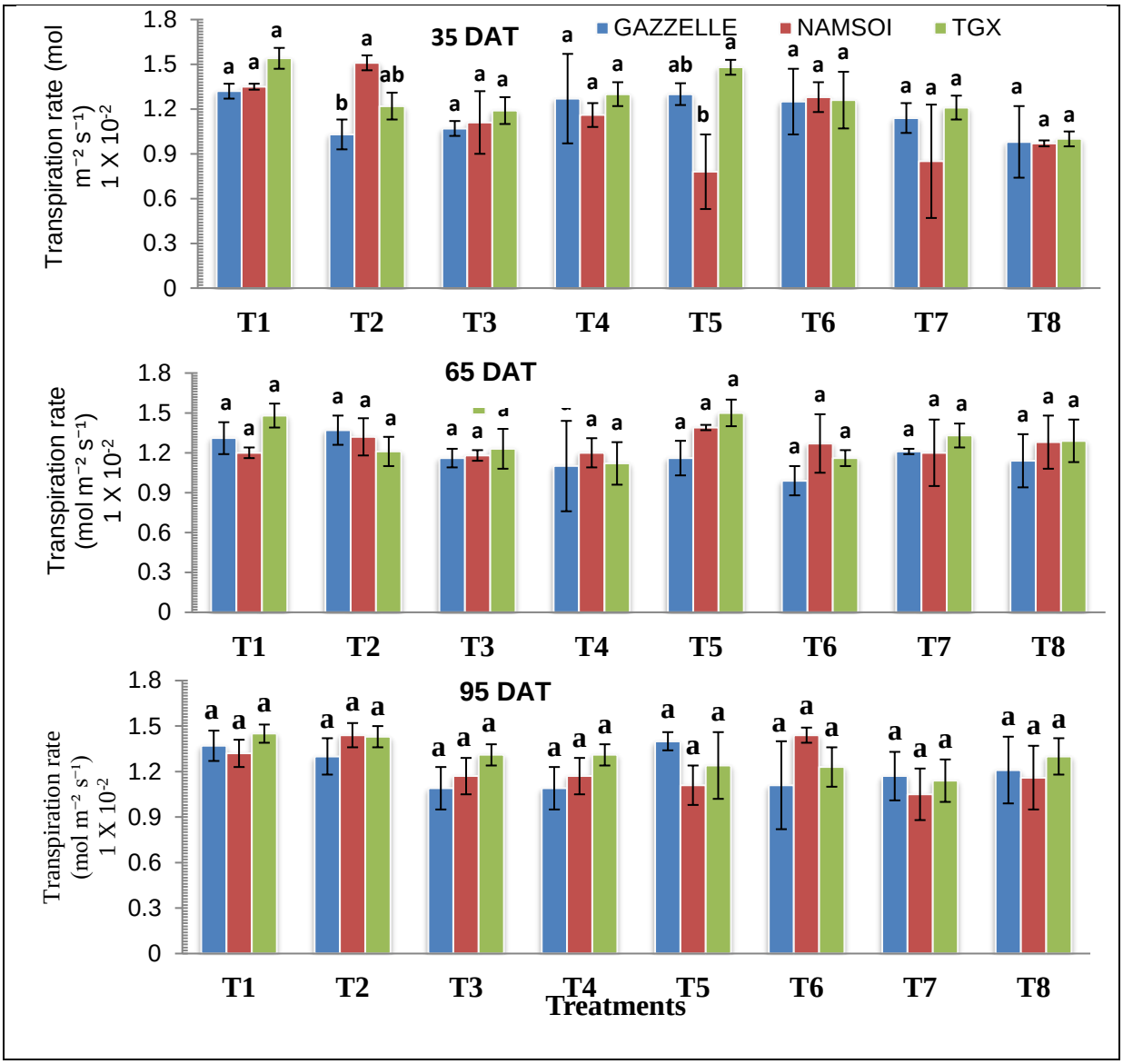

Fig. 5. Transpiration rate (*E*) in plants of the three soy bean genotypes on 35 DAT, 65 DAT and 95 DAT subjected to various treatments. Values are means of three replicates±SEs. Means with the same latter are not significantly different. Control (Water)*Inoculated (T1), 480µM Al*Inoculated (T2), 750µM Al*Inoculated (T3) and 960µM Al*Inoculated (T4), Control (T5), 480µM Al (T6), 750µM Al (T7) and 960µM Al (T8)

A three way analysis of variance run to determine the effects of *Rhizobia* treatments, aluminium treatments and genotypes on Chl. *a* in plants on 39 day after treatment showed that there was a statistical significant (p<.01) three way interaction amongst them. Mean of for control (2.37 ml/g) treatment was significantly higher than those means at 480 µM Al (2.10 mg.g^{-1}), 750 µM Al (2.01 mg.g^{-1}) and 960 µM Al (1.61 mg.g^{-1}), respectively. Mean of Chl. *a* at 960 µM Al was significantly lower than those at 480 µM Al and 750 µM Al, respectively. However, mean of USDA-inoculated (2.22 mg.g^{-1}) plants was not significantly different from that of non-inoculated (1.82 mg.g^{-1}). Mean of Chl. *a* for GAZZELE (2.90 mg.g^{-1}) soy bean genotype treated with *Rhizobia* and Al was significantly higher than those of TGX (1.77 mg.g^{-1}) and NAMSOI (1.39 mg.g^{-1}), respectively.

A three way analysis of variance run to determine the effects of *Rhizobia* treatments, aluminium treatments and genotypes on Chl. *a* in plants on 73 day after treatment showed that there was a

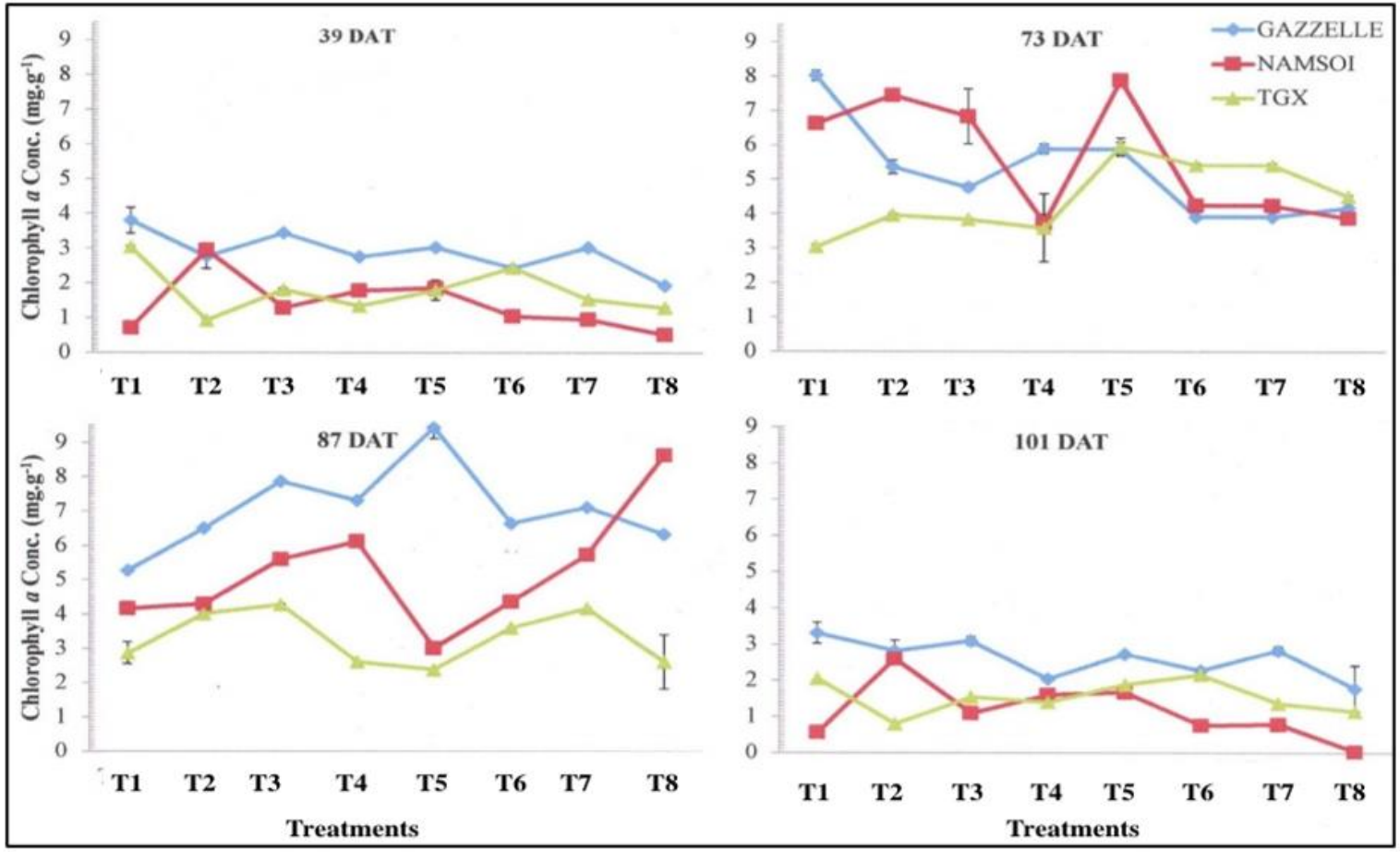

Fig. 6. Chlorophyll *a* concentration in plants of the three soy bean genotypes on 39 DAT, 73 DAT, 87 DAT and 101 DAT subjected to various treatments. Values are means of three replicates±SEs. Control (Water)*Inoculated (T1), 480 µM Al*Inoculated (T2), 750 µM Al*Inoculated (T3) and 960µM Al*Inoculated (T4), Control (T5), 480 µM Al (T6), 750 µM Al (T7) and 960 µM Al (T8)

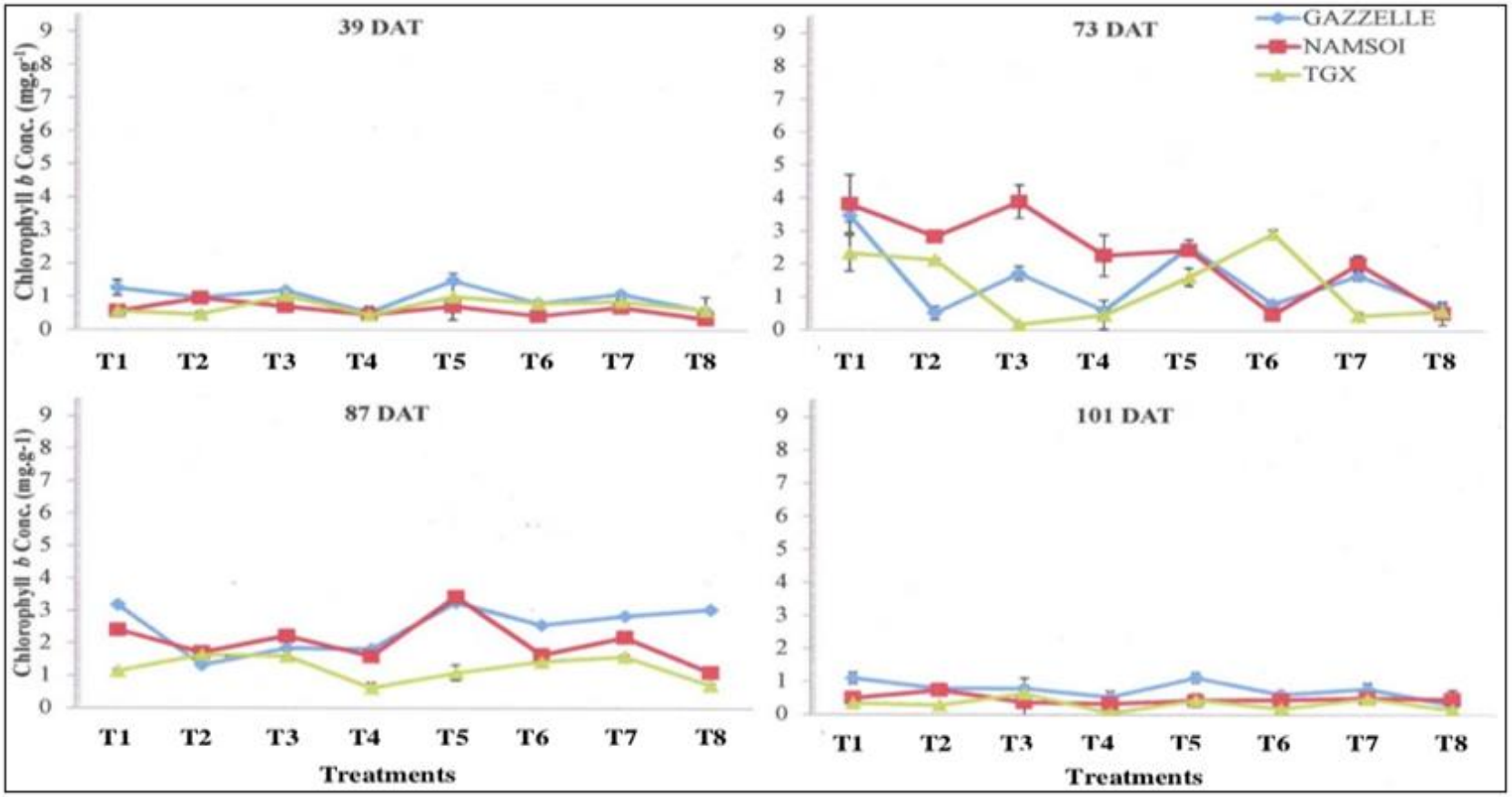

Fig. 7. Chlorophyll *b* concentration in plant of three soy bean genotypes on 39 DAT, 73 DAT, 87 DAT and 101 DAT subjected to various treatments. Values are means of three replicates±SEs. Control (Water)*Inoculated (T1), 480 µM Al*Inoculated (T2), 750 µM Al*Inoculated (T3) and 960 µM Al*Inoculated (T4), Control (T5), 480 µM Al (T6), 750 µM Al (T7) and 960 µM Al (T8)

statistical significant (p<.01) three way interaction amongst them. However, chlorophyll *a* concentration showed a significantly higher (p<.01) interaction between treatments and the genotypes**.** Mean of Chl. *a* at Control (6.24 $mg.g^{-1}$) was significantly higher than treatment means at 480 µM Al (5.05 $mg.g^{-1}$), 750 µM Al (4.84 $mg.g^{-1}$) and 960 µM Al (4.34 $mg.g^{-1}$), respectively. Moreover, mean for GAZZELE (5.50 $mg.g^{-1}$) soy bean genotype was significantly higher than that of NAMSOI (5.32 $mg.g^{-1}$) and TGX (4.53 $mg.g^{-1}$), respectively.

A three way analysis of variance run to determine the effects of *Rhizobia* treatments, aluminium treatments and genotypes on Chl. *a* in plants on 87 day after treatment showed that there was a statistical significant (p<.01) three way interaction amongst them. Means of Chl. *a* at treatments 750 µM Al (5.81 $mg.g^{-1}$) and at 960 µM Al (5.62 $mg.g^{-1}$) were significantly higher than those of treatments 480 µM Al (4.92 $mg.g^{-1}$) and control (4.53 $mg.g^{-1}$). Mean of USDA-inoculated (5.35 $mg.g^{-1}$) was significantly higher than that of non-inoculated (5.08 $mg.g^{-1}$). Mean for GAZZELE (7.07 $mg.g^{-1}$) genotype was significantly higher than that of NAMSOI (5.25 $mg.g^{-1}$) and TGX (3.33 $mg.g^{-1}$), respectively.

A three way analysis of variance run to determine the effects of *Rhizobia* treatments, aluminium treatments and genotypes on Chl. *a* in plants on 101 day after treatment showed that there was a statistical significant (p<.01) three way interaction amongst them. On DAT 101, chlorophyll *a* concentration in plants was significantly higher (p<.01) among eight treatments, genotypes and also when treatments interacted with genotypes**.** Mean of Chl *a* at control (2.04 $mg.g^{-1}$) was significantly higher than mean treatments at 480 µM Al (1.90 $mg.g^{-1}$), 750 µM Al (1.79 $mg.g^{-1}$) and 960 µM Al (1.40 $mg.g^{-1}$). Mean of Chl *a* at 960 µM Al was significantly lower than means at 480 µM Al and 750 µM Al. Mean of USDA-inoculated (1.91 $mg.g^{-1}$) plants was also significantly higher than that of non-inoculated (1.66 $mg.g^{-1}$). Moreover, mean of genotype GAZZELE (2.61 $mg.g^{-1}$) was significantly higher compared to mean TGX (1.55 $mg.g^{-1}$) and NAMSOI (1.20 $mg.g^{-1}$), respectively.

Chlorophyll *b* concentration: Fig. 7 shows chlorophyll *b* concentration in the three soy bean genotypes on 39 DAT, 73 DAT, 87 DAT and 101 DAT. The figure shows that mean of Chl. *b* for GAZZELLE genotype was significantly higher than that of NAMSOI and TGX on 39 DAT for treatments T1, T3 and T5; on 73 DAT for treatment T5; on 87 DAT for treatments T1, T5 and T8 and on 101 DAT for treatments T1, T3 and T5. Mean Chl. *b* for NAMSOI genotype was significantly higher than GAZZELLE and TGX on 73 DAT for treatment T3 and on 87 DAT for treatment T3.

A three way analysis of variance run to determine the effects of *Rhizobia* treatments, aluminium treatments and genotypes on Chl. *b* in plants showed that there was a statistical significant (p=.05) three way interaction amongst them. Mean for Chl. *b* at control (1.68 $mg.g^{-1}$) treatment was significantly higher than those at 750 µM Al (1.30 $mg.g^{-1}$), 480 µM Al (1.15 $mg.g^{-1}$) and 960 µM Al (0.79 $mg.g^{-1}$), respectively. Mean at 960 µM Al was significantly lower than treatment means at 750 µM Al and 480 µM Al. However, mean at USDA-inoculated (1.27 $mg.g^{-1}$) had no significant difference compared to non-inoculated (1.19 $mg.g^{-1}$) treatment. Contrarily, means of Chl*. b* for GAZZELE (1.43 $mg.g^{-1}$) and NAMSOI (1.35 $mg.g^{-1}$) soy bean genotypes treated with *Rhizobia* and Al was significantly higher than that of TGX (2.80 $mg.g^{-1}$), respectively. Analysis of variance run to determine the effects of aluminium treatments and genotypes on Chl. *b* in plants on day 39 after treatment showed that there was a statistical significant (p<.01) interaction between them. Mean at 960 µM Al (0.49 $mg.g^{-1}$) was significantly lower than those at control (0.93 $mg.g^{-1}$), 750 µM Al (0.92 $mg.g^{-1}$) and 480 µM Al (0.74 $mg.g^{-1}$), respectively. However, mean of non-inoculated (0.78 $mg.g^{-1}$) was not significantly different from that of USDA-inoculated (0.76 $mg.g^{-1}$) soy bean plants. Mean of *Chl. b* for GAZZELE (0.99 $mg.g^{-1}$) soy bean genotype treated with *Rhizobia* and Al was significantly higher than those of TGX (0.72 $mg.g^{-1}$) and NAMSOI (0.61 $mg.g^{-1}$), respectively.

Analysis of variance run to determine the effects of *Rhizobia* treatments, aluminium treatments and genotypes on Chl. *b* in plants on day 73 after treatment showed that there were statistical significant (p<.01) interaction between *Rhizobia* and genotype and, between aluminium treatments and genotypes. Means for Chl. *b* at control (2.69 $mg.g^{-1}$) was significantly higher than those of treatments 480 µM Al (1.62 $mg.g^{-1}$) and 960 µM Al (0.86 $mg.g^{-1}$), respectively. Mean at 960 µM Al was significantly lower than means at 750 µM Al and 480 µM Al. Moreover, mean of USDA- inoculated (2.02 $mg.g^{-1}$) soy bean plants was significant higher than that of non-inoculated (1.4 $mg.g^{-1}$g) plants. Mean of Chl. *b* for NAMSOI

(2.29 mg.g^{-1}) soy bean genotype treated with *Rhizobia* and Al was significantly higher than those of GAZZELE (1.50 mg.g^{-1}) and TGX (1.34 mg.g^{-1}) genotypes, respectively. A three way analysis of variance run to determine the effects of *Rhizobia* treatments, aluminium treatments and genotypes on Chl. *b* in plants on day 87 after treatment showed that there was a statistical significant (p<.01) interaction amongst them. Means of Chl. *b* for control (2.42 mg.g^{-1}) was significantly higher than means at treatments 750 µM Al (2.05 mg.g^{-1}), 480 µM Al (1.72 mg.g^{-1}) and 960 µM Al (1.48 mg.g^{-1}), respectively. Mean at 960 µM Al was significantly lower than that at 750 µM Al and 480 µM Al, respectively. Similarly, mean of Chl. *b* in non-inoculated (2.08 mg.g^{-1}) plants was significantly higher than that of USDA-inoculated (1.76 mg.g^{-1}) plants. Mean of Chl*. b* for GAZZELE (2.49 mg.g^{-1}) soy bean genotype treated with *Rhizobia* and Al was significantly higher than those of TGX (2.04 mg.g^{-1}) and NAMSOI (1.23 mg.g^{-1}) genotypes, respectively.

A three way analysis of variance run to determine the effects of *Rhizobia* treatments, aluminium treatments and genotypes on Chl. *b* in plants on day 101 after treatment showed that there was a statistical significant (p<.01) interaction amongst them. However, the parameter was significantly higher among genotypes (p<.01). Mean at treatment 960 µM Al (0.32 mg.g^{-1}) was significantly lower than at control (0.66 mg.g^{-1}), 750 µM Al (0.60 mg.g^{-1}) and 480 µM Al (0.51 mg.g^{-1}), respectively. Mean for USDA-inoculated (0.54 mg.g^{-1}) plants was not significantly different from non-inoculated (0.51 mg.g^{-1}). However, mean of *Chl. b* for GAZZELE (0.76 mg.g^{-1}) soy bean genotype treated with *Rhizobia* and Al was significantly higher than those of NAMSOI (0.48 mg.g^{-1}) and TGX (0.33 mg.g^{-1}) genotypes, respectively.

Chlorophyll *a*/*b* ratio; Fig. 8 shows chlorophyll *a*/*b* ratio of the three soy bean genotypes on 39 DAT, 73 DAT, 87 DAT and 101 DAT. The figure shows that mean for Chl. *a*/*b* ratio of genotype GAZZELLE was significantly higher than for NAMSOI and TGX on 39 DAT at treatments T3 and T5, and on 87 DAT at treatment T3. The mean for TGX was similarly significantly higher than those of GAZZELLE and NAMSOI on 39 DAT at treatments T1 and T8. However, means for genotypes TGX and NAMSOI were significantly higher than GAZZELLE on 87 DAT at treatment T8.

A three way analysis of variance run to determine the effects of *Rhizobia* treatments, aluminium treatments and genotypes on Chl. *a/b* in plants showed that there was a statistical significant (p=.05) interaction amongst them. Mean Chl. *a/b* at control (4.82) was significantly higher than means at 960 µM Al (3.73), 750 µM Al (3.59) and 480 µM Al (3.31), respectively. Mean Chl. *a/b* at treatments 960 µM Al and 750 µM Al were significantly higher than at 480 µM Al. However, mean of USDA-inoculated (4.11) plants was not significantly different from non-inoculated (3.61). Mean of *Chl. a/b* for TGX (5.07) soy bean genotype treated with *Rhizobia* and Al was significantly higher than those of GAZZELE (3.62) and NAMSOI (2.90), respectively. A three way analysis of variance run to determine the effects of *Rhizobia* treatments, aluminium treatments and genotypes on Chl. *a/b* in plants on day 39 after treatment showed that there was a statistical significant (p=.05) interaction amongst them. Means at control (3.4) was significantly higher than those at treatments 960 µM Al (2.90), 480 µM Al (2.85) and 750 µM Al (2.11), respectively. Mean at treatment 750 µM Al was significantly lower than those at 960 µM Al and at 480 µM Al. However, mean Chl. *a*/*b* ratio of USDA-inoculated (3.09) soy bean plants was not significantly different than of non-inoculated (2.54). Mean of Chl. *a/b* for GAZZELE (3.09) soy bean genotype treated with *Rhizobia* and Al was significantly higher than those of TGX (3.02) and NAMSOI (2.33). The mean for TGX was significantly higher than NAMSOI genotype.

Analysis of variance run to determine the effects of aluminium treatments and genotypes on Chl. *a/b* in plants on day 73 after treatment showed that there was a statistical significant (p=.05) interaction between them. Mean chl. *a/b* at control (6.99) was significantly higher than those at 750 µM Al (6.3), 960 µM Al (5.50) and 480 µM Al (2.89), respectively. Mean at treatment 480 µM Al was also significantly lower than those of 960 µM Al and 750 µM Al, respectively. Contrarily, mean of USDA-inoculated (5.43) soy bean plants was not significantly different than non-inoculated (5.4). Moreover, mean for TGX (7.97) was significant higher than for GAZZELE (4.83) and NAMSOI (3.80), respectively. Analysis of variance run to determine the effects of aluminium treatments and genotypes on Chl. *a/b* in plants on day 87 after treatment showed that there was a statistical significant (p=.05) interaction between them. Means at control (3.37) was significantly higher than at 480 µM Al (2.95), 750 µM Al (2.88) and 960 µM Al (2.37),

respectively. Mean at 960 µM Al was significantly lower than that of 750 µM Al and 480 µM Al, respectively. Mean for USDA-inoculated (3.13) was also significantly higher than mean for non-inoculated (2.64) treatments. Mean of Chl. *a/b* for GAZZELE (3.07) soy bean genotype treated with *Rhizobia* and Al was significantly higher than those of TGX (3.01) and NAMSOI (2.60). Furthermore, the mean for TGX was significantly higher than NAMSOI genotype. On day 101 after treatment, ANOVA run to determine the effects of genotypes on chlorophyll a*/b* ratio in plants showed that there was a significant ($p<0.05$) difference. The mean of Chl. *a/b* for genotype TGX (6.28 $mg.g^{-1}$) was significant higher than for GAZZELE (3.82) and NAMSOI (2.87), respectively.

Total chlorophyll concentrations; Fig. 9 shows total chlorophyll concentration of the three soy bean genotypes on 39 DAT, 73 DAT, 87 DAT and 101 DAT. The figure shows that mean for Chl. *a+b* of GAZZELLE soy bean genotypes was significantly higher than for NAMSOI and TGX on 39 DAT at treatments T1, T3 and T8; on 73 DAT at treatment T1); on 87 DAT at treatment T1, T3 and T5; and on 101 DAT at treatment T1, T3, T5 and T8. Furthermore, mean for genotype NAMSOI was significantly higher than means for genotypes GAZZELLE and TGX on 73 DAT at treatments T3 and T5 and on 87 DAT at treatment T8. A three way analysis of variance run to determine the effects of *Rhizobia* treatments, aluminium treatments and genotypes on total Chl. in plants showed that there was a statistical significant ($p=.05$) interaction amongst them. The total chlorophyll mean of USDA-inoculated (4.97 $mg.g^{-1}$) plants was significantly higher than mean of non-inoculated (4.58 mg/g). Means for GAZZELE (5.92 $mg.g^{-1}$), NAMSOI (4.55 $mg.g^{-1}$) and TGX (3.84 $mg.g^{-1}$) were not significantly different from each other.

A three way analysis of variance run to determine the effects of *Rhizobia* treatments, aluminium treatments and genotypes on total Chl. in plants on day 39 after treatment showed that there was a statistical significant ($p=.05$) interaction amongst them. The mean of total chlorophyll at control (3.12 $mg.g^{-1}$) was significantly higher than those of treatments 750 µM Al (2.94 $mg.g^{-1}$), 480 µM Al (2.84 $mg.g^{-1}$) and 960 µM Al (2.16 $mg.g^{-1}$), respectively. Mean at 960 µM Al treatment was significantly lower than 750 µM Al and 480 µM Al, respectively. Mean at USDA-inoculated (2.98 $mg.g^{-1}$) was also significantly higher than that of non-inoculated (2.58 $mg.g^{-1}$) treatments. Moreover, mean of *Chl. a+b* for GAZZELE (3.89 mg/g) soy bean genotype treated with *Rhizobia* and Al was significantly higher than those of TGX (2.47 $mg.g^{-1}$) and NAMSOI (2.00 $mg.g^{-1}$), respectively. A three way analysis of variance run to determine the effects of *Rhizobia* treatments, aluminium treatments and genotypes on total Chl. in plants on day 73 after treatment showed that there was a statistical significant ($p=.05$) interaction amongst them.

A three way analysis of variance run to determine the effects of *Rhizobia* treatments, aluminium treatments and genotypes on total Chl. in plants on day 87 after treatment showed that there was a statistical significant ($p<.01$) interaction amongst them. The mean of total chlorophyll at treatments 960 µM Al (7.86 $mg.g^{-1}$) and 750 µM Al (7.82 $mg.g^{-1}$) were significantly different than mean at either 480 µM Al (6.64 $mg.g^{-1}$) and control (6.04 $mg.g^{-1}$), respectively. Mean at control was significantly lower compared to mean at 480 µM Al. Mean at 480 µM Al was significantly higher compared to mean at either 960 µM Al and 750 µM Al. Moreover, mean at non-inoculated (7.34 $mg.g^{-1}$) was significantly higher than mean at USDA-inoculated (6.84 $mg.g^{-1}$) plants. Furthermore, mean for GAZZELE (9.56 $mg.g^{-1}$) was significantly higher than mean for NAMSOI (7.29 $mg.g^{-1}$) and TGX (4.42 $mg.g^{-1}$), respectively.

A three way analysis of variance run to determine the effects of *Rhizobia* treatments, aluminium treatments and genotypes on total Chl. in plants on day 101 after treatment showed that there was a statistical significant ($p=.05$) interaction amongst them. The mean of total chl. at control (2.64 $mg.g^{-1}$) was significantly higher compared to mean than those of 480 µM Al (2.41 $mg.g^{-1}$), 750 µM Al (2.39 $mg.g^{-1}$) and 960 µM Al (1.72 $mg.g^{-1}$), respectively. Mean for non-inoculated (2.46 $mg.g^{-1}$) was significantly higher than for USDA-inoculated (2.12 $mg.g^{-1}$). Likewise, mean of Chl*. a+b* of GAZZELE (3.40 $mg.g^{-1}$) soy bean genotype treated with *Rhizobia* and Al was significantly higher than those of TGX (1.83 $mg.g^{-1}$) and NAMSOI (1.65 $mg.g^{-1}$), respectively.

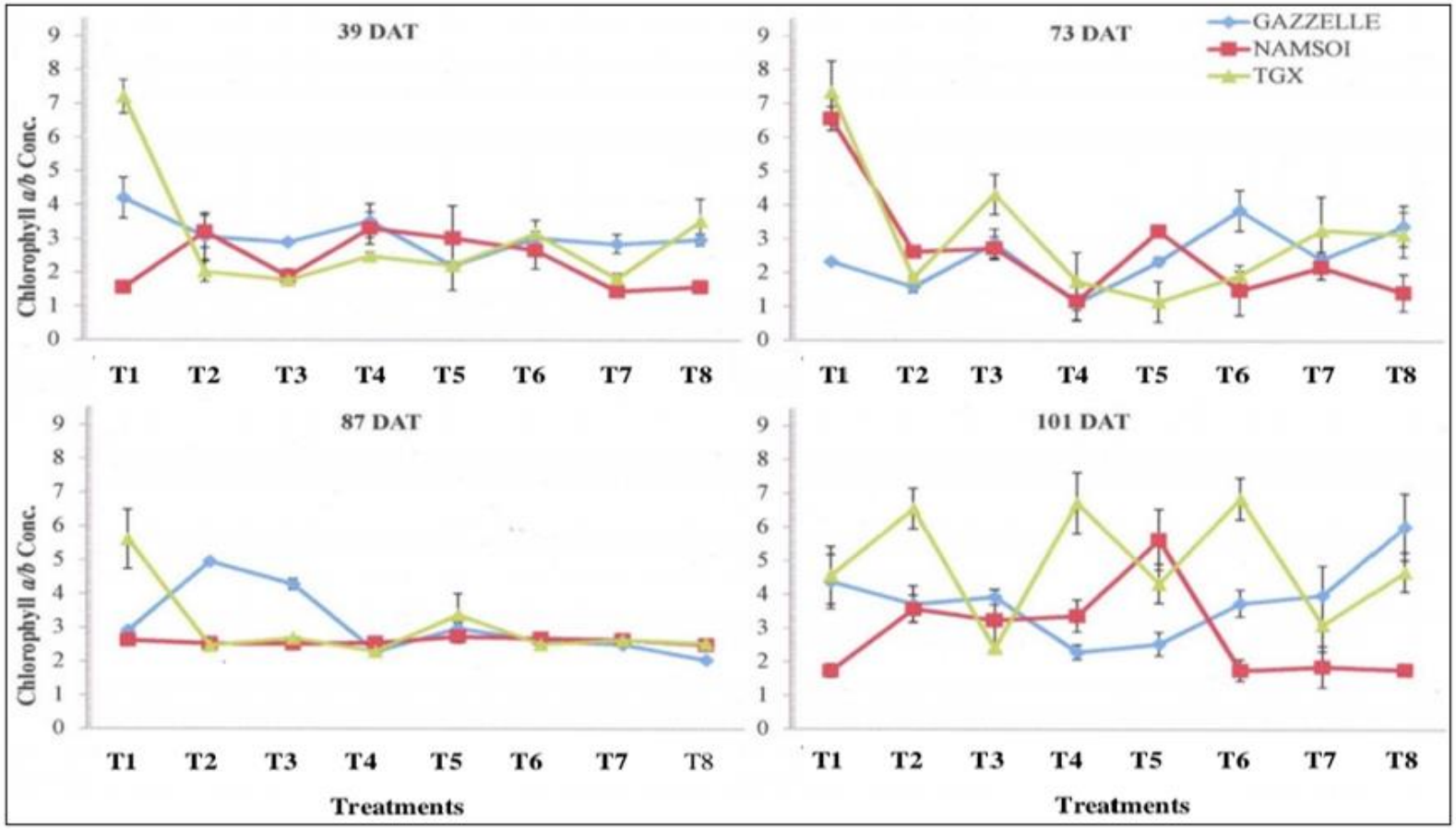

Fig. 8. Chlorophyll *a/b* concentration in plants of hree soy bean genotypes on 39 DAT, 73 DAT, 87 DAT and 101 DAT subjected to various treatments. Values are means of three replicates±SEs. Control (Water)*Inoculated (T1), 480µM Al*Inoculated (T2), 750 µM Al*Inoculated (T3) and 960 µM Al*Inoculated (T4), Control (T5), 480 µM Al (T6), 750 µM Al (T7) and 960 µM Al (T8)

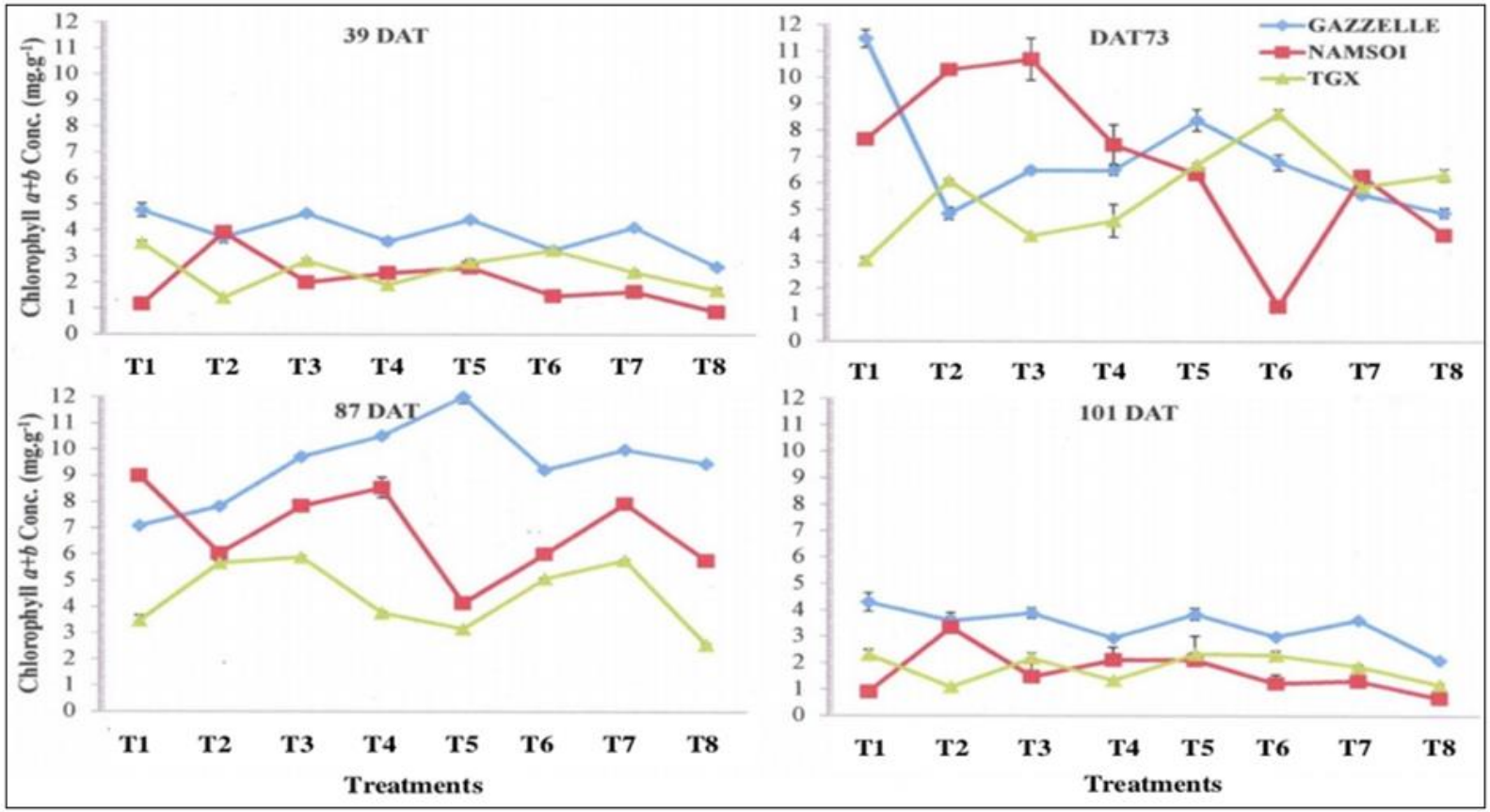

Fig. 9. Chlorophyll *a+b* concentration in plants of three soy bean genotypes on 39 DAT, 73 DAT, 87 DAT and 101 DAT subjected to various treatments. Values are means of three replicates±SEs. Control (Water)*Inoculated (T1), 480 µM Al*Inoculated (T2), 750 µM Al*Inoculated (T3) and 960µM Al*Inoculated (T4), Control (T5), 480 µM Al (T6), 750 µM Al (T7) and 960µM Al (T8)

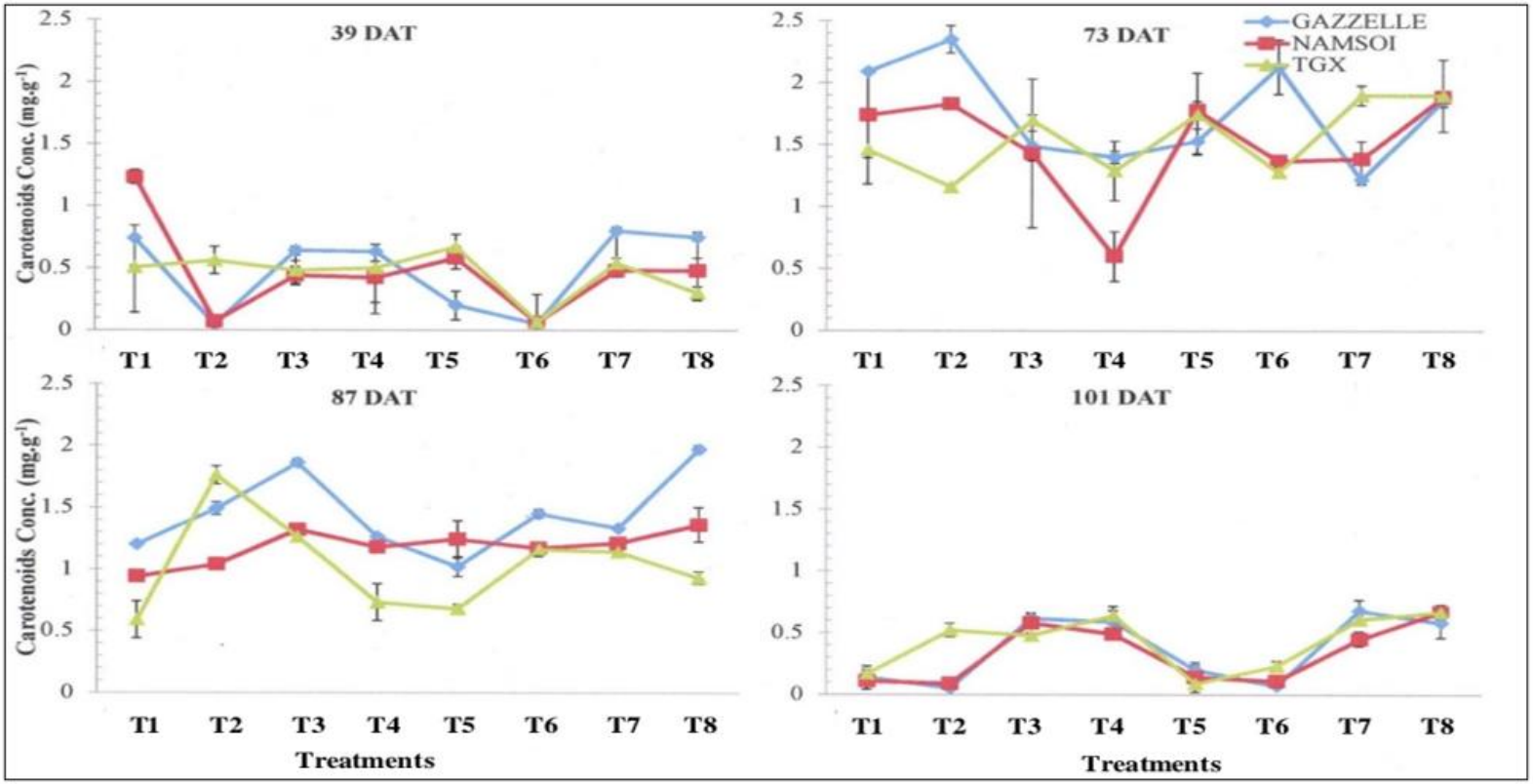

Fig. 10. Carotenoids concentration in plants of three soy bean genotypes on 39 DAT, 73 DAT, 87 DAT and 101 DAT subjected to various treatments. Values are means of three replicates±SEs. Control (Water)*Inoculated (T1), 480 µM Al*Inoculated (T2), 750 µM Al*Inoculated (T3) and 960 µM Al*Inoculated (T4), Control (T5), 480 µM Al (T6), 750 µM Al (T7) and 960µM Al (T8)

Carotenoids Concentrations: Fig. 10 shows results for carotenoids concentrations in three soy bean genotypes on 38 DAT, 73 DAT, 87 DAT and 101 DAT. Mean carotenoids concentrations for TGX was significantly higher than mean for NAMSOI and GAZZELLE on 39 DAT at treatment T3, respectively. Mean for GAZZELLE was also significantly higher than mean for NAMSOI and TGX on 87 DAT at treatment T1, T3, T5 and T8.

A three way analysis of variance run to determine the effects of *Rhizobia* treatments, aluminium treatments and genotypes on carotenoid concentrations in plants showed that there was a statistical significant (p=.05) interaction amongst them. On 39 DAT, mean for carotenoid concentration at 750 µM Al (0.86 $mg.g^{-1}$) was significantly higher compared to mean at control (0.54 $mg.g^{-1}$), 960 µM Al (0.51 $mg.g^{-1}$) and 480 µM Al (0.14 $mg.g^{-1}$). There were no significant differences in carotenoid concentration between USDA-inoculated (0.61 $mg.g^{-1}$) and non-inoculated (0.42 $mg.g^{-1}$) treatment. Mean of carotenoid concentration for NAMSOI (0.60 $mg.g^{-1}$), GAZZELE (0.49 $mg.g^{-1}$) and TGX (0.45 $mg.g^{-1}$) were not significantly different.

A three way analysis of variance run to determine the effects of *Rhizobia* treatments, aluminium treatments and genotypes on carotenoid concentrations in plants on 73 day after treatment showed that there was a statistical significant (p=.05) interaction amongst them. Mean of carotenoids for GAZZELE (1.88 $mg.g^{-1}$) soy bean genotype treated with *Rhizobia* and Al was significantly higher than those of TGX (1.56 $mg.g^{-1}$) and NAMSOI (1.50 $mg.g^{-1}$), respectively. Analysis of variance run to determine the effects of *Rhizobia* treatments, aluminium treatments and genotypes on carotenoid concentration in plants on day 87 after treatment showed that there were statistical significant (p=.05) interactions between *Rhizobia* treatments and aluminium treatments, and between aluminium treatments and genotypes.

A three way analysis of variance run to determine the effects of *Rhizobia* treatments, aluminium treatments and genotypes on carotenoid concentrations in plants on day101 after treatment showed that there was statistical significant (p=.05) interaction amongst them. Mean of 960 µM Al (0.61 $mg.g^{-1}$) and 750 µM Al (0.57 $mg.g^{-1}$) were significantly higher than that of 480 µM Al (0.18 $mg.g^{-1}$) and control (0.14 $mg.g^{-1}$), respectively. Similarly, mean of carotenoids for TGX (0.43 $mg.g^{-1}$) soy bean genotype treated with *Rhizobia* and Al was significantly higher than those of GAZZELE (0.37 $mg.g^{-1}$) and NAMSOI (0.33 $mg.g^{-1}$), respectively.

3.3 Plant Secondary Metabolites

Total Flavonoids Concentration: Fig. 11 shows total flavonoids concentration for the three soy bean genotypes. The figure shows that means of flavonoids concentration of NAMSOI, GAZZELLE and TGX were not significantly different from each other for the eight treatments. Flavonoids concentration means were not significantly at treatments 960 µM Al (54.05.l^{-1}), control (54.30 µg.l^{-1}), 750 µM Al (52.73 µg.l^{-1}) and 480 µM Al (51.62 µg.l^{-1}). Flavonoids concentration mean of USDA-inoculated (54.25 µg.l^{-1}) soy bean plants was not significantly different than that of non-inoculated (53.08 µg.l^{-1}). Similarly, genotype means of flavonoid concentration for TGX (54.87 µg.l^{-1}), NAMSOI (53.97 µg.l^{-1}) and GAZZELE (52.18 µg.l^{-1}) did not show significant differences among themselves.

Anthocyanin concentration: Fig. 12 shows anthocyanin concentration of the three soy bean genotypes. The figure shows that mean of anthocyanin concentration for NAMSOI and TGX soy bean genotypes were significantly higher than that of GAZZELLE genotype at treatment T3. Mean of anthocyanin concentration for NAMSOI was significantly lower than those for TGX and GAZZELLE genotypes at treatment T4. Mean for NAMSOI genotype was significantly higher than those of GAZZELLE and TGX genotypes at treatment T5 (Fig. 12). TGX genotype had generally higher anthocyanin concentration as Al treatments levels increased except at treatment T2 and T3. A three way analysis of variance run to determine the effects of aluminium treatments, *Rhizobia* and genotypes on anthocyanin concentration in plants showed that there was a statistical significant (p<.01) three way interaction amongst them. However, a significantly higher (p<.01) interaction was shown between treatments and genotypes. Mean of anthocyanin concentration at 480 µM Al (222.16 µg.l^{-1}) significant higher compared to mean at either 960 µM Al (208.65 µg.l^{-1}), 750 µM Al (205.01 µg.l^{-1}) and control (170.78 µg.l^{-1}), respectively. Mean of anthocyanin concentration at treatments 960 µM Al and 750 µM Al were significantly higher than mean at control. However, mean of non-inoculated (214.90 µg.l^{-1}) was not significant to that of USDA-inoculated (188.41 µg.l^{-1}) plant. Means of

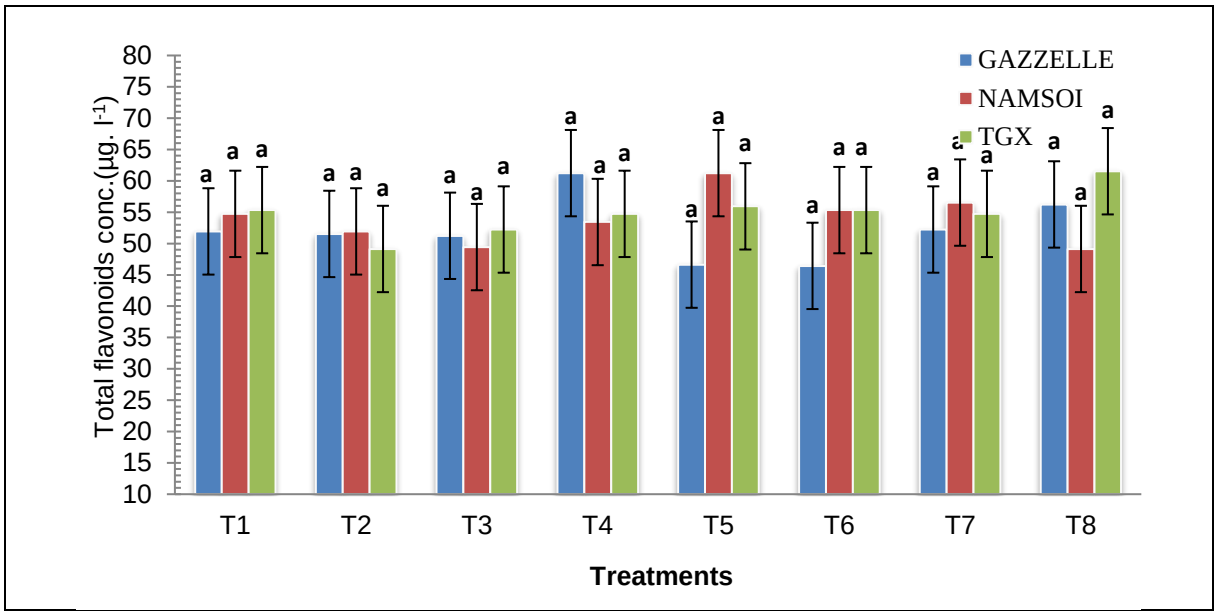

Fig. 11. Total flavonoids concentrations in plants of the three soy bean genotypes at maturity subjected to various treatments. Values are means of three replicates±SEs. Means with the same latter are not significantly different. Control (Water)*Inoculated (T1), 480 µM Al*Inoculated (T2), 750 µM Al*Inoculated (T3) and 960 µM Al*Inoculated (T4), Control (T5), 480 µM Al (T6), 750 µM Al (T7) and 960 µM Al (T8)

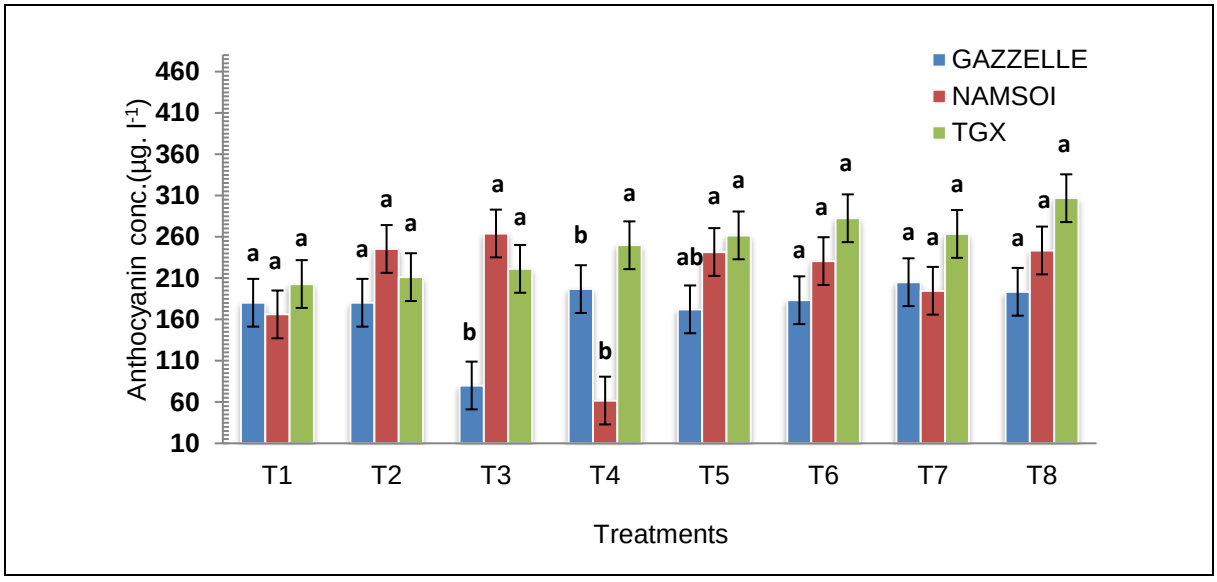

Fig. 12 Anthocyanin concentrations in plants of the three soy bean genotypes at maturity subjected to various treatments. Values are means of three replicates±SEs. Means with the same latter are not significantly different. Control (Water)*Inoculated (T1), 480 µM Al*Inoculated (T2), 750 µM Al*Inoculated (T3) and 960 µM Al*Inoculated (T4), Control (T5), 480 µM Al (T6), 750 µM Al (T7) and 960 µM Al (T8)

anthocyanin concentration for genotypes TGX (224.94 µg.l^{-1}), NAMSOI (206.03 µg.l^{-1}) and GAZZELE (173.99 µg.l^{-1}) were not significantly different among each other.

Phenolic Compound Concentration: Fig. 13 shows phenolic compound concentration determined in the three soy bean genotypes. The figure shows that the mean phenolic compound

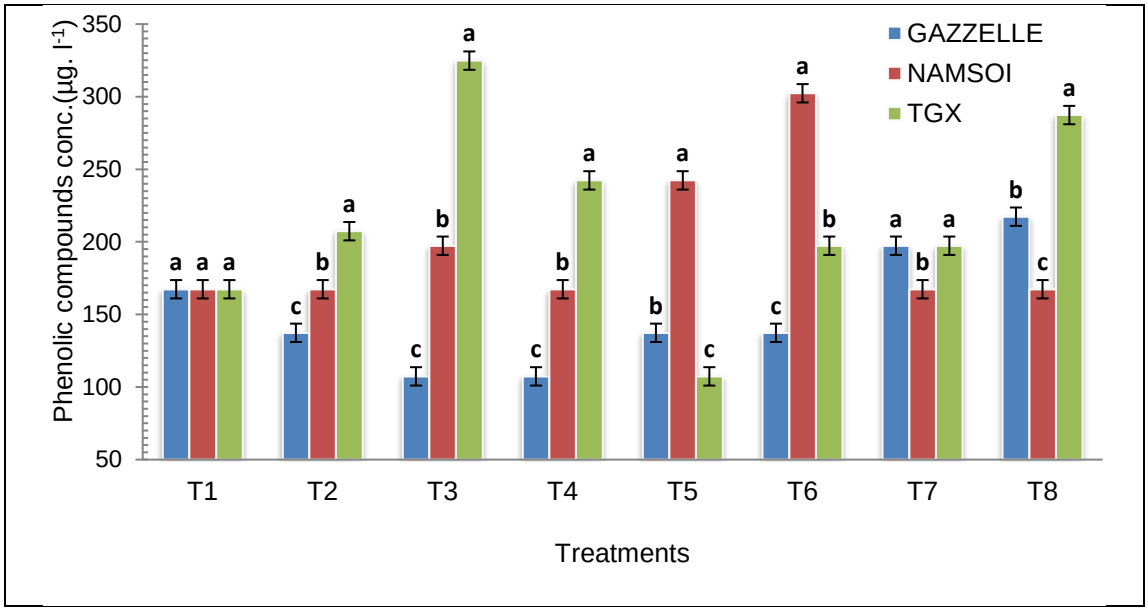

Fig. 13 Phenolic compounds concentrations in plants of the three soy bean genotypes at maturity subjected to various treatments. Values are means of three replicates±SEs. Means with the same latter are not significantly different. Control (Water)*Inoculated (T1), 480 µM Al*Inoculated (T2), 750 µM Al*Inoculated (T3) and 960 µM Al*Inoculated (T4), Control (T5), 480 µM Al (T6), 750 µM Al (T7) and 960 µM Al (T8)

concentration for TGX was significantly higher than that for GAZZELLE and NAMSOI at treatments T2, T4 and T8, respectively. A three way analysis of variance run to determine the effects of aluminium treatments, *Rhizobia* and genotypes on total phenolic compounds concentration in plants showed that there was a statistical significant ($p<0.01$) three way interaction amongst them. Phenolic compound concentration means at either 750 µM Al (198.58 $\mu g.l^{-1}$), 960 µM Al (194.83 $\mu g.l^{-1}$) and 480 µM Al (191.50 $\mu g.l^{-1}$) were significantly higher than that at control (169.83. Similarly, mean for non-inoculated (194.83 $\mu g.l^{-1}$) was significantly higher than for USDA-inoculated (182.54 $\mu g.l^{-1}$) treatment. Means for TGX (220.15 $\mu g.l^{-1}$), NAMSOI (201.08 $\mu g.l^{-1}$) and GAZZELE (144.83 $\mu g.l^{-1}$) were significantly different from each other.

4. DISCUSSION

Effects of Aluminium Application and *Rhizobia* Inoculation on Gas Exchange: Net carbon assimilation rate decreased in genotypes NAMSOI, GAZZELLE and TGX with Al application regardless of *Rhizobia* inoculation. Previous studies using Al alone for example in citrus by [23,11], also found reduction in net assimilation with aluminium application. For instance, [24] in their review noted that CO_2 assimilation in four citrus rootstocks differentially reduced as Al was increased gradually from 0 to 400 µM. Therefore, what is common in the findings herein with others is that the rate at which Al decrease CO_2 assimilation varies with genotypes. For instance, TGX and NAMSOI had higher CO_2 assimilation at almost all days of treatment. Net carbon assimilation rate is higher in the two genotypes under Al application. This might be due to the fact that *Rhizobia* reduced the suppressing effect of Al to nitrogenase activity, hence plants acquired more nitrogen [25].

Net photosynthetic rate of soy bean plants was remarkably affected by Al toxicity for TGX and NAMSOI regardless of USDA inoculation. On the contrary, a previous research found tolerant sorghum genotypes to be more affected by Al toxicity [24]. In this study, Al-treated plant leaves had higher intercellular carbon concentration at controls while in other cases there was no difference. However, Al reduced stomatal

conductance. The phenomena that led [24] to conclude that CO_2 assimilation due to Al toxicity is because of factors that are not related to stomatal behaviour. On the contrary, stomatal closure had minimal contribution to low CO_2 assimilation in Al-stressed plants of *Thinopyrum bessarabicum* [25]. Therefore a similar trend as found in the present study may corroborate the fact that, thylakoid destruction was increased by aluminium application, that even low aluminium concentration of 10 µM may have damaged thylakoids [24] and severely injured chloroplasts hence decrease in photosynthesis of soybean plants. Moreover, chloroplast damage may have decreased PSII performance and ETR [26] that greatly affect this physiological process in soy bean plant production.

Net photosynthetic rate was significantly higher in most cases for plants under USDA inoculation. *Rhizobia* inoculation may have increased light interception capacity due to increased leaf area and number at these earlier stages [27] as found on 35 DAT and 65 DAT. There may have been increased light penetration that led to effective and efficient use of resources [28] by soy bean plants on DAT 95. This would suggestively imply that photosynthetic rate would occur even in Al treated and inoculated plants, hence no significant differences. High capacity of TGX as compared to either GAZZELLE and NAMSOI to assimilate CO_2 was therefore likely reason that increased yield in this genotype. Similarly, improvement in net photosynthesis in *Rhizobia* inoculated soy bean plants can be due to adequate N symbiotic fixation. Therefore, nitrogen may have synthesized rubisco as a macromolecule necessary for carbon assimilation. This explains why TGX genotypes with quality and active nodules were also found to have high net photosynthesis. Additionally, this enzyme is so vital in CO_2 fixation and synthesis of light harvesting chlorophylls that enhance photosynthesis in soy beans. This may have contributed to increased yield. Similarly, *Rhizobia* may have enriched the soy bean plants with N leading to a positive shift in metabolism. Eventually, initiating high gaseous exchange in early developmental stages [3].

Rhizobia inoculated plants significantly increased stomatal conductance in NAMSOI genotype as compared to GAZZELLE and TGX at T2 on 35 days after treatment. Similarly, for GAZZELLE when compared to TGX and NAMSOI at T8 on 65 days after treatment. Ayalew [28] similarly reported significant increase for stomatal conductance due to *Rhizobia* inoculation in cow peas. USDA inoculum with *Bradyrhizobium* may have improved water use efficiency that led to an increase stomatal conductance in NAMSOI and GAZZELLE. This *Rhizobia* strain might have been residentially found in soils used within the study thus leading to the difference at T8. Moreover, a difference in leaf stomatal conductance among soy bean genotypes may have increased transpiration rate [28]. Therefore, this led to increased water loss and thus decreased rate of CO_2 diffusion for photosynthesis. Further, this would lead to lower grain yield [26] in TGX genotypes that had highest transpiration rate.

Effects of Aluminium Application and *Rhizobia* Inoculation on Plant Photosynthetic Pigments Concentrations: Soy bean plants treated with Al had lower concentration of photosynthetic pigments as previously found by [29]. Consequently, Al may have impaired chloroplasts which decreased light absorption and photochemical activity [22]. Chlorophyll *a* concentration in soy beans was found to have reduced on 39, 73, 87 and 101 days after treatment when Al treatments increased. This has been found in other plants, namely soy bean, sorghum, barely and citrus as found in [29]. In this study, USDA-inoculated plants accumulated higher chlorophyll *a*. A higher concentration of Chl. *a* in GAZZELLE soy bean genotype compared to the others may indicate that the plant genotype exhibits some tolerant qualities to Al stress. Considerably, genotype GAZZELLE was found to concentrate higher chlorophyll *a* as compared to NAMSOI and TGX at T3 and T8 on both 39, 87 and 101 days after treatment.

A reason that might have decreased chlorophyll *a* concentration in Al treated and non-inoculated plants is that Al destroy monopyrrole porphobilonogen and cytochromes [29]. This is because it inhibits aminolevulinic acid dehydratase responsible for formation of this molecule [30]. In maize, chlorophyllase activity was impaired by Al together with 5-Monopyrrole porphobilonogen [23]. Therefore, PSII photochemistry and enzymatic machinery distribution may have decreased CO_2 assimilation in TGX [31] than it was for genotype GAZZELLE even when USDA-inoculated seeds were applied to with Al treatments.

GAZZELLE varied significantly with higher concentration of Chl. *a* compared to NAMSOI and TGX. This meant that the TGX and NAMSOI

genotypes experience the above damages more adversely than GAZZELLE. Rhizobial inoculation increased Chl. *b* concentration with Al treatment. According to [29], this increase suggests chlorophyll *b* was highly assisting in the uptake of light and reducing the harmful effects of light in the photosynthetic apparatus. Additionally, as accessory pigments Chl. *b* may have avoided damage to photosynthetic membranes as chl. *a* pigment captured light.

Chl. *a* and *b* were found to be significantly higher in GAZZELLE genotype than in the NAMSOI and TGX genotypes for some treatments. Zhou [32] studied the effects of *Rhizobia* and nitrogen on soy bean plants, their research linked lower concentration of chlorophylls as in TGX and NAMSOI to decreased Mg concentrations with increasing Al treatment. Therefore, such soy bean plants had reduction in PAR utility efficiency that limited photosynthetic capacity to a significant level [10]. High Al mobility in non-inoculated soy bean plants may have caused abundance of Al in plants that replaced Mg in chlorophyll molecule structure. This lead to formation of a non-functional chlorophyll-Al complex which disrupt the overall structure of the chloroplast membrane and affect the structure of other membrane bound proteins [10]. Therefore, this leads to a reduced photosynthetic efficiency due to chlorosis. Further, in this scenario there is reduced capacity to absorb light, obstruct ETR and block Calvin cycle to reduce photosynthesis [30]. Al replaces key co-factors and metallic ions in such vital molecules in photosynthesis [10]; there is a detrimental effect to acyclic photophosphorylation. It is what prevents the key chlorophyll *a* molecule from maximum absorption of light.

The ratio of Chl. a/b is markedly used to quantify antenna chlorophyll size in PSI and PSII [30]. Seemingly in this study, the chl. *a*/*b* ratio was lower under aluminium treatment regardless of inoculation in soy beans genotypes. Therefore, control plants with higher chl. *a*/*b* ratio indicate a higher antenna pigment complex size and less photo-inhibition [22]. A significant amount of energy may be delivered into photosystems of such plants with large antenna pigment complex [10]. Chl. *a*/*b* ratio increased significantly in GAZZELLE than NAMSOI and TGX at T3 in early days after treatment. Therefore, in early stages of vigorous development GAZZELLE might have had little photoinhibition. On the contrary, the chlorophyll *a*/*b* increased variably when Al was increased in soy bean, rice and 'Cleopatra' tangerine in studies by [24] and by [23] respectively. In this study it appears that leaves contained lower Chl. *a* compared to Chl. *b* when they were exposed to Al. Therefore, inoculation may have raised Chl. *a* compared to Chl. *b*. There might have been magnified oxidation within reaction centers regardless of aluminium, a process that is marginally irreversible [24]. Concomitantly, photosynthetic pigments become much excited and interact with O_2 species producing superoxide (O^{2-}) among other free radicals, which in turn damage the same pigments.

Chl. *a*/*b* ratio was high in GAZZELLE in early days after treatment while TGX had higher mean values on day 87 and 101 after treatment. However, in another study on 'Sour pummelo' it was found that Chl. *a*/*b* ratio remained unchanged in response to Al treatments [24]. Li-Song [24] also found changes in the ratio of blue berry genotypes under hydroponic Al stress. According to their study, the chlorophyll antenna size also reduced slightly in some genotypes at the beginning. Later, it was found higher in others like in TGX to maintain PSII. [10] suggested that there might be slow acclimatization to photosynthetic apparatus to aluminium stress in those genotypes that show high Chl. *a*/*b* ratio in later days after treatments. In this study, this phenomenon might have taken place in genotypes TGX and NAMSOI. Therefore, Chl. *a*/*b* ratio size changed with days after treatment to reduce light absorption. This may have been an adaptive strategy in such genotypes to avoid possible damage to the photosystems by aluminium stress [32].

Chlorophyll *a*+*b* decreased for GAZZELLE, NAMSOI and TGX genotypes under Al treatments, regardless of inoculation. This decrease in total chlorophyll became much pronounced in NAMSOI and TGX at T8 and T3 (Fig. 9). Majorly this suggests, photo bleaching being experienced in PSI and PSII biochemistry by this Al treated plant even if it was inoculated plants [31]. Therefore this would mean a smaller fraction of absorbed light energy was available to drive electron transport as also found by [22] when studying barley.

Carotenoids are accessory pigments, which include carotenes and xanthophylls. In this study, Al treatment increased carotenoids concentration for the three genotypes. Carotenoids concentrations were also higher in non-inoculated plants compared to inoculated. High

carotenoid concentrations indicate increased transfer of energy. They act as filters of light, protecting PS [10] in such plants. This phenomenon might have helped such genotypes to dissipate heat formed due to excessive light into the antenna pigment complexes [10]. Cunha-neto [29] also found a similar trend in some members of Fabaceae when studying Al and lead effects to carotenoids. Occasionally, then TGX had higher concentration of carotenoids even when inoculated and treated with Al compared to NAMSOI and GAZZELLE. This was also found by [31] when studying the effects of plant-derived extracts, microbial, and potassium silicate on zucchini plants. The plants of this genotype had decreased photochemical parameters, indicating they favoured heat dissipation pathway to avoid photoinhibition that might have been caused by photo-bleaching [10]. Further, it is known that absorbed energy damage chlorophyll and thylakoid membrane by photo oxidation [32]. Carotenoids may have been produced to a greater extent in such plant as a strategy to prevent this damage in Al treated plants that were not inoculated. Additionally, pigments such as antheraxanthin and zeaxanthin may have decreased in soy bean plants that were inoculated and stressed with Al suggesting a reduction in thermal energy dissipation. However, if carotenoid content is stabilized in plants, this may lead to a higher tolerance level to Al metal ions [29].

Effects of Aluminium Application and *Rhizobia* Inoculation on Secondary Metabolites: There [1] found differences that were not significant in tartary buckwheat and blueberry genotypes respectively. Plants of genotypes that accumulate more secondary metabolites like TGX and GAZZELLE may have been more tolerant to Al stress [1]. Therefore, three soy bean genotypes displayed diverse profile of secondary metabolites which may be dependent on the genetic adaptation to Al stresses [10]. There was high accumulation of secondary metabolites in plants inoculated with USDA. For instance, high accumulation of total flavonoids that play crucial role in physiological adaptation process [12]. In addition, infection of soy bean by USDA *Rhizobium* led to increased phenolics in TGX genotype at T2 and T4. According to [12] inoculation caused synthesis and release of phenolics. Phenolics bind to soil particles and organic matter forming aggregates which holds roots stronger [33] and therefore better performance is expected in TGX. Further, there may be improved soil structure for plant growth due to better soil drainage dictated by the aggregates formed [34]. The quantity of phenolics varies from genotypes to genotypes [12] hence genotype difference in their release levels into the soils.

Aluminium treatments caused an increase in anthocyanin and phenolic compounds. In comparisons, [11] found that, phenol concentration after Al treatment was higher whereas flavonoid concentration remained constant as opposed to this study where they both increased. According to a review paper by [35], this trend can be explained that there is change in several gene expressions due to increased Al application that is genotype specific. Anthocyanin was significantly accumulated in TGX, suggestively it possesses strong antioxidant properties which helped plants of this genotype protected from oxidative stress caused by Al toxicity [36]. They protected cellular components from damage by scavenging harmful free radical [8]. Contrarily, GAZZELLE genotype had reduced ability in absorbing harmful UV radiations and therefore increased risk of DNA damage by photoinhibition [14]. It accumulated lower anthocyanin which act as a natural sunscreen to shield sensitive young leaves and flowers from UV radiations [37-40].

5. CONCLUSION

Genotype differences found for photosynthesis parameters measured indicate their relationship to both growth and mineral nutrition when soy bean genotypes are grown under aluminium application and inoculation. C*i*, *A* and g_i showed GAZZELLE and TGX to perform better in photosynthesis. Therefore, the two genotypes when inoculated with USDA-*Rhizobia* will have a potential alternative benefit to face problems caused by Al in acidic soils due to their genotype effects on plant development and photosynthesis. The study recommends use of C_i, *A* and g_i. These parameters showed consistent mean differences that were significantly higher for GAZZELLE genotype compared to NAMSOI at various treatments. Inoculating GAZZELLE genotype with *Bradyrhizobium japonicum* increases photosynthetic performance, therefore recommended for growth under Al prone acidic soils. The current studies concentrated on gas exchange activities for plants subjected to Al stress and *Rhizobia* inoculation. These measurements could not establish the effects of Al on the internal activities on the photosynthetic

apparatus. Therefore future research should concentrate on such activities, using chlorophyll fluorescence parameters such as NPQ, ETR, on inoculated soy bean genotypes under Al.

Photosynthetic pigments (Chl. *a*, Chl. *b* and Total Chl.) measured showed GAZZELLE to have had high concentrations. Generally non-inoculated plants treated with Al were lowly concentrated with chlorophyll *a*, chlorophyll *b,* chlorophyll *a*/*b,* total chlorophyll and carotenoids. Therefore inoculation ameliorates the effects of aluminium to some significant level. Under even the higher Al concentration, inoculated soy bean plants are able to concentrate normal amounts of these pigments. Photosynthetic pigments were shown to be highly accumulating in GAZZELLE. In this genotype, chlorophyll *b* is recommended to be measured under Al application and USDA inoculation. It normally captured and transferred light energy to chlorophyll *a*. High accumulation of carotenoids indicate that GAZZELLE genotype highly protected reaction centers in photosystems and therefore recommended to be grown in soils where Al toxicity cause high light irradiance in plants. Determination of chlorophylls and carotenoids could not clearly give insight on mechanisms underlying the phenomena at which antheraxanthin, zeaxanthin and carotenoids are involved in photoprotection. Therefore this can be studied in future when evaluating soy bean genotypes grown in Al prone soils under USDA-inoculation.

USDA-inoculation as an agricultural practice can enhance substantial yield in genotypes GAZZELLE which accumulated lots of flavonoids and TGX which accumulated high anthocyanin and total phenolic compounds. These compounds might be exuded to form complexes with Al in acidic rhizosphere thus limiting Al uptake by plants. Significant differences in genotypes at various treatments showed GAZZELE and TGX to be mostly accumulating most of secondary metabolites measured. Suggestively, these genotypes highly form Al complexes in soil Rhizosphere. Therefore, recommended to tolerate Al when USDA-inoculated. Future studies should also determine concentrations of sugars, amino acids, organic acids, enzymes and hormones as they are source of carbon for *Rhizobia* in soy beans.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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