Scientia Agriculturae
www.pscipub.com/SA
E-ISSN: 2310-953X / P-ISSN: 2311-0228
DOI: 10.15192/PSCP.SA.2015.10.2.8494

Sci. Agri.
10 (2), 2015: 84-94
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Chlorophyll fluorescence parameters and photosynthetic pigments of four Glycine max varieties under Aluminium chloride stress

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Paper Information

Received: 7 February, 2015

Accepted: 11 April, 2015

Published: 20 May, 2015

Citation

Mmayi MP, Musyimi DM, Netondo GW, Sikuku PA. 2015. Chlorophyll fluorescence parameters and photosynthetic pigments of four Glycine max varieties under Aluminium chloride stress. Scientia Agriculturae, 10 (2), 84-94. Retrieved from www.pscipub.com
(DOI: 10.15192/PSCP.SA.2015.10.2.8494)

ABSTRACT

Soil acidity limits agricultural production globally. Different kinds of stresses, mainly Al stress, generated from acid soils affect plant growth and result in food shortage and production. General effects of Al toxicity in plants include plant growth alterations, reduction of photochemical efficiency of photosystem II (PS II), reduced photosynthesis and inhibition of synthesis of photosynthetic pigments. There is need to improve food security in Kenya by encouraging the growing of soybean crop, however there is lack of information on physiological response of soybean varieties grown in Kenya to aluminium chloride toxicity. There is need to investigate the effects of aluminium toxicity to soy bean Varieties (*Glycine max* (L.) commonly grown in Kenya with a view of identifying the tolerant varieties among them to be recommended for growing in areas prone to aluminium toxicity. Such varieties include SB 97, SB 19, SB 20 and SB123. The experiment was conducted at Maseno University under greenhouse conditions. Randomized complete block design factorial was used with three replicates and five levels of 0(control), 25mg/l, 50 mg/l, 75mg/l and 100 mg/l aluminium concentration in tap water. Parameters determined included dry weight, chlorophyll fluorescence and photosynthetic pigment contents. SAS software was used to analyse the data by subjecting it to ANOVA. Tukey`s HSD tests at 5% was used to separate treatment and Variety means. Plant dry weights decreased with increased aluminium chloride concentration. Variety SB 123 had the highest dry weight compared to the other three soy bean varieties. Generally maximum quantum yield (Fv/Fm ratio) and Effective quantum yield (ϕPSII) reduced with increasing aluminium chloride concentration in all the varieties. Maximum quantum yield and effective quantum yield, and chlorophyll *a* and *b* contents were highest in variety SB 20 but measured low NPQ values. Variety SB 123 had higher carotenoids contents at 100 mg/l aluminium chloride solution in comparison to other three soybean varieties. Photochemical parameters of PSII and photosynthetic pigments parameters measured were found to be quite sensitive to $AlCl_3$ solution stress. Soybean varieties SB 20 and SB 19 were found to be more tolerant to aluminium chloride solution stress under the current study.

Key words: *Aluminium chloride toxicity, chlorophyll fluorescence, photosynthetic pigments, Soybeans.*

Introduction

Soy bean (Glycine max L.) is a commonly grown legume crop in Kenya. Soy bean grains are among the world's most important in terms of protein content of 35-40 % and oil content of 15-22%. Soy bean grains are also rich in essential amino acids, vitamins and minerals (Alvim et al., 2012). Considerable attention has been focused on assessing the impact of aluminium (Al) stress on cultivated plants because its stress is often the primary factor limiting crop production in acid soils (Kochian, 1995). Soluble aluminium is toxic to the roots of most plants leading to reduced growth and reduces plant production rate. Aluminium reaches the photosynthetic cells posing negative effects on photosynthetic accessory pigments associated with both photosystem I and II (Cai et al., 2011). Cai et al. (2011) observed that Aluminium affects plants physiologically including the quantity of chlorophyll pigments, suppression of photosynthetic activities at the photosynthetic apparatus and plant mineral nutrient uptake. The use of aluminium-tolerant plants is part of crop management strategy for agricultural production in acid soils (Alvim et al., 2012). In Kenya, Western region led in soy bean production with nearly 50% in 2003. The area that is potentially suitable for soybean production in Kenya ranges from 157,000 ha to 224,000 (Chianu et al., 2008). At low pH, the release of toxic aluminium soluble forms (particularly

Al^{3+}) is enhanced by the dissolution of Al-containing compounds, thus becoming available to interact with plants and other organisms (Samac and Tesfaye, 2003; Alvim et al., 2012. Very little research on the effects of aluminium chloride stress has been done on soy bean varieties grown in Kenya. Growing aluminium tolerant soybean varieties can overcome the problems of aluminium toxicity and hence improve the food insecurity problem in Kenya. This will also reduce the expenditure on soil amendments practices like liming, and nitrogen fertilizer application (Cai et al., 2011). The results of this study can be used by plant breeders to develop aluminium chloride tolerant varieties. The objective of this study therefore was to assess dry weights, chlorophyll fluorescence parameters and photosynthetic pigment contents of four soy bean varieties grown in Kenya under aluminium chloride stress conditions in order to identify the tolerant ones that can be recommended for aluminium prone soils.

Material And Methods

Greenhouse Conditions and Soil characteristics

The research was carried out at Maseno University under Polythene covered Green house conditions located at the University Research farm. Soils had pH range of 4.5-5.4. Maseno receives both short and long rain averaging to 1750mm per annum with a mean temperature of 28.7^0C. Latitude extent $0^0\ 1^/$ N – $0^0\ 12^/$ S; Longitude extent $34^0\ 25^/$ E – $34^0\ 47^/$ E is its location at approximate 1500m above sea level. Greenhouse growth conditions were 25^0C-40^0C/20^0C-30^0C (day/night) temperature, 14/10-h (light/dark) photoperiod, and 64-77% relative humidity. Tap water with a pH of 5.6 was used for irrigation.

Plant Materials and Treatments

Seeds of Soy bean; SB 97, SB 19, SB 20 and SB 123 varieties were obtained from the International Centre for Tropical Agriculture/ Tropical Soil Biology and Fertility (CIAT/TSBF), Maseno. Seeds were sterilized using 0.1% sodium hypochlorite solution, washed with distilled water and planted in PVC pots filled with soil from Maseno University Research farm. Calcium superphosphate (71.4kg/ha, 15.5% P_2O_5) was applied before planting then DAP (35.7kg/ha, 20.6% N) applied ten days after germination. Potassium sulphate (119kg/h, 48%K_20) was applied at twenty five days after planting. A Randomized Complete Block Design (RCBD) comprising of five levels of aluminium chloride treatments (0 (control), 25, 50, 75 and 100 mg/l) replicated three times was used. The Al concentrations were adopted from Rafia and Sehrish (2008). Aluminium chloride was dissolved in tap water and treatments were initiated 21 days after germination at the rates of 800 ml per pot. Treatments were applied at three day interval according to Villagarcia et al. (2001) for five weeks.

Determination of dry weights

At the end of the experiment (after five weeks), the plants were harvested in each treatment. The harvested plants were rinsed with tap water, and the roots immersed in a bucket of water to remove soil that adhered to the root surface. Plants were separated into shoots and roots before fresh weight (FW) were determined using a weighing balance (Denver instrument XL-3100D). The roots and shoots were then dried in an oven for at least 72 hours at 70°C to constant weights for dry weight determination.

Determination of chlorophyll fluorescence parameters

Leaf chlorophyll fluorescence was determined using a portable pulse-amplitude modulated fluorometer (FMS 2; Hansatech Instruments, King’s Lynn, UK). Minimal fluorescence (Fo) was determined in dark-adapted (15 minutes) leaf by applying a weak modulated light (0.4 mmol-$m^{-2}s^{-1}$), and maximal fluorescence (Fm) was induced by a short pulse (0.8 s) of saturating light (9000 mmol-m^{-2} s^{-1}). After 10 second, actinic light (120 mmol-m^{-2} s^{-1}) was turned on to obtain fluorescence parameters during steady-state photosynthesis. Saturating pulses were applied after steady-state photosynthesis had been reached to determine maximal fluorescence in light-adapted leaves (Fm’) and steady-state fluorescence (Fs). Finally, the actinic light was turned off and a 5 second far-red (FR) pulse was immediately applied to obtain minimal fluorescence in light-adapted leaves (Fo’). The fluorescence parameters were estimated as described by Maxwell and Johnson (2000) and these included; automatically calculated maximum quantum yield of the PSII (Fv/Fm), Effective quantum yield (ϕPSII), and non-photochemical quenching (NPQ). Effective quantum yield (ϕPSII) was manually calculated as (Fm’ – Fs)/Fm’ and is the indicator of the effective quantum yield of PSII (Genty et al., 1989). Non-photochemical quenching was manually calculated using the formula, NPQ = (Fm – Fm’)/Fm’ (Kate et al., 2000; Maxwell and Johnson, 2000; Marjorie et al., 2010).

Photosynthetic pigments content determination

Chlorophyll content was determined according to Coombs et al. (1985) as described by Netondo (1999) where the third youngest leaf was sampled from all treatments. In the lab 0.5g of the fresh leaf tissue was weighed and cut into small pieces into specimen bottle. Ten millilitres of 80% acetone was added and the set kept in the dark for 4 days for the chlorophyll to be extracted by the acetone. Absorbance of the chlorophyll of the solution was measured using a spectrophotometer (Novaspec II, Pharmacia Biotech, and Cambridge, England) at 480, 645 and 663nm to determine the carotenoids and chlorophyll a and b content. The respective chlorophyll content in mg of chlorophyll per gram of the leaf collected was calculated using the formula of Arnon (1949) as follows: -

Chl a = 12.7 (D663) - 2.67 (D645) × V/1000 × W [mg Chl a g^{-1} leaf tissue];

Chl b = 22.9 (D645) - 4.68 (D663) × V/1000 × W [mg Chl b g^{-1} leaf tissue] and

Total chlorophyll content and chlorophyll a/b ratio was then calculated as; Chl a + Chl b, Chl a /Chl b respectively.

Carotenoids were measured in mg per gram according to the method described by Yadegari et al. (2007) in Musyimi (2011) as follows: -

C_{X+C} = 1000 (D480) - 2.270 (chl a) – 81.4 (chl b)/ 227 [mg C_{X+C} g^{-1} leaf tissue]

Where:

Chl a and chl b are chlorophylls a and b concentrations respectively; C_{X+C} are Carotenoids concentration ($_X$= xanthophylls and $_C$= carotenes); D= absorbance measured at wavelengths 645nm, 480 and 663nm; V= volume in ml of acetone extract used and W= fresh weight (g) of leaf from which the extract was made.

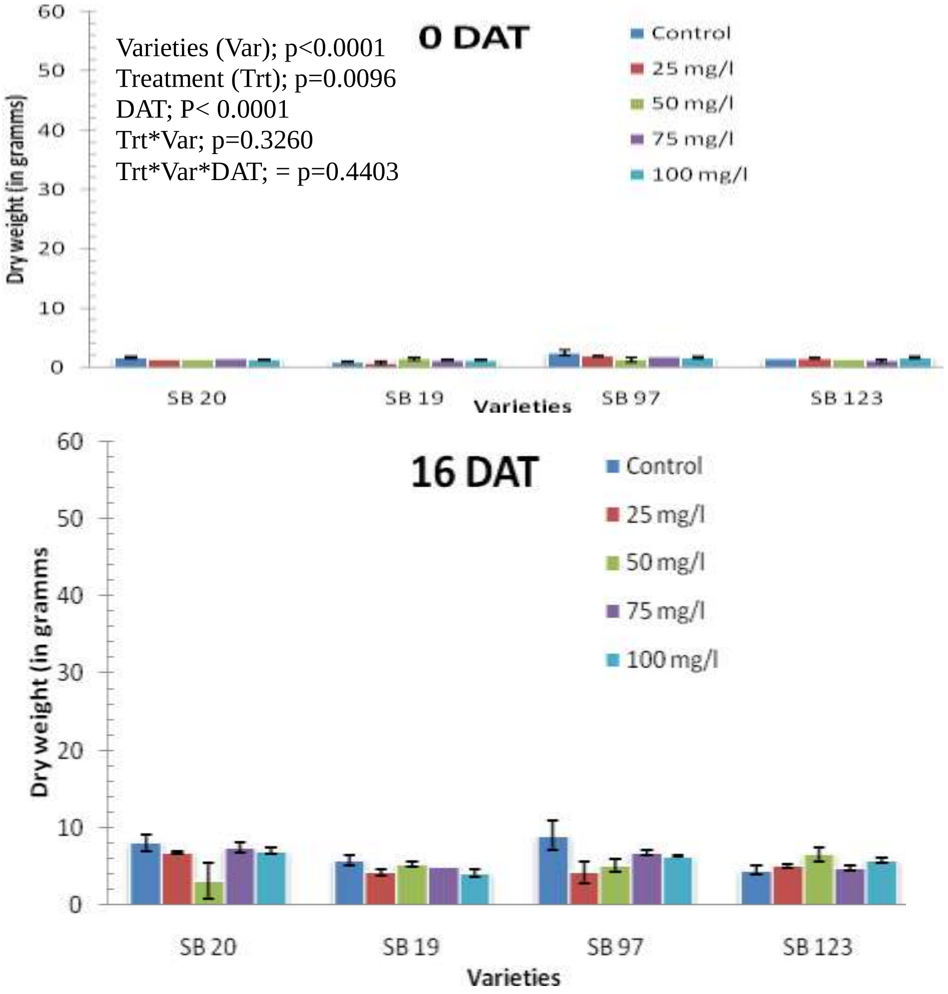
Statistical Data Analysis

The data were subjected to Factorial ANOVA using SAS statistical computer package (Steel et al., 2006). Measurements for parameters were repeated for one factor (cultivars) (Quinn and Keough, 2006). Tukey's HSD test at 5% level was used to separate and to compare the treatment means.

Results

Total dry weight

The dry weight of soy bean varieties decreased on 16 DAT and 56 DAT with aluminium chloride concentration apart from SB 123 (Fig. 1) on 16 DAT. There was also a significant difference among varieties ($P \leq 0.05$) and treatments. However, their interaction was non-significant ($P > 0.05$). The interaction among DAT, treatments and varieties was also non-significant. Variety SB 123 had a greater mean (16.99) dry weight value for varieties followed by SB 20 (14.19), SB 97 (10.84) then SB 19 (9.79). The highest treatment mean value was found at 0 mg/l (15.51) followed by 100 mg/l (13.03) 75 mg/l (12.73), 25 mg/l (12.27) then at 50 mg/l (11.26).



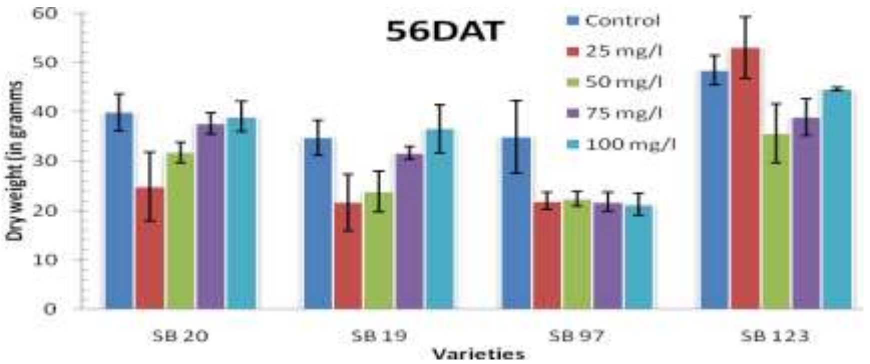


Figure 1.Total dry weight of soy bean varieties subjected to various concentrations of $AlCl_3$ solution. Values are means of three replicates ±STD DEV. Varieties (LSD=2.77), treatments LSD=3.29 and DAT LSD=2.19

Chlorophyll fluorescence Parameters

Maximum quantum yield (Fv/Fm)

The Fv/Fm ratio was high at the control compared to the other aluminium chloride treatments apart from SB 20 at 25 DAT and 38 DAT, SB 19, SB 97 at 25 DAT and SB 123 at 5 DAT (Table 1). There was a significant difference ($p \leq 0.05$) in DAT. There was no significantly difference ($p \geq 0.05$) in the Fv/Fm ratio among varieties and treatment. The interaction between varieties and treatments was also not significant as well as that among DAT, treatment and varieties. Variety SB 20 had a higher mean (0.56) Fv/Fm value followed by SB 19 (0.54), SB123 (0.51) and then SB 97 (0.50). For treatments, the highest mean value was found at 0 mg/l (0.56) followed by 50 mg/l (0.54), 100mg/l (0.53), 25mg/l (0.52) then at 75mg/l (0.49).

Effective quantum yield (ϕPSII)

Effective quantum yield reduced with increased aluminium chloride concentration in most cases for varieties and DAT (Table. 2). There was no significant difference ($p \geq 0.05$) among varieties, treatment and for the interaction between varieties and treatments, but a significant difference ($p \leq 0.05$) in DAT was shown. There was also no significant difference in the interaction among DAT treatments and varieties. Variety SB 20 had higher mean (0.55) value of ϕPSII followed by SB 19 (0.52), SB 97 (0.50) and then SB 123 (0.48). For treatments, the highest mean (0.54) value was found at 0 mg/l followed by 100mg/l (0.53), 25mg/l (0.51), 75 mg/l (0.50) then at 50mg/l (0.48).

Non-photochemical quenching (NPQ)

Heat dissipated in most cases for all varieties was generally more in $AlCl_3$ treated leaves compared to the control in both days (Table. 3). There was no significant difference ($p \geq 0.05$) among varieties, treatment and for the interaction between varieties and treatments as well as for the interaction among DAT, treatments and varieties. However, there was a significant difference ($p \leq 0.05$) in DAT. Variety SB 123 had a high mean (0.84) value for energy being dissipated followed by SB 97 (0.83), SB 19 (0.63) and then SB 20 (0.54). For treatments, the highest mean value was found at 75 mg/l (0.85) followed by 25 mg/l (0.85), 0 mg/l (0.66), 100 mg/l (0.61) then at 50 mg/l (0.59.

Table 1. Maximum quantum yield (Fv/Fm) of soy bean varieties subjected to various $AlCl_3$ treatments. Values are means of three replicates ±STD DEV.

		Fv/Fm (Relative Units)			
	Al Treatments	Varieties			
DAT	(mg/l)	SB 20	SB 19	SB 97	SB 123
5	control (0)	0.69±0.03	0.62±0.02	0.63±0.04	0.63±0.05
	25	0.73±0.06	0.57±0.02	0.56±0.01	0.51±0.06
	50	0.59±0.04	0.48±0.08	0.55 ±0.02	0.50±0.04
	75	0.59±0.04	0.64±0.03	0.62±0.03	0.50±0.04
	100	0.63±0.01	0.66±0.05	0.50±0.05	0.67±0.08
25	control (0)	0.53±0.02	0.54±0.00	0.46±0.00	0.52±0.05
	25	0.53±0.02	0.47±0.05	0.51±0.03	0.49±0.04
	50	0.55±0.00	0.48±0.04	0.34±0.08	0.40±0.03
	75	0.73±0.12	0.45±0.06	0.50±0.03	0.41±0.02
	100	0.42±0.09	0.74±0.15	0.50±0.03	0.38±0.04
38	control (0)	0.53±0.02	0.50±0.01	0.50±0.03	0.56±0.03
	25	0.57±0.05	0.53±0.02	0.45±0.00	0.54±0.02
	50	0.47±0.02	0.53±0.03	0.49±0.02	0.47±0.04
	75	0.44±0.04	0.35±0.10	0.46±0.01	0.51±0.01
	100	0.48±0.01	0.55±0.04	0.37±0.06	0.51±0.00

Mean varieties = SB 20 (0.56±0.02), SB 19 (0.54±0.01), SB 123 (0.51±0.01), SB 97 (0.50±0.02)
Mean DAT = 5 DAT (0.60±0.05), 25 DAT (0.50±0.02), 38 DAT (0.49±0.03)

Mean treatment = 0 mg/l (0.56±0.02), 100 mg/l (0.54±0.01), 25 mg/l (0.52±0.00), 75 mg/l (0.49±0.01), 50 mg/l (0.49±0.03). Varieties (Var); p=0.3759, Treatments (Trt); p=0.6815, DAT; p=0.0062, Trt*Var; p=0.9692, Trt*Var*DAT; p=9971.
LSD varieties = 0.11; LSD Treatments = 0.13 and LSD DAT = 0.09

Table 2. Effective quantum yield (ϕPSII) of soy bean varieties subjected to various $AlCl_3$ treatments. Values are means of three replicates ±STD DEV.

		ϕPSII (Relative Units)			
	Al Treatments	Varieties			
DAT	(mg/l)	SB 20	SB 19	SB 97	SB 123
5	control (0)	0.61±0.01	0.60±0.05	0.55±0.02	0.70±0.08
	25	0.64±0.02	0.41±0.09	0.50±0.01	0.50±0.04
	50	0.54±0.06	0.51±0.02	0.36±0.12	0.55±0.02
	75	0.63±0.01	0.52±0.01	0.55±0.03	0.54±0.03
	100	0.68±0.04	0.62±0.04	0.62±0.07	0.63±0.03
25	control (0)	0.54±0.02	0.33±0.13	0.48±0.01	0.48±0.08
	25	0.57±0.04	0.58±0.05	0.39±0.08	0.29±0.06
	50	0.38±0.10	0.47±0.03	0.47±0.02	0.44±0.05
	75	0.65±0.10	0.57±0.04	0.58±0.06	0.28±0.07
	100	0.43±0.06	0.62±0.08	0.54±0.04	0.36±0.09
38	control (0)	0.58±0.05	0.51±0.02	0.58±0.07	0.56±0.06
	25	0.54±0.01	0.66±0.13	0.48±0.00	0.52±0.03
	50	0.51±0.00	0.39±0.06	0.44±0.03	0.44±0.03
	75	0.39±0.09	0.34±0.10	0.42±0.04	0.46±0.02
	100	0.53±0.02	0.48±0.00	0.49±0.01	0.43±0.04

Mean varieties = SB 20 (0.55± 0.03), SB 19 (0.52± 0.01), SB 97 (0.50±0.01), SB 123 (0.48±0.02)
Mean DAT = 5 DAT (0.57±0.04), 38 DAT (0.49±0.02), 25 DAT (0.47±0.02)
Mean treatment = 0 mg/l (0.54±0.02), 100 mg/l (0.53± 0.01), 25 mg/l (0.51± 0.002), 75 mg/l (0.49 ±0.01), 50 mg/l (0.49 ± 0.01). Varieties (Var); p=0.4283, Treatments (Trt); p=0.6278, DAT; p=0.0423,Trt*Var; p=0.9326, Trt*Var*DAT; p=9867 . LSD varieties = 0.11; LSD Treatments = 0.14 and LSD (DAT) = 0.09

Table 3. Non-photochemical quenching (NPQ) of soy bean varieties subjected to various $AlCl_3$ treatments. Values are means of three replicates ±STD DEV.

		NPQ (In Relative Units)			
	Al Treatments	Varieties			
DAT	(mg/l)	SB 20	SB 19	SB 97	SB 123
5	control (0)	0.70±0.36	0.75±0.26	0.96 ±0.36	0.53±0.59
	25	0.88±0.06	0.62±0.18	0.82±0.06	0.98±1.18
	50	0.64±0.11	0.50±0.26	0.84±0.07	0.60±0.54
	75	0.63±0.36	0.77±0.62	0.86±0.31	0.99±0.60
	100	0.88±0.05	0.99±0.08	0.86±0.27	0.60±0.65
25	control (0)	0.50±0.03	0.85±0.05	0.96±0.00	0.25±0.12
	25	0.30±0.06	0.64±0.17	0.62±0.05	0.99±0.68
	50	0.12±0.06	0.30±0.07	0.38±0.22	0.16±0.18
	75	0.73±0.06	0.97±0.11	0.76±0.09	0.98±0.17
	100	0.24±0.02	0.35±0.03	0.67±0.36	0.64±0.22
38	control (0)	0.90±0.06	0.65±0.41	0.98±0.25	0.72±0.34
	25	0.37±0.18	0.34±0.25	0.93±0.00	0.97±0.35
	50	0.46±0.11	0.49±0.14	0.76±0.55	0.36±0.26
	75	0.53±0.60	0.87±0.07	0.96±0.16	0.94±0.30
	100	0.26±0.25	0.74±0.04	0.54±0.07	0.57±0.30

Mean varieties = SB 123 (0.84±0.09), SB 97 (0.83±0.08), SB 19 (0.63±0.06), SB 20 (0.54±0.1)
Mean DAT = 5 DAT (0.99±0.2), 38 DAT (0.74±0.01), 25 DAT (0.44±0.2)
Mean treatment = 75 mg/l (0.85± 0.1), 25 mg/l (0.85±0.1), 0 mg/l (0.66±0.04), 100 mg/l (0.61±0.07), 50 mg/l (0.59±0.09). Varieties (Var); p=0.2846, Treatments (Trt); p=0.5499, DAT; p=0.0022, Trt*Var; p=0.3146, Trt*Var*DAT; p=1905.
LSD varieties = 0.48; LSD Treatments = 0.57 and LSD DAT = 0.3

Plant photosynthetic pigment content

Chlorophyll a content

Figure 2 indicate that there was a decrease in chlorophyll a content in all the varieties as the $AlCl_3$ concentration in the growth medium increased apart from SB 19, SB 97 on 0 DAT and on 25 DAT for SB 123. Varieties did not show significant difference ($p \geq 0.05$) in chlorophyll content when they were subjected to $AlCl_3$ treatments. There was however a significant difference ($p \leq 0.05$) in this parameter among treatments and the interaction between varieties and treatments as well as DAT. There was no significant difference in the interaction among DAT, treatments and varieties. SB 20 had the highest mean (1.48) value for chlorophyll a content followed by SB 97 (1.28), SB 123 (1.23) and then SB 19 (1.21). For treatments, the highest mean value was found at 0 mg/l (1.64) followed by 25 mg/l (1.25) 100 mg/l (1.23), 75 mg/l (1.21) then at 50 mg/l (1.17).

Chlorophyll b content

The general trend was that chlorophyll b content decreased with increasing aluminium chloride concentration in the growth medium for all varieties except SB 97 on 5 DAT and 38 DAT (Fig. 3.). There was no significant difference ($p \geq 0.05$) among varieties, treatments and their interaction between varieties and treatments but there was a significant difference within DAT. The highest chlorophyll b mean value content was found in SB 19 (0.79) followed by SB 20 (0.78), SB 97 (0.77) and then SB 123 (0.69). For treatments, the highest mean value was found at 0 mg/l (0.93) followed by 50 mg/l (0.73), 100 mg/l (0.72), 75 mg/l (0.71) then at 25 mg/l (0.71).

Total chlorophyll content

Total chlorophyll content decreased with increasing aluminium chloride concentration in the growth apart from SB 97 on 5 DAT (Fig.4). There was a significant interaction difference ($p \leq 0.05$) between varieties, treatment and DAT. There was no significance difference ($p \geq 0.05$) among varieties and treatments as well as for the interaction among DAT, treatments and varieties. SB 20 (2.26) had generally higher total chlorophyll content compared to other varieties (SB 97; 1.96, SB 19; 1.96 and SB 123; 1.90). For treatments, the highest mean value was found at 0 mg/l (2.33) followed by 50 mg/l (1.98), 25 mg/l (1.97), 75 mg/l (1.94) then at 100 mg/l (1.93).

Chlorophyll a/b ratio

There was a clear decrease in chlorophyll a/b ratio with increasing aluminium chloride concentration on 25 DAT and 38 DAT apart from SB 97 and SB 123 on 25 DAT (Fig. 5). There were no significant difference ($p \geq 0.05$) among varieties and treatments (Appendix 4). There was a significant difference ($p \leq 0.05$) among DAT. Varieties and treatments as well as DAT, treatments and varieties did not significantly interact ($p \geq 0.05$) for the chlorophyll a/b ratio. The highest mean content in this parameter was found in SB 20 (1.90) followed by SB 123 (1.90), SB 97 (1.88) and then SB 19 (1.69). For treatments, the highest mean value was found at 25 mg/l (2.00) followed by 0 mg/l (1.99), 75 mg/l (1.82), 100 mg/l (1.82) then at 50 mg/l (1.61).

Carotenoid content

There was an increase in carotenoids content with increasing aluminium chloride concentration in varieties SB 20 and SB 123 for all DAT, SB 97 on 25 DAT and SB 19 on 38 DAT (Fig. 6). There was no significant difference ($p \geq 0.05$) among DAT, varieties, treatments and for the interaction between varieties and treatments as well as for the interaction among DAT, treatments and varieties. The highest mean content value in this parameter was found in SB 97 (0.33) followed by SB 19 (0.30), SB 123 (0.26) and then SB 20 (0.23). For treatments, the highest mean value was found at 0 mg/l (0.35) followed by 50 mg/l (0.31), 75 mg/l (0.28), 25 mg/l (0.25) then at 0 mg/l (0.23).

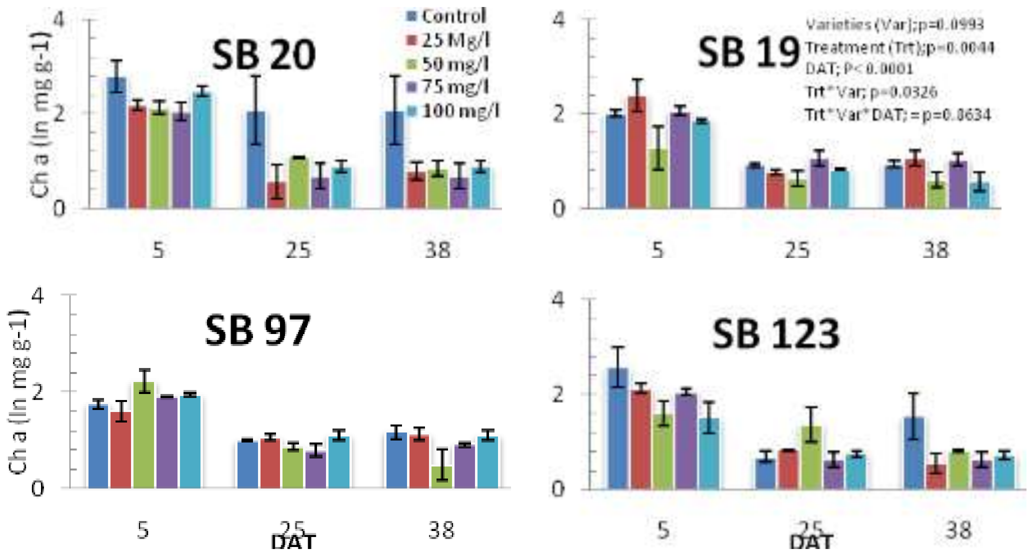


Figure 2. Chlorophyll a content of soy bean varieties subjected to various concentrations of $AlCl_3$ treatments. Values are means of three replicates ± STD DEV. Varieties LSD=0.31, treatments LSD=0.37 and DAT LSD=0.25.

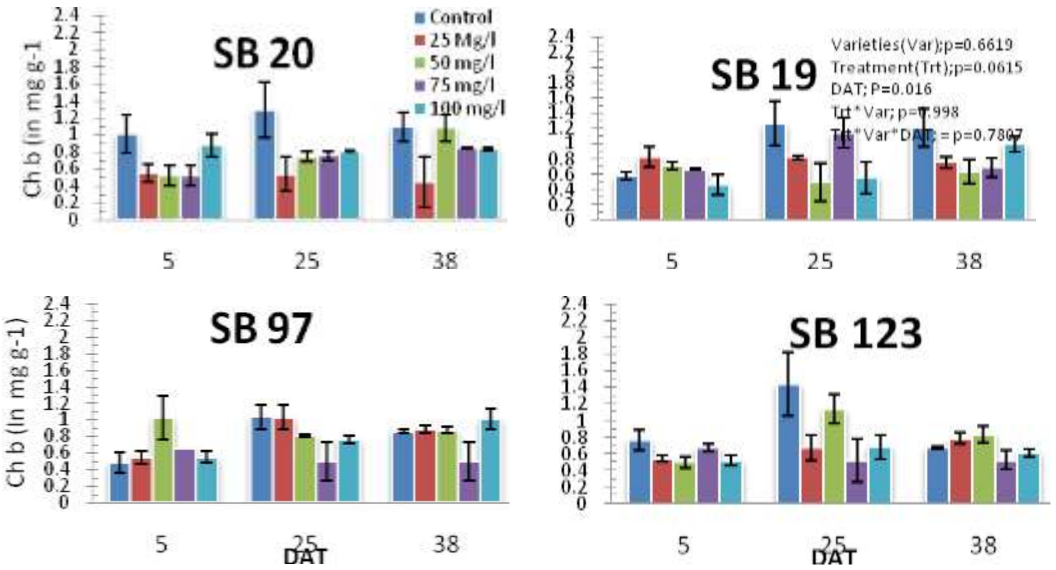


Figure 3. Chlorophyll b content of soy bean varieties subjected to various concentrations of $AlCl_3$ treatments. Values are means of three replicates ± STD DEV. Varieties LSD=0.21, treatments LSD=0.25 and DAT LSD=0.17.

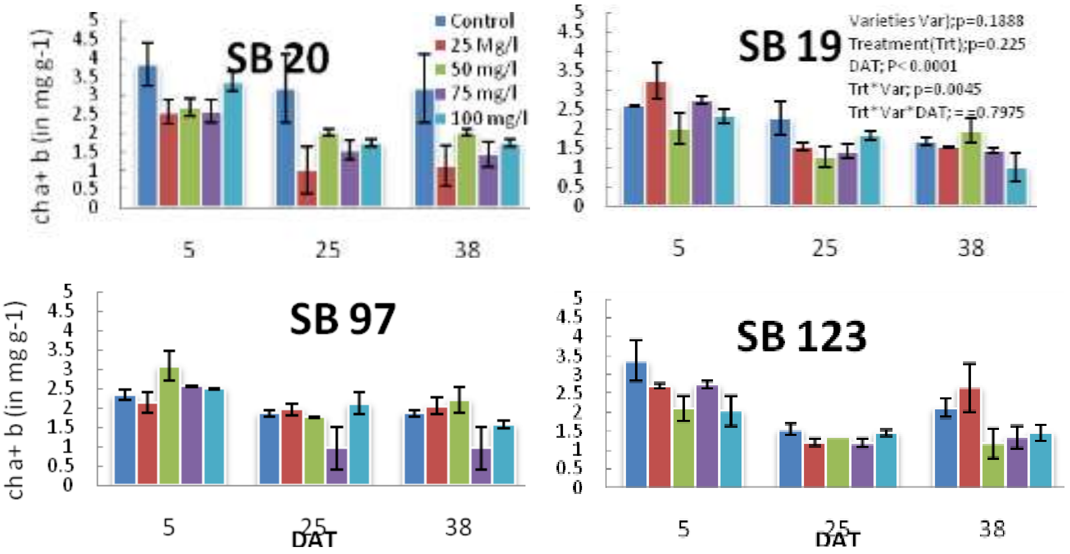
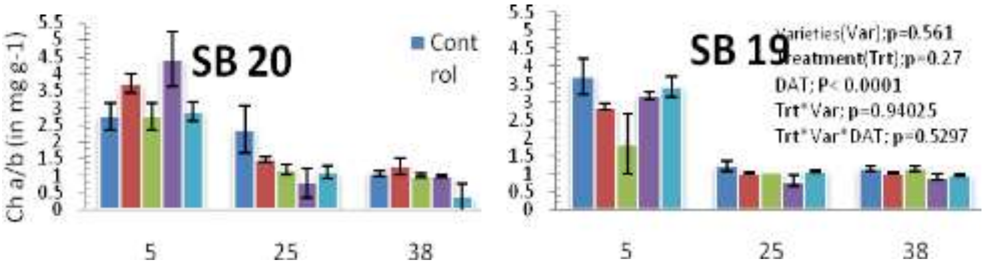


Fig. 4. Total chlorophyll content of soy bean varieties subjected to various concentration of $AlCl_3$ treatments. Values are means of three replicates ± STD DEV. Varieties LSD=0.47, treatments LSD=0.55 and DAT LSD=0.37.



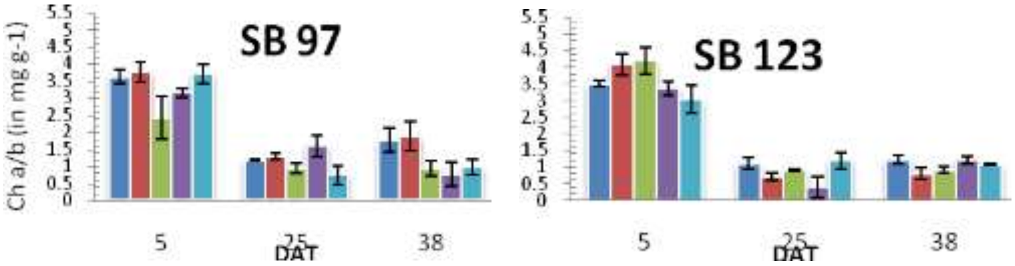


Figure 5. Chlorophyll a/b content of soy bean varieties subjected to various concentrations of $AlCl_3$ treatments. Values are means of three replicates ± STD DEV. Varieties LSD=0.48, treatments LSD=0.57 and DAT LSD=0.38.

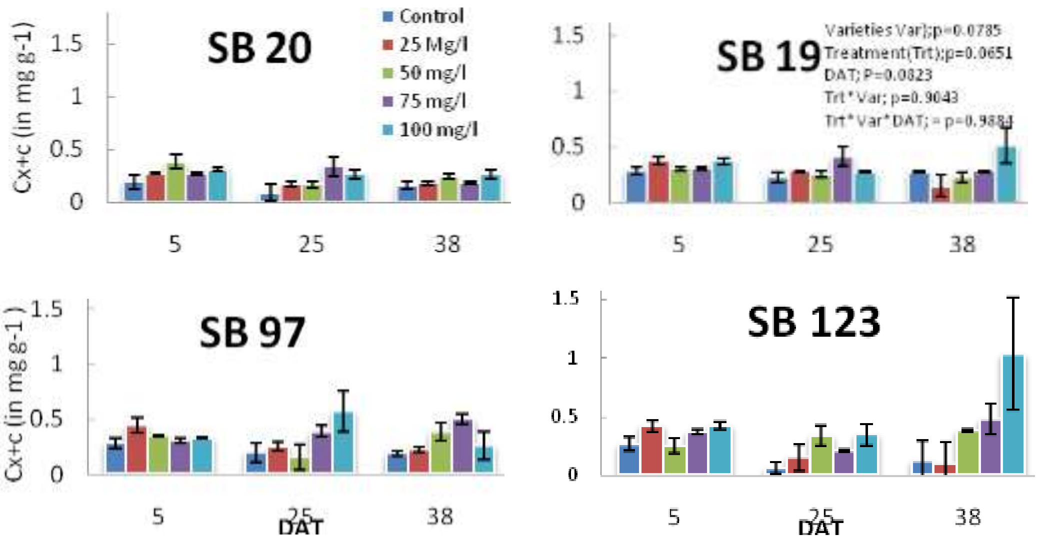


Figure 6. Carotenoid content of Soy bean varieties subjected to various concentrations of $AlCl_3$ treatments. Values are means of three replicates ± STD DEV. Varieties LSD=0.10, treatments LSD=0.12 and DAT LSD=0.08

Discussion

Dry weights

Aluminium chloride reduced the plant dry weight of soybean varieties under study. Faster growth as noted in varieties SB 20 and SB 19 might have contributed to a larger dry weight compared to the other two varieties (Fig .1). Similar findings were reported by Marjorie et al. (2010) where they found a decrease in the general dry weight with aluminium stress in blueberry genotypes. Cordovilla et al. (1999) found that roots were more sensitive than shoots to aluminium stress in faba beans. However, Bayuelo-Jimenez (2002a and 20002b) reported that aluminium-tolerant species of Phaseolus maintained relatively high root growth even in a nutrient solution containing 180 mM aluminium chloride. Dry weight of varieties studied significantly reduced in response to increasing concentration of $AlCl_3$, as earlier found by Ketan et al. (2005) working with Butea monosperma. Rapid dry weight reduction in tap roots might contribute to a major share to total root mass (Frantzius et al., 2000). Dry weights of the varieties evaluated were adversely reduced as the concentration of aluminium chloride solution increased. Aluminium in the soil affects water uptake by plants (Hong et al., 2006). Under Al stress the plants might spend more photosynthetic energy on root production in search of water (Kafkafi, 1991). This might have led to SB 123 gain more dry weight compared to the other varieties. Avoidance of $AlCl_3$ by intensive root development is dependent on the genotypes (Kuo et al., 2003).

Chlorophyll fluorescence parameters

Photochemical efficiency of PSII (Fv/Fm

The treatment of aluminium chloride differentially reduced the photochemical efficiency of PSII (Fv/Fm), ϕPSII and increased the NPQ of the varieties of soy beans investigated. Similar results were found in citrus leaves, where, aluminium chloride caused a significant decrease in photochemical efficiency of PSII (Chen et al., 2005a and 2005b). Photochemical parameters of PSII are indicative, under aluminium condition on how the overall rate of photosynthesis is affected (Genty et al., 1989). They gave the potential to estimate photosynthetic performance and, thereby, plant productivity under different environmental conditions (Maxwell and Johnson, 2000; Sikuku et al., 2010).

Maximum quantum yield (Fv/Fm)

The Fv/Fm ratio measured in the four varieties of soy bean after exposure to progressively increasing aluminium concentrations showed a non significant ($p \geq 0.05$) difference. The mean treatment values were high at the control treatment compared to aluminium treated plants. This was in agreement with the results of Ambrosio et al. (2003) in maize. This shows that photosynthetic apparatus of the plants were somehow affected by aluminium exposure (Pereira et al., 2000). The Fv/Fm values found in this study did not show a consistent reduction with aluminium chloride during all days of measurement. Similar low Fv/Fm values of 0.5 to 0.62 (in Al-treated leaves) and high Fv/Fm values of 0.78-0.8 (untreated leaves) were reported by Chen et al. (2005b) in citrus leaves. In another study, Fv/Fm has been found to be in the normal ranges of between 0.7–0.8 for healthy blueberry Al treated plants (Marjorie et al., 2010). According to Bjoȑkman and Demmig (1987) and Kate and Giles (2000), Fv/Fm ratio for normal plants have an optimal value of 0.83.

Effective quantum yield (ɸPSII)

Effective quantum yield (ɸPSII) had a low mean value at the control compared to aluminium chloride treated soy bean plants under investigation. These photochemical parameters in SB 123 were lower even under the control conditions compared with the other varieties. The variety SB 123 may be intrinsically less efficient in managing its energy for photochemical processes than the other varieties (Giannakoula et al., 2008). This cultivar-specific behaviour indicates that it might have lower productivity with respect to the other varieties. The high values of ΦPSII in SB 20 showed that the photochemical activity was the main way to dissipate safely the excess of excitation energy. This indicates that electron transport rate was never saturated which is an indication that other sinks apart from the assimilatory process were likely to accepting these electrons. The excess energy of excitation is dissipated by photochemical activity avoiding the over reduction of PSII reaction centres (Ambrosio et al., 2003). Hoshino et al. (2000) observed Mehler reaction in mesophyll chloroplasts of C4 species and proposed that they play a role in the production of extra ATP for the pseudocyclic photophosphorylation.

Non-photochemical quenching (NPQ)

Thermal energy dissipation measured as NPQ in the four soy bean cultivars did not have a clear pattern of reduction with increasing aluminium chloride concentration (Table 3). Mostly NPQ was high in aluminium chloride treated plants compared to control plants. The highest NPQ mean value was found at 75 mg/l. Similar results were observed in Artemisia anethifolia (Lu et al., 2003) and blueberry genotypes (Marjiorie et al., 2010) when subjected to aluminium. In these cases other metabolic pathways such as the photorespiration in aluminium chloride treated leaves may have been upregulated to cope with the increased excess of excitation energy (Osmond and Grace, 1995; Marjiorie et al., 2010). Bilger and Bjoȑkman (1990) and Demmig-Adams and Adams (1996) reported that changes in NPQ correlate closely and directly with changes in carotenoids pigments; however, it has also been found that carotenoids may be unrelated to NPQ (Chen et al., 2005b; Johnson et al., 1993). It is accepted that PSII is the most vulnerable part of the photosynthetic apparatus to stress-induced damage (Marjorie et al., 2010). Aluminium treated leaves therefore might have used a smaller fraction of the absorbed light in electron transport compared with control leaves which had more excess excitation energy just as found by Chen and Cheng (2003) in citrus leaves. A higher concentration of aluminium chloride treated leaves contributed to excess of thermal energy of dissipation (NPQ). This gives evidence that apart from photochemistry, fluorescence strategy have been adopted to dissipate energy. Low Non-photochemical quenching in plants is an indication that less thermal energy is dissipated (Chen and Cheng, 2003). It has been suggested that, excess absorbed light can be harmlessly dissipated as heat through xanthophyll cycle-dependent thermal energy dissipation in the antenna pigment complexes of PSII (Demmig-Adams and Adams, 1996; Niyogi et al., 1998; Li-song et al., 2010). The up-regulation of enzymatic and non-enzymatic antioxidants may have increased as a requirement for scavenging reactive oxygen species in aluminium stressed leaves due to increased closure of PSII reaction centres, as indicated by increased NPQ (Li-Song et al., 2010).

Total chlorophyll (a + b), Chlorophyll a and b and Chlorophyll a /b ratio

Aluminium-induced decrease in chlorophyll content. This has been also reported in other plant species, such as citrus (Chen et al., 2005a; Jiang et al., 2008, 2009a and 2009b), soy beans (Milivojevi et al., 2000; Ying and Liu, 2005), sorghum (Peixoto et al., 2002), rice (Oryza sativa) (Kuo and Kao, 2003); wheat (Okhi, 1986), beech (Ridolfi and Garrec, 2000) and barely (Hordeum vulgare) (Abdalla, 2008). Although it should be noted that a decrease in chlorophyll content to a greater extent in response to aluminium was probably not the primary factor to limit photosynthetic ability of the plants (Jiang et al., 2008, 2009a and 2009b; Li-Song et al., 2010). In the four soy bean varieties under study, it is of noteworthy that the other factors other than decreased synthesis of chlorophyll may have contributed to adverse effects on growth and development of the four soybean varieties (Chen et al., 2005b). Decrease in chlorophyll a and chlorophyll b (Figs. 2, 3 and 4) with increasing aluminium chloride concentration in the leaves of the four soy bean varieties may be attributed to the inhibition of the activity of - aminolevulinic acid (-ALA) dehydratase (Pereira et al., 2006). Chlorophyll a/b ratio decreased markedly under aluminium treatment in the four soy bean varieties (Fig. 5). These decreases reflect a reduction in the chlorophyll antenna size of the photosystems and might protect the photosystems from photoinhibition by reducing energy delivery to the reaction centres (Adams et al., 2004; Marjiorie et al., 2010). Contrary to these findings in ‘Sour pummelo’ (Citrus grandis) it was shown that chlorophyll a/b ratio remained unchanged (Jiang et al., 2008). Change in chlorophyll antenna size (Chlorophyll a/b) is probably a strategy to reduce light absorption and avoid possible damage to the photosystems of the soy bean varieties (Azmat and Hasan, 2008). According to Marjiorie et al. (2010) reduction in chlorophyll antenna size under aluminium chloride stress is meant to maintain ɸPSII.

Carotenoids

The photoprotective carotenoids increased within varieties under investigation with increasing aluminium concentration (Fig. 6); in agreement with the findings of Marjorie et al. (2010). This increase in carotenoids play an essential role in protecting the photosynthetic apparatus against the harmful effects of light and oxygen, dissipating the excess light as heat in the antenna pigment complexes (Krupa and Baszynski, 1995; Niyogi et al., 1998). Carotene functions as a passive light protecting filter and has got the role of accessory pigments transferring energy and oxygen (Adams et al., 1998). A slight increase of carotenoids in SB 20 with a decrease of photochemical parameters, suggests that this SB 20 variety may favour the heat dissipation pathway and thus avoid PSII photoinhibition as is also suggested by Demmig-Adams and Adams (1996) and Marjorie et al. (2009). In this study carotenoids pigments may have prevented chlorophyll and thylakoid membrane from the damage by the absorbed energy through photo oxidation (Sükran et a., 1998).

Conclusion

Intervarietal differences were observed in relationship to dry weight accumulation, chlorophyll fluorescence and photosynthetic pigments contents. The chlorophyll fluorescence and photosynthetic pigment parameters indicated that SB 20 was more tolerant to aluminium chloride stress, followed by variety 19 and hence recommended for growing in acidic soils.

Acknowledgments

Appreciation goes to TSBF-CIAT (Maseno) for providing the soy been seeds used in this study.

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