SELECTED PHYSIOLOGICAL AND GROWTH RESPONSE OF FOUR SOY BEAN VARIETIES TO ALUMINIUM CHLORIDE STRESS

BY

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DECLARATION

I certify that this thesis has not been previously presented for a degree in Maseno University or in any other University. The work reported herein is my original work and all sources of information have been supported by relevant references.

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DEDICATION

This work is dedicated to my family, because their love has kept me going. Through the years I have learned how love, friendship, sacrifice and unity in a family can be a strong base of commitment, inspiration and accomplishment. Highly dedicated to my very own youngest brother Xavier Mulinya, baby friend Sasha and Wanga Erick whom we share similar dream.

ABSTRACT

Soy bean varieties commonly grown in Kenya`s acidic soils that contain aluminium are SB 97, SB 19, SB 20 and SB 123. Soy bean grains have high protein content, vitamins and used to manufacture industrial products. Aluminium toxicity affect growth and physiology of plant growth. The effects of aluminium chloride solution on plant growth, uptake of mineral nutrients and distribution in vegetative parts of soy beans are still not fully understood. The objective of this study was to investigate the effects of aluminium chloride stress on four varieties of soy bean grown in Kenya with a view of identifying the tolerant varieties among them to be recommended for growing in areas prone to aluminium stress. The experiment was done under greenhouse conditions. Seeds were planted in 20 litre PVC pots filled with soil. Randomized Complete Block Design in a factorial way, with three replicates and five levels of 0 (control), 25 mg/l, 50 mg/l, 75 mg/l and 100 mg/l aluminium chloride concentrations in tap water was used. Growth and physiological parameters; including mineral concentration in leaves, chlorophyll fluorescence and photosynthetic pigment contents were measured. The data was subjected to factorial Analysis of Variancy and Tukey`s HSD tests at 5% was used to separate treatment-Variety means. Growth parameters measured mostly showed clearly the tolerance difference levels of the varieties. Aluminium reduced water absorption hence decreasing productivity. Variety SB 20 concentrated more aluminium in leaves. Some varietal difference which were not significant were observed in mineral accumulation. This indicate that varieties could be behaving differently in absorption and accumulation of nutrients. Maximum quantum yield and effective quantum yield had the highest mean value in SB 20. Non-photochemical quenching was highest in SB 123. These implies that varieties behaved differently in PSII impairment activity. Major decrease in total chlorophyll with increased aluminium chloride concentrations was observed in SB 19, SB 97, and SB 123 suggesting selective chlorophyll photobleaching showing that smaller amounts of energy was delivered for electron transport. There was a marked decrease in chlorophyll a/b ratio under aluminium chloride solution treatment. SB 123 had a larger mean value of carotenoids in comparison to SB 20, SB 19 and SB 97. Carotenoids concentration was more at 100 mg/l aluminium chloride concentration. It was to assist in transfer of energy and oxygen in accessory pigments. Tukey`s HSD tests showed no significant difference ($p \geq 0.05$) within varieties for physiological parameters measured. Variety SB 20 and SB 19 were identified to be more tolerant to aluminium stress and hence recommended for growing in aluminium prone soils. Mineral nutrients accumulation, photochemical parameters of PSII and photosynthetic pigments parameters measured were found to be sensitive to aluminium chloride treatments: the later two parameters showed that $AlCl_3$ affects the overall rate of photosynthesis.

LIST OF ABBREVIATIONS AND SYMBOLS

``Cont``:- Continuation
AAS:- Atomic Absorption Spectrophotometer
CEC:- Cation Exchange Capacity
Chl a* + *b:-Total chlorophyll
***Chl a*:-** Chlorophyll *a*
Chl *b*:- Chlorophyll b
CIAT:- International centre for tropical Agriculture
COD:- Cytochrome-oxidase isozymes
$\mathbf{C_{X+C}}$:- Carotenoid concentration
DPSB:- Dual Purpose Soybean Bean
Eco-SSL:- Ecological Soil Screening Level
ETR:- Electron transport rate
F0:- Initial fluorescence
F0':- Fluorescence after light induction
Fm:- Maximal fluorescence
Fs:- Steady-state fluorescence
FW:- Fresh weight
KARI:- Kenya Agricultural Research Institute
OJIP:- Chlorophyll fluorescence
PI:- Photosystem I
PII:- Photosystem II
POD:- Peroxidase isozymes
ϕPSII:- Actual efficiency of PSII or quantum yield of non- cyclic photosynthetic electron transport
qN:- Non-photochemical
qP: - Photochemical
R: S:- Root: Shoot ratio
RGR:- Relative growth rate
SOD:- Superoxide dismutase
TGx:- Tropical Glycine crosses series
TSBF:- Tropical soil Biology and Fertility
VFE:- The visual food encyclopedia

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CHAPTER ONE
INTRODUCTION

1.1 Background information

Soy bean (*Glycine max* L.) is a commonly grown legume crop in Kenya (Mahasi *et al*., 2010). The species is classified in the Order Fabales and Family Fabaceae (Helio *et al*., 2013). The grains are among the world's most important in terms of high protein content of 35-40 % and oil of 15-22 %. In addition, soy bean seeds are rich in essential amino acids, vitamins and minerals. Soy beans are used in the manufacture of foods, for instance; fermented and dried products like milk, tofu, soya sauce and bean sprouts (VFE, 1996). Medically, it's used to improve the function of the heart, liver, kidneys, stomach, and bowels and to cure various diseases and ailments (Merritt *et al*., 2004). It is also important as a source of oil which is extracted and used in manufacturing salad oil, cooking oil, and margarine for food and for numerous industrial purposes (Merritt *et al*., 2004). Soy bean meal is extensively used as an ingredient in livestock feed (Monty and Gary, 2003).

Crops grown on 90% of arable lands experience one or more environmental stresses (Mona, 2008). Considerable attention has focused on assessing the impact of aluminium chloride stress on cultivated plants, because its stress is often the primary factor limiting crop production in many acid soils (Kochian 1995; Alvim *et al*., 2012). Furthermore, the use of aluminium chloride resistant plants is part of crop management strategy for agricultural production in acid soils, since this factor is particularly common in the world (Larsen *et al*.,1996; Alvim *et al*., 2012). Western Kenyan soils were screened and most were found to have high aluminium saturation and low phosphorous (P) availability (Gudu *et al.*, 2001). This was found to be remarkably affecting maize production by Gudu *et al.* (2001). Soy bean and other crops are also grown in the same areas; hence face a problem of aluminium stress. Mahasi *et al*. (2010) noted that Aluminium have led to low soy bean production in Kenya compared to other countries such as USA. The productivity of soy beans therefore needs to be increased in Kenya by planting aluminium chloride tolerant varieties of soy beans to cater for these demands. On contrary such tolerant varieties have not been identified and therefore need to be identified.

Africa had 0.4 – 1% of total world production of soy bean during the last decade.

Nigeria, contributed 50% of Africa's output, which accounted for 0.3% of the world soy bean production (Chianu *et al*., 2008). After 1990, data suggests that production, area and yield in Kenya have remained almost stagnant, with little annual change (FAOSTAT, 2008). The then Western province led in soy bean production in Kenya, with nearly 50% (Chianu *et al*., 2008). However, this would be much higher in relation to other former provinces if aluminium chloride tolerant varieties are cultivated in this regions. The main soy bean producing districts in former Western province are Butere/Mumias, Busia, Bungoma, Teso, Kakamega, Mount Elgon, Lugari, and Vihiga. Butere/Mumias, Busia, and Bungoma districts accounted for approximately 80% of the total soy bean production within the province (Mahasi *et al*., 2010). Unfortunately the same regions were screened by Gudu *et al.* (2001) and found to have aluminium in the soil. Nyanza and Central provinces, produced 11-12% (Gok, 2007; Chianu *et al*., 2008). Estimates of area potentially suitable for soybean production in Kenya ranges from 157,000 ha to 224,000 ha as indicated by ministry of agriculture and lake victoria basin development authority (Chianu *et al*., 2008). Former Nyanza province accounts for 11–15% of Kenyan land area potentially suitable to soy bean cultivation, former Western province accounts for 9–13%. Trans Nzoia, Siaya, and Bungoma counties account for the largest proportion of land potentially good for soybean production in western parts compared to other regions of Kenya .

Productivity of soy bean in Kenya is low (450 kg/ha) compared to Brazil and USA which produce 1301 to 2033 kg/ha (Gudu *et al*., 2001). This low productivity is a problem because Kenya needs more soy beans to satisfy a growing demand for stock feed and to improve the human nutrition (GoK, 2007). The reasons for poor production in Western Kenya have been clearly identified to be absence of desirable traits such as tolerance to extreme high temperatures and tolerance to acidic soils, which are often high in exchangeable aluminium (Mahasi *et al*., 2010). Higher-yielding varieties better suited to the aluminium stress conditions in the growing areas of Kenya are needed to alleviate this problem. Overall, Kenya Agricultural Research Institute (KARI) four grain varieties (931/5/34, Nyala, EAI 3600 and Gazelle) have been evaluated in terms of yield and found to be among the best varieties across East Africa (Mahasi *et al*., 2010). On the other hand, through farmer evaluation activity, CIAT-TSBF (Maseno) have identified varieties DPSP 19 (TGX 1740-2F), SB 20, EAI 3600 (SB 97) and SC Squire (SB 123) to be among the best performing within Nyanza

and Western regions (Chianu *et al*., 2008). This was through farmers own identification programmes. However, little information exist on the tolerance levels of the varieties grown in these areas to aluminium chloride stress has not been established.

Aluminium constitute about 8% of soil minerals and is the most abundant on earth (Alvim *et al*., 2012). Levels of free aluminium in soils are increased by acidification of the soils. Natural waters contain up to 48µM Al (Isabel *et al*., 2003). Aluminium ion (Al^{+3}) is found in approximately 40% of the arable soils of the world (Mona, 2008) and acidic soils favour the dissolution of minute quantities of Al^{+3} from metal oxides. Aluminium stress is a major factor in limiting growth in plants grown in most acid soils (Mona, 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). Aluminosilicates, including the feldspars, micas, and clay minerals, are the most common primary and secondary minerals in soils. Aluminium oxide (Al_2O_3) occurs as corundum and emery. The hydroxide ($Al(OH)_3$, occurs as gibbsite. Aluminium also occurs in interlayer positions in clays, often forming complete layers to which the term chloride is sometimes applied (Eco-SSL, 2003). Therefore Al does not occur as a single element but in compound form with other elements in soils (Xiao-Bin *et al*., 2007) and thus it`s individual stress effects in plants can`t be detected unless when supplied in compound form (Alvim *et al*., 2012).

Quantitative information on the uptake and on the cellular distribution of aluminium is required to understand the mechanisms of aluminium stress; the mechanistic basis of aluminium transport and the overall subcelluler distribution in many plants remain speculative (Mossor-Pietraszewka, 2001) and unknown in soy beans. Different plant species and genotypes show distinct variation in tolerance and/or sensitivity to Al^{3+} generating a broad spectrum of responses to Al^{3+} exposure (Ezaki *et al*., 2000; Alvim *et al*., 2012). Studies have identified varietal differences in aluminium tolerance in rice, alfalfa, tomato, snap bean, cotton, maize, sunflower, pea, and sweet potato (Voegelet, 2001). Research on aluminium availability and aluminium stress effects on maize plant varieties was carried out by Gudu *et al.* (2001). However, no research data on effects of aluminium stress on soy bean varieties grown in Kenya has been reported.

Soil acidification, may results from air pollution by acidic nitrogen and sulphur oxides which leads to acid rain formation. High rainfall affects the rate of soil acidification depending on the rate of water percolation through the soil profile. Aluminium is hydrolyzed contributing to soil acidity because it's a major constituent in a large number of primary and secondary minerals (Hede *et al*., 2001). Primary minerals are minerals used to assign a classification name to the rock and the accessory minerals present in lesser abundance. Secondary minerals form at a later time through processes such as weathering and hydrothermal alteration. Soil acidification is intensified by the removal of cations through the harvesting of crops and burning of plant wastes on farms (Arunakumara, 2012). Organic matter decaying to form carbonic acid and other weak acids also contributes to acidification (Heru, 2014). Then, under these acid soil conditions, primary and secondary minerals dissolve to a high extent, releasing aluminium into the soil solution (Hede *et al*., 2001). Over half a million hectare of arable lands may have been acidified as noted by Okalebo *et al.* (1997) in Kenya through this processes.

Acidic soils will therefore have low levels of nutrients, especially phosphorus (P) and nutrient cations, but high levels of potentially exchangeable aluminium (Mamun *et al.*, 2014). When these soils are strongly acidic, aluminium becomes soluble and interacts with phosphorus, calcium and other minerals (Mundayatan *et al*., 2008). This interaction makes the nutrients available to plants in low amounts but the differences in rates of nutrient availability to the chosen soy bean varieties under study and grown in Kenya is not known. Soluble aluminium is toxic to the roots of most plants leading to reduced growth in terms of diameter, height and leave number (Mamun, 2014). These soils are considered to be plant stressing and reduces plant production rate. Aluminium reaches the photosynthetic cells posing an effect on photosynthetic pigments associated to both photosystem I and II (Cai *et al*., 2011). Literature herein indicates that, there is less concern on the effects of Al to photosynthetic pigments and photosynthesis process of the soybean varieties grown in Kenya (Mahasi *et al*., 2010).

Aluminium availability in the plant tissues therefore is expected to decrease production of soy bean crops cultivated on acidified soils unless special management strategies are employed (Mossor-Pietraszewka, 2001). These special strategies implies that more money is needed for management thus straining developing country`s economy when aluminium chloride tolerant variety cultivation can easily medicate the problem better and cheaply. Acidic soils can

be rehabilitated by liming, increasing soil pH to 5.5 eliminate aluminium toxicity problem and increases the range of crop types that can be grown (Luciana *et al*., 2007). Nutrient additions to the soils are still required to achieve good crop productivity. Liming does not correct the acidity of the subsoil below 0-20 cm (Hede *et al*., 2001). At this region root growth is restricted in susceptible cultivars causing susceptibility to drought and restricted nutrient uptake (Hede *et al*., 2001). In developing countries, most areas have acidified soils which have constraint impact to crop production (Cai *et al*., 2011). This is because agricultural lime and fertilizer inputs are not readily available or affordable. In Kenya, there is, therefore, need to determine the most tolerant varieties of soy bean to aluminium stress and other species of crop. Measuring the selected physiological parameters on soybean varieties subjected to aluminium stress may lead to recommending varieties that are tolerant to be grown in Western Kenya. In fact other authors such as Marjorie *et al.* (2010) have used this approach to determine tolerance levels in other plants varieties.

Aluminium has effects on plant dry weight and morphological parameters (Gary *et al*., 1998). Marjiorie *et al.* (2010) noted that the tolerant varieties accumulate more aluminium in leaves. Al ions interfere with uptake and transport of mineral nutrients (Luciane *et al*., 2007). Owing to this documented information it was found interesting to study aluminium effects to growth, aluminium accumulation and mineral nutrients accumulation in the soy bean varieties. Aluminium also affects photochemical efficiency of photosystem II (PSII) since it has been found to have a significant effects to PSII parameters and to photosynthetic pigments in some plants such as citrus (Chen et *al*., 2005b)

Bioactive forms of aluminium, particularly monomeric aluminium, has been found to be toxic to plants (Zsoldos *et al*., 2003). Growing aluminium chloride tolerant soybean varieties can overcome the problems of aluminium toxicity. This will limit the expenditure on the soil amendments like liming, and nitrogen fertilizer application that exceed plant uptake, which is costly (Cai *et al*., 2011).

1.2 Problem statement

Gudu *et al.* (2001) found that acidic soils with aluminium occur in most regions of Kenya. Aluminium chloride stress is a serious environmental problem that contributes to low production of soy bean. Soil amendments needed to ameliorate the aluminium stress initiated to soy bean plants by the saturated aluminium in Kenyan soils are an expense to the rural

poor farmers and may not be sustainable. Information on the effects of aluminium chloride on growth, uptake of nutrients, concentration in leaves, PSII parameters and to photosynthetic pigment concentration for the four selected soy beans grown in Kenya is missing. Moreover, tolerance levels of the soy bean varieties recommended for cultivation on Kenyan aluminium prone soils need to be investigated since it is not known. No research on growth, chlorophyll fluorescence, mineral nutrients and photosynthetic pigment contents has been done on the selected soy bean varieties under study and grown in Kenya. It was necessary to assess the selected physiological and growth parameters of different four varieties of soy bean selected for study so as to identify the tolerant ones that can be recommended for aluminium prone lands.

1.3 Justification of the research problem

The varieties EAI 3600 (SB 97), TGX 1746-2F (SB 19), SB 20 and SC Squire (SB 123) are recommended for most regions in Kenya basing on farmers own evaluation programmes under TSBF programmes. Over half a million hectare of arable lands are acidic in Kenya. Unfortunately, aluminium availability in the soils of these areas has negative impact on crop production, including soy bean as identified by Mahasi *et al*. (2010). Identification of the most tolerant varieties to aluminium chloride give an insight that will lead to breeding of aluminium tolerant varieties of soy bean. Aluminium tolerant varieties identified herein will be recommended to farmers in order to overcome yield reduction. Photosynthesis and photosynthetic pigment contents was chosen to be studied in soy bean varieties under study since it is the most important metabolic event on earth and is certainly the most important process to understand in attempting to maximize crop productivity and minimize the side-effects of soil contamination. However, it is a physiological process affected by environmental factors, including various stresses such as aluminium chloride stress understudy.

There is high demand for nutrient rich foods to meet the ever increasing population in Kenya. Soy beans meet such requirements due to it`s high protein content. It`s increased production through planting aluminium chloride tolerant varieties will therefore meet the nutritional requirements of the ever growing population, improve food security and save on foreign exchange. However, tolerance level to aluminium stress have never been determined for the varieties of soy beans that are widely grown in Kenya. There is therefore need to

determine the tolerance levels of soy bean varieties grown in Kenya to aluminium stress in order to identify the tolerant varieties that can be recommended to be grown by farmers in areas prone to aluminium stress.

1.4 Objectives of the study

1.4.1 General objective

The general objective of this study was to assess the selected physiological and growth response of four selected soy bean varieties (SB 20, SB 19, SB 97 and SB 123) grown in Kenya to aluminium chloride stress.

1.4.2 Specific objectives

i) To determine the effects of aluminium chloride stress on growth parameters of four soy bean varieties grown in Kenya.

ii) To determine the effects of aluminium chloride stress on aluminium accumulation and NPK, Mg and Ca uptake as mineral elements by the four soy bean varieties grown in Kenya.

iii) To determine the effects of aluminium chloride stress on chlorophyll fluorescence of the four soy bean varieties grown in Kenya.

iv) To determine the effects of aluminium chloride stress on photosynthetic pigments content of the four soy bean varieties grown in Kenya.

1.5 Hypotheses

i) Aluminium chloride stress has effects on growth of the four soy bean varieties grown in Kenya.

ii) Aluminium chloride stress has effects on aluminium accumulation and mineral elements uptake in the four soy bean varieties grown in Kenya.

iii) Aluminium chloride stress has effects on photosynthesis of the four soy bean varieties grown in Kenya.

iv) Aluminium chloride stress has effects on photosynthetic pigment contents of the four varieties of soy beans grown in Kenya.

CHAPTER TWO
LITERATURE REVIEW

2.1 Soy bean

In sub-Saharan Africa, small-scale farmers grow soy bean as a sole crop or as mixture with sorghum, maize or cassava. They are grown on paddy-rice bunds too (Mahasi *et al*., 2010). To prevent a build-up of soil-borne diseases, soy bean should not be grown on the same site for more than two years. Crop rotation of 3 to 4 years is also used to control disease (Helio *et al*. 2015). The plant grows best in a rotation after maize or small grains but should not follow edible beans, rape, or sunflowers because white mould disease can be carried over.

Soy bean grows best from the Equator to latitude 55° N or 55° S and from below sea level to altitudes close to 2000 m. The crop thrives best at soil pH 6.0-6.5. Temperatures below 21°C and above 32°C can reduce floral initiation and pod set (Da Mota, 1978). Soy bean varieties for different agro-ecological zones in Kenya and their parameters have been described by Wafula and Nassiuma (2001). This includes;

Homabay which is a warm temperate region recommended for "Duicker", "EAI 3600" and "Nyala",

Bukura, Kakamega, Embu are moderate temperate regions, varieties "SCS I", "Duicker", "Nyala" and "Gazelle",

Cool temperature sites e.g. Bahati, Baraton, Menengai; varieties "Sable", "SCS I", "Nyala" and "Gazelle",

"Gazelle", "EAI 3600", "Nyala" and "Sable" are varieties in marginal rainfall sites such as Matayos, Gachoka, Makueni, and Ol Rongai.

These varieties grown in Kenya, posses different plant characteristics. High production rate have been found to be in varieties EAI 3600 (SB 97), DPSB 19 (TGX 1740-2F) (SB 19), SB 20 and SC Squire (SB 123) through farmers own identification programme by International Centre for Tropical Agriculture (CIAT) as noted by Jonas and Bernard, (2006). SB 123 has its origin from the Seedco company. Variety TGX1740-2F has its origin in Uganda and is the only variety that can be recommended across locations in Kenya due to its high promiscuous nodulation. It is clearly better than the existing farmers' own variety, Nyala and EAI 3600 which are KARI product with EAI 3600 having its origin in USA (Jonas

and Bernard, 2006). TSBF-CIAT have since 1990`s developed and tried the multipurpose soy bean varieties (specifically the TGX series). The TGX series is characterized by high promiscuous nodulation. The varieties are called 'dual-purpose' they produce grains like the traditional varieties. Secondly the properties; poverty alleviation, income generation, soil fertility among others make them earn the name and be desirable in Kenya (Jonas *et al*., 2008). Trials by TSBF in Western Kenya have demonstrated higher yielding for the dual-purpose varieties due to increased dry weight production and higher net nitrogen addition into the soils.

Varieties chosen for study are among the yielding in terms of grains; EAI 3600 (SB 97) is an early maturing variety just like SB 19 which is promiscuous with more but smaller leaves. SB 97 is early maturing (123 days). SB 20 is the late maturing (137 days) with more and broad leaves. It achieves an average height just like SB 123 (matures at 137 days) and SB 19. SB 20 and SB 19 are small seeded varieties compared to SB 123 and 97. Soy bean seed loose viability within 6-10 months. Soy beans may be sown without tillage in rice stubble after each harvest in rows with spacing of 25 x 25 or 20 x 20 cm. In tilled fields, soy bean are sown in rows 40-50 cm apart and within rows the seeds are planted 10 cm apart. Seed rate is 60-70 kg / ha (Wafula and Nassiuma, 2001).

Irrigation at flowering and during seed filling is essential to gain optimum yield. More frequent irrigation is needed in sandy, well-drained soils than in heavy clay soils. Where soy beans have not been grown before it may be beneficial to treat the seed with Soy bean inoculum (*Rhizobium japonicum*) at a rate of 100g/15kg seed before planting to allow maximum nitrogen fixing throughout the growing season (Mahasi *et al*., 2010). Nitrogen added during planting delays nodulation and when applied during the vegetative stage results in poor nodule formation in proportion to the rates applied (Mahasi *et al*., 2010). Some early-maturing cultivars can be harvested for grain 70 days after planting and late maturing cultivars need up to 180 days. Vegetable soy beans are harvested when the pods are still green but when the seeds have filled the pod. Most small scale farmers achieve yields of about 500-1000 kg/ha, though 3000kg/ha is possible with good husbandry practices and recommended varieties (Mahasi *et al*., 2010).

2.2 Aluminium stress and growth

Aluminium toxicity is an important growth-limiting factor for plants in acid soils below pH 5.0 but can occur at pH levels as high as 5.5 in mine spoils (Rasiane *et al*., 2014). This toxicity affects various organs of the plant body. Excessive concentrations of Al lead to decreased dry weight. Aluminium stress inhibits cell elongation by reducing plant-water status (Rout and Das, 2001). Nevertheless exposure of plants to Al for either a short period (30 minutes to 2 hours) or low concentrations has been found to be beneficial to plant growth, by accelerating root formation, root growth and elongation, shoot growth and an overall plant growth stimulation (Mona, 2008). Under acidic conditions aluminium is toxic to plant roots and their capacity to absorb water and nutrients. Aluminium toxicity limits plant growth mainly through its adverse effects on root growth and development. Plants grown in acid soils as a result of aluminium solubility at low pH have undeveloped root system and exhibit a variety of nutrient-deficiency symptoms leading to decreased yields (Haider *et al*., 2006). This growth parameters as affected by aluminium stress in the selected Kenyan grown soy bean have not been determined.

In most plants, the foliar symptoms resemble those of phosphorous deficiency (overall stunting, small, dark green leaves and late maturity, purpling of stems, leaves, and leaf veins, yellowing and death of leaf tips). In some cases, aluminium toxicity appears as an induced calcium (Ca) deficiency or reduced Ca transport (Figure 2.3) problem (curling or rolling of young leaves and collapse of growing points or petioles). Information on how aluminium stress affects accumulation of macronutrients in soy beans grown in Kenya is lacking. Excess Al induces iron (Fe) deficiency symptoms in rice (*Oryza sativa* L.), sorghum and wheat (Rout and Das, 2001). Such symptoms have hardly been studied in the selected soy bean.

Root growth is inhibited 2-4 days after germination by aluminium toxicity in barley plant, having greater signs of damage compared to other parts (Abdalla, 2008). Lateral roots become thickened and turn brown, stubby and lack fine branching and toxicity can also be observed in the root tips (Nadine *et al.,* 2010). The cells of the epidermis and outer cortex of maize (Al-sensitive one) in the portion approximately 1 cm from the root-tip are damaged. Their walls are abnormal and partially detached in barley plants highly sensitive to Al (Hede, 2001). Pronounced abnormality and detachment of the cell walls to cell membranes involves almost the whole cortex. Few cortex cells remain alive in oats (Al-tolerant ones) after 6 days'

exposure to the Al treatment. Aluminium was absorbed in large amounts in the tip portion of the root with decrease in potassium (K) content and almost constant Ca content (Miguel *et al.*, 2013). Aluminium has been associated with the collapse of the conducting tissue of the stele and disintegration of the outer cells of the root (Rout and Das, 200).

Several studies have provided evidence that the root apoplast plays a critical role in the tolerance mechanisms, based on efflux of organic acids such as malate and citrate (Ma, 2005; Alvim *et al*., 2012). These substances are able to form a strong complexes with aluminium, thus chelating Al^{3+} and rendering aluminium to a non-phytotoxic state. Ma *et al*. (2002); Yang *et al*. (2008); Alvim *et al*. (2012) reported that root organic acid secretion does not appear to contribute to differential Al-resistance among rice cultivars, and root cell walls may play an important role in excluding Al^{3+} specifically from the rice root. Another response is callose (1→3, β-D-glucan) formation, which is synthesized by plants in response to biotic and abiotic stress (Verma and Hong, 2001; Alvim *et al*., 2012). Aluminium ions can elicit callose formation, indicating Al^{3+}-injury to roots (Sivaguru and Horst 1998; Alvim *et al*., 2012). Its deposition is considered as a marker for Al^{3+} stress (Rincon and Gonzales 1992; Alvim *et al*., 2012) and is believed to mediate Al^{3+} stress in plants (Alvim *et al*., 2012). Moreover, the impact caused by Al^{3+} can provoke alterations in morphology and physiology of plants (Alvarez *et al*., 2012) such as soy bean which needs to be studied critically.

In spite of the progress made, Al^{3+} exclusion mechanisms of some of the most Al^{3+}-resistant plant species such as some varieties of rice (*Oryza sativa* L.) (Cai *et al*., 2011) and soy bean (Fathy *et al*., 2015) are still far from being well understood and the cause of Al^{3+} stress needs further investigation (Alvim *et al*., 2012). Plants tolerate aluminium stress by external tolerance mechanisms that facilitate aluminium exclusion from the root apex and internal tolerance mechanisms that confer the ability to tolerate aluminium in the plant symplasm (Fathy *et al.*, 2015). Due to the common assumption that most aluminium in the root is apoplasmic and that very low penetration of aluminium into the root by symplasm is expected, there is less research on internal tolerance mechanisms (Hede, 2001). It has been demonstrated that aluminium can be present in the symplasm after only 30 minute exposure to a solution containing aluminium (Jianjun *et al*., 2009). The most important internal tolerance is chelation in the cytosol, compartmentalization in the vacuole, formation of Al tolerant enzymes, and elevated enzyme activity (Taylor, 1995). Substantial experimental

evidence supports the synthesis of Al-binding proteins (Basu *et al*., 1997). Several external tolerance mechanisms have been suggested which includes exudation of organic acids, Immobilization at the cell wall, exudation of phosphate (Xiao *et al*., 2003a and 2003b), active Al efflux across the plasma membrane, production of root mucilage, Al exclusion via alterations in rhizosphere pH, and selective permeability of the plasma membrane (Hede, 2001).

Plant species and varieties vary widely in tolerance to excess aluminium in the growth medium (Cristina *et al.*, 2009). In several species, these differences are mostly genetically controlled (Xiao-Bin *et al*., 2007). Closely related genotypes as the ones used herein are valuable tools for studying the physiological mechanisms of aluminium toxicity or tolerance (Rout and Das, 2001). Sorghum accessions from Kenya have been characterized for aluminium tolerance mechanisms, including organic acid exudation and gene expression (Gudu *et al.*, 2001). Some sorghum genotypes have been shown to be highly tolerant through aluminium exclusion and citrate exudation in root tips. Studies on aluminium tolerance in barley revealed decreased shoot dry weight by 28.6% for tolerant and 14.1% for sensitive cultivar (Foy, 1996). Length of root and shoot, mean number of leaves and lateral roots and mean fresh and dry weights highly decrease with increase in soil aluminium concentration (Mona, 2008). Some external mechanisms that limit the uptake of metals by roots can help plants tolerate a certain amount of toxic metal in soil (Knörz er *et al.*, 1996). One of them is the formation of non-toxic metal-ligand chelates in rhizosphere involving organic acids and other substances exuded from roots of crops (Knörz er *et al.*, 1996).

Aluminium is a non essential element and therefore it is a non nutritive element to the plant (Luciane *et al.*, 2007). Adapted plants accumulate larger amounts of aluminium without injury. Little or no change in the Al contents in the foliage (Stanislava *et al.*, 2015) can be found by plants during toxicity. Aluminium accumulation within plant tissues can be used to group them (Heru, 2014). Aluminium concentrations are lower in certain tolerant cultivars of wheat, barley, soybean and pea (Rout and Das, 2001). Aluminium tolerance in such plants is by an exclusion mechanism. In others; there is less aluminium in plant shoots and more Al in roots or both, as experienced in wheat, barley and potato (Mundayatan 2009). In a third group, the plants of high internal aluminium tolerance accumulate aluminium directly in shoot. These plants include; pine trees, tea and mangroves (Arunakumara *et al.*,2012). There

is no evidence of studies on aluminium accumulation in soy bean that are grown in Kenya and hence the tolerance mechanisms are yet to be ascertained.

2.3 Effects of aluminium stress on aluminium accumulation

Acidification can also bring many other changes of chemical properties in the soil which affects plant growth and development. Aluminium toxicity is manifested as a deficiency of N, P, K, Ca and Mg (Rout and Das, 2001).

Many of the biochemical effects of aluminium on plants are probably associated with the alteration of root membrane structure and function (Fathy *et al*., 2015). Plant membranes are visualized as arrangements of semi fluid, proteins and lipids. Aluminium can bind either proteins or lipids, depending on pH and other conditions (Rout and Das, 2001). Cristina *et al.* (2009) reported that chlorosis due to aluminium induces interference in the uptake and/or use of iron, copper and potassium. Therefore aluminium sensitive cultivars are characterized by chlorosis, decreased Fe concentrations in tops, decreased Ca, K and Mg in both shoots and roots (Graham, 2001). Aluminium (100 mM) inhibits the influx of calcium (69%), ammonium (40%) and potassium (13%) and enhances the efflux of nitrate (44%) and phosphate (17%). This needs to be studied in the selected soy beans. Young seedlings are more susceptible to aluminium than older plants (Rout and Das, 2001).

2.4 Effects of Aluminium on mineral nutrients (NPK, Mg and Ca) uptake

Rout and Das (2001) confirmed that aluminium toxicity at ambient ammonium – N is reduced by elevating the level of NO_3 – N or NH_4 – N. Nitrogen content of maize shoots and longan leaves decreases significantly with increasing aluminium concentration (Xiao *et al.* 2003a; Chen, 2006). Nitrogen content of longan stems increases when aluminium concentration in nutrient solution increases, but up to a point of further aluminium increase, it decreases (Xiao *et al.* 2003b). Nitrate Reductase activity is higher in aluminium tolerant cultivars (Rout and Das, 2001; Graham, 2001).

Aluminium interferes with water supply to plants (Xiao *et al.* 2003a). This leads to a problem with cell division in plant roots, as they fix phosphorous in less available forms in the soil, decrease root respiration, interfere with certain enzymes governing the deposition of polysaccharides in cell walls and increase in cell wall rigidity (cross-linking pectins). Aluminium increase P content of roots and decreases P content of shoots (Liang *et al*., 2001;

Quartin *et al*.. 2001). There may be formation of P and Al complexes in root, which inhibits P transport from root to shoot leading to this phenomenon. Al-induceses decrease in the activity of ATP-dependent H^+ transport system (Liang *et al*., 2001). Xiao *et al*. (2003b) reported that Al increases P content of both roots and stems and decreases P content of longan leaves. When studying the effects of Al and P interaction on soybean root growth and root organic acid exudation Hong *et al.* (2006) found out that addition of P significantly increased aluminium tolerance in four soybean genotypes that differs in P efficiency.

Aluminium competes with K for root uptake sites and depresses K uptake in roots and shoots (Graham , 2002; Liang *et al*., 2001) and therefore there might exist differences in rates of K depression. There is a net K^+ efflux and H^+ influx at one centimetre from the tip of the root apex, whereas in the rest of the root these fluxes are reversed (Yang *et al*., 2012). However, K content of both roots and shoots of 5 citrus rootstocks increased when Al concentration in nutrient solution increases from 4 to 178 μmol/l, then decreases as Al concentration increases further (Meriño-Gergichevich *et al*., 2010).

Magnesium content of the leaves of peach 'Nemaguard'(Simon *et al*., 1994a), 3 triticale cultivars (Graham, 2001), tomato (Quartin *et al*., 2001), both leaves and stems of longan (Xiao *et al*., 2005) and of the shoots of 2 maize cultivars (Chen, 2006) was decreased due to high Al. Fourteen weeks of Al treatment did not influence, magnesium content of *Quercus glauca* leaves (Chen, 2006). Magnesium content of maize shoots did not change significantly after both 9mg/l and 21mg/l Al treatments, but was decreased by up to 81% under 81mg/l Al treatment (Lidon *et al*., 1999).

Aluminium stress in low pH soils decreases cytosolic calcium (Fig. 2.3). It has been observed to affect Ca uptake in different plant species, and to reduce Ca content of both roots and shoots (Xiao *et al*., 2003a and 2003b). Plant roots responds to external low pH by a sustained elevation in cytosolic free calcium ion (Ca^{2+}) concentration (Plieth *et al*., 1999). Aluminium interferes with the binding of the cations in the cell wall by the same order of magnitude as their respective influx (Luciane *et al*., 2007). Meristems are sensitive to Al ions and more affected. Aluminium ions induces inhibition of ion fluxes (Fig. 2.3). In particular Ca^{2+} which play an important role in mechanisms of reducing Al^{3+} toxicity as it binds off

cations or screen negative charges on the plasma membrane. This reduces the activity of Al^{3+} close to the cell surface (Isabel *et al*., 2003).

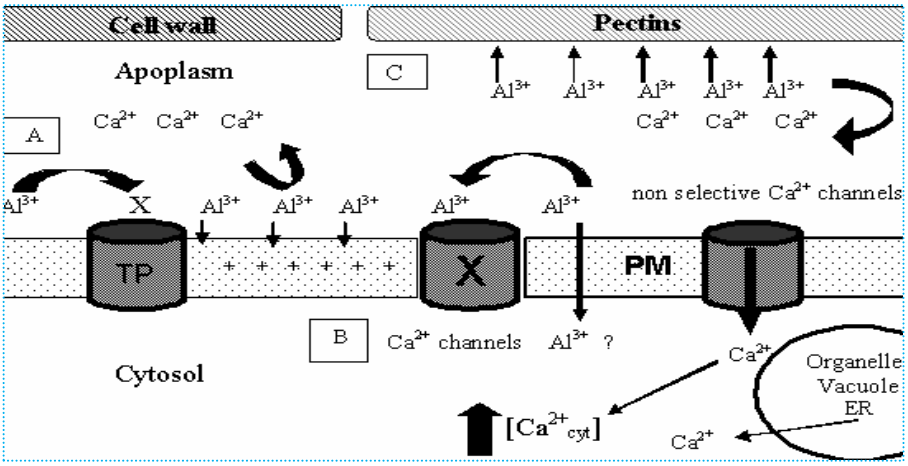

Fig. 2.3. Three mechanisms of Ca-Al interactions at cellular level;

(A) shows Inhibition of Ca^{2+} transport via symplasm by Al^{3+}; (B) Disruption of Ca^{2+} homeostasis in cytoplasm by Al^{3+} and (C), Ca^{2+} displacement by Al^{3+} from apoplasm. (ER) Endoplasmatic reticulum, (PM) plasma membrane, (TP) transport protein.

Source: Meriño-Gergichevich *et al*. (2010)

Jianjun *et al*. (2009) documented that Fe, Mn, and Zn contents of *Pinus massoniana* needles is decreased with Al increase. Molybdenum, Fe and Mn contents of peach leaves also decreases in response to high aluminium. Copper (Cu) and Zn contents show insignificant changes in response to increased aluminium (Graham, 2001; Möttönen *et al*., 2001). Aluminium decreases Fe and Zn contents of maize shoots, but increases their Cu and Mn contents (Andreas and Heinz, 2011). The inconsistency in results of these researchers illustrates the fact that the effects of Al on micro-elements in plant shoots depend on Al concentrations and plant species.

2.5 Aluminium stress effects on chlorophyll fluorescence

Aluminium affects plants physiologically (Cai *et al*., 2011), including the quantity of chlorophyll pigments and suppression of photosynthetic activities at the photosynthetic apparatus. There is induction of chloroplast malformations as much as less amounts of Al reaches within the leaves (Moustakas *et al*., 1995). This, therefore leads to a total chlorophyll content decrease and a partial inhibition of photosynthetic electron transport in photosystem II (PSII) (Peixoto *et al*., 2002; Chen, 2006). Aluminium stress inhibited the Hill reaction in rice chloroplasts and photosphorylation in the chloroplasts of both rice and longan (Xiao *et*

al., 2005). Chen *et al*. (2005b) observed that, In 'Cleopatra' tangerine, photochemical quenching coefficient (qP) and photosynthetic electron transport rate through PSII were greatly decreased by aluminium. Similar results have been obtained for wheat (Moustakas *et al*., 1995) and *Thinopyrum bessarabicum* (Moustakas *et al*., 1995).

Lidon *et al*. (1999) found that F_0 and maximum quantum yield (Fv/Fm) of photosystem II (PSII) in maize remained unchanged, while photosynthetic capacity, electron transport rate through photosystem I (PSI) and the contents of cytochromes f and b565 were lower above 9 mg/l Al treatment. This suggest that the Al-induced decrease in photosynthesis was associated with a reduction in photosynthetic electron transport rate through PSI. Photochemical parameters of PSII are widely used in studying effects of abiotic stress on plants hence their choice for this study. Photosystem II for some reason is the most vulnerable to aluminium stress induced damage. This shows that the photochemical parameters of PSII are indicative of many stress conditions (Marjorie *et al*., 2009). These enable approximation of plant productivity under different environmental conditions through photosynthetic performance (Maxwell and Johnson, 2000).

Jiang *et al*. (2008) studied the Al-induced effects on PSII photochemistry in 'Sour pummelo' leaves assessed by the *Chl a* transient fluorescence. Both control and aluminium treated plant leaves showed a typical rise in Chlorophyll fluorescence just as observed by Xiao-Bin *et al*. (2007). Aluminium stress decreased total electron carriers per reaction centre, yields and damaged all of the photochemical and non-photochemical reactions that were measured here, as indicated by the decreases in performance index and total performance index. In respect to the Al-untreated leaves, Al-treated citrus leaves accumulate Al with a concomitant decrease of the photochemical efficiency of PSII (Chen *et al*., 2005a).

The Fv/Fm was found to be within the normal ranges (0.7–0.8) for healthy blue berry plants for the three cultivars under aluminium stress treatment (Marjorie *et al*., 2009). Marjorie *et al*. (2009) also found out that 'Bluegold' blue berry cultivar was the most negatively affected by the Al treatments. Aluminium accumulating in leaves differentially affects the photochemical efficiency of PSII {effective quantum yield (ϕPSII) and electron transport rate (ETR)} of different cultivars. It`s clear that electron transport capacity

accompanied by the lack of reducing equivalents were the main factor contributing to the decreased CO_2 assimilation in Al stressed leaves.

Non-photochemical quenching (NPQ) which measures thermal energy dissipation increased with increasing aluminium chloride treatments with exception of tolerant varieties (Marjorie *et al.,* 2009). In such tolerant varieties, a decrease of NPQ at all treatments and exposure times is found. This has been observed in the salt-acclimated halophyte *Artemisia anethifolia* (Lu *et al.*, 2003) as well as in Blueberry (Marjorie *et al.*, 2009). It is postulated that other metabolic pathways (water-water cycle, Mehler reaction, and photorespiration) in Al-treated leaves may be upregulated to cope with the increased excess of excitation energy (Lu *et al.*, 2003; Marjorie *et al.*, 2009). This is supported by previous results that aluminium increases non-photochemical quenching (NPQ) in coffee leaves (Konrad *et al.*, 2005) and coefficient of non-photochemical quenching (qN) in *Thinopyrum bessarabicum* leaves (Moustakas *et al.*, 1995). The foregoing literature indicates that aluminium stress has an effect in all chlorophyll fluorescence parameters and may be applicable to soy bean varieties just as they have been applied to other plants.

2.6 Effects of aluminium stress on photosynthetic pigments

A negative correlation between total chlorophyll and aluminium treatment in leaves was reported in all blueberry varieties (Marjorie *et al.*, 2009). Peixoto *et al.* (2002) also found that in *S. bicolor* cultivars total chlorophyll is substantially decreased after 48 h of Al exposure. This decrease in total chlorophyll due to aluminium stress suggests chlorophyll photo bleaching. Consequently, smaller fraction of light energy for electron transport is involved in photochemistry (Adams *et al.*, 2004). 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; Marjorie *et al.*, 2009).

The change in chlorophyll antenna size is probably an adaptive strategy to reduce light absorption and avoid possible damage to the photosystems due to aluminium stress in plants (Marjorie *et al.*, 2009). This was expected to occur in soy bean varieties under study. In some varieties, at the beginning of the aluminium treatment, the chlorophyll antenna size will reduce, but after 48 h of aluminium treatment, chlorophyll antenna size becomes similar

to the controls (Marjorie *et al.*, 2009). The reason is to maintain ΦPSII. Such varieties have favourable root growth to support a faster acclimation of photosynthetic apparatus to Al stress by increasing water absorption and nutrient uptake. This is a photo-protective strategy. This mechanism has been reported in evergreen species under stress (Savitch *et al*., 2002) but have not been reported to be true for soy beans.

In most varieties, carotenoids increase is related with a decrease of photochemical parameters, suggesting that the variety can favour the heat dissipation pathway and thus avoid PSII photoinhibition (Demmig-Adams and Adams, 1996). Carotenoids protect the photosynthetic apparatus from harmful effects of light and oxygen. The excess light is dissipated by carotenoid as heat in the antenna pigment complexes (Niyogi *et al*., 1998). The less tolerant variety has carotenoid contents that reflect a similar concept when treated with aluminium chloride (Marjorie *et al*., 2009). The relationship between NPQ and carotenoid content sometimes comes out to be controversial in most papers. For example, Bilger and Björkman (1990) documented that changes in NPQ correlate closely and directly with changes in carotenoid pigments while Chen *et al*. (2005b) found that carotenoids were unrelated to NPQ.

More excess excitation energy existed in aluminium stressed leaves when compared with controls under high photon flux at midday (Chen *et al*. 2005a, b). Excess absorbed light is therefore harmlessly dissipated as heat through xanthophylls cycle-dependent thermal energy dissipation in the antenna pigment complexes of PSII (Demmig-Adams and Adams, 1996; Niyogi *et al*., 1998). It is for this reason, aluminium stressed 'Cleopatra' tangerine leaves were found to use a smaller fraction of the absorbed light in electron transport (Chen *et al*. 2005a, b).

Since thermal dissipation of excitation energy, measured as NPQ, is slightly decreased by Al, it might not be the main cycle (Chen *et al*., 2005a). An alternative route for energy dissipation and consumption of photosynthetic electrons in soy bean may be directly in the water to water cycle or indirectly in the photorespiration (Asada, 1999).

CHAPTER THREE
MATERIALS AND METHODS

3.1 Field site and soil characteristics

The research was carried out at Maseno University within the months of October 2012- April 2013 under Polythene covered Green house conditions located at the University Research farm. Njau (2001) Classified Maseno soils as acrisol deep reddish brown clay and well drained with a pH range of 4.5-5.4. Maseno receives both short and long rain averaging to 1750 mm 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.

3.2 Plant materials and treatments

Seeds of Soy bean; SB 20, SB 19 (TGX 1740-2F), SB 97 (EAI 3600) and SB 123 (SC Squire) 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 20 litre PVC pots filled with soil from Maseno University Research farm. Twelve seeds were planted per pot then thinned to leave six best performing seeding for enough growth space. Calcium superphosphate (71.4 kg/ha, 15.5% P_2O_5) was applied before planting then Diammonium phosphate (35.7 kg/ha, 20.6% N) applied ten days after germination. Potassium sulphate (119 kg/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/litre) and three replicates was used. These Al concentrations levels were chosen as they were used by Rafia and Sehrish (2008) and Chen (2006) while studying other plants. Al-treatment concentrations in milligram per litre have been widely used, for instance Miguel *et al*. (2013) and Nagarajan *et al*. (2014). Aluminium chloride was dissolved in tap water as the same tap water was used for irrigation. Tap water comes with certain substances but was used for all plants as $AlCl_3$ treatment levels were varied. 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.

3.3 Measurement of parameters

3.3.1 Plant growth parameters

Plant growth parameters were determined at 0, 16, 43 and 56 days after treatment (DAT) apart from dry mass which was determined on days 0, 16 and 56.

3.3.1.1 Determination of number of leaves

Number of leaves per selected plant in each pot were counted and recorded.

3.3.1.2 Determination of shoot height

Shoot height of the selected plant in each pot was measured from the soil level to the tip of the shoot by a meter rule.

3.3.1.3 Determination of stem diameter

A vernier calliper was used to measure stem diameter of the selected plant in each pot at five centimetre distance from the ground.

3.3.1.4 Determination of leaf area

Thirty centimetre ruler was used to measure the length along all the leaf blades of the selected plant in each pot and the width across the same blades. All leaf-let areas per plant were calculated following the formula of Otusanya *et al*. (2007) with little modifications as indicated below.

LA = 0.5 (L× W); Where L= Length of the leaf-let, W= width of leaf-let and LA= Leaf-let area. All leaf-lets per plant average was then calculated to get leaf area.

3.3.1.5 Determination of total dry weights

At the end of the experiment, 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 and dried in an oven for at least 72 hours at 70°C to constant weights for dry weight determination using a weighing balance (Denver instrument XL-3100D. Total dry weights was determined by weighing roots and shoots.

3.3.1.6 Determination of relative growth rate

Relative growth rate (RGR) was determined as the rate of increase in total dry weight per unit of plant weight according to Hunt (1982), thus:

RGR = (l_n W_2 - l_n W_1/t_2-t_1): RGR in $g.g^{-1}$ day^{-1}; Where :- W_1 is initial day plant weight, W_2 is final day plant weight and t is the time in days (Gama *et al.*, 2006).

3.3.1.7 Determination of root: shoot ratios

The data obtained in 3.3.1.5 was used to calculate R: S ratio by the formula that was earlier used by Kabel *et al*. (2006). Where;

R:S ratios = (Root dry weight) ÷ Shoot dry weight).

3.3.2 Determination of aluminium and mineral nutrient elements (NPK, Mg and Ca) in leaves

The following mineral nutrients were determined at harvest.

3.3.2.1 Aluminium concentration

Plank (1992) procedure was used to determine Al content at plant harvest for each pot. Dry ashing by high-temperature oxidation was used to destroy the organic matter. The plant samples were ashed at 500 °C by placing 0.5 g of the sample in a silica crucible and heating it in a muffle furnace for 4 hours. The ash was allowed to cool in the muffle furnace before being dissolved. Buffer solution was then prepared by adding 50g lithium carbonate (Li_2CO_3) into a 1-L volumetric flask. Then 20 ml concentrated nitric acid (HNO_3) was slowly added to the same flask. This solution in the flask was left to cool and later diluted to 50 ml using deionized water.

Five millilitres of buffer solution was added to the crucible and gently swirled to dissolve the ash. Aluminium was then quantified using a simultaneous multi-element atomic absorption spectrophotometer (model 969; UNICAM, Cambridge, UK) as described by Sadzawka *et al*. (2004) and Marjorie *et al*. (2010).

3.3.2.2 Nitrogen (N) concentration

Motsara and Roy (2008) procedure was used to determine N content at plant harvest for each pot. Plant sample of 0.5 gram was wet digested in di-acid mixture, placed in a Kjeldahl flask. 0.7 g of copper sulphate and 1.5 g of potassium sulphate were added, followed by 30 ml of 0.05M H_2SO_4 . The solution was boiled briskly until it was clear then further digested for 15 minutes by adding 30 ml of 0.05M H_2SO_4. The flask was cooled before adding 50 ml of deionized water and transferred to a distilling flask. Twenty three millilitres

of hydrochloric acid (0.1M HCl) was placed accurately in a receiving conical flask. Three drops of methyl red indicator was then added before tap water was run through the condenser. Thirty millilitres of 35 % NaOH was added in the distilling flask and heated for 15 minutes. Excess acid in the distillate was titrated with 0.1M NaOH. The same quantity of 0.1M HCL acid in a receiving conical flask was used to make a blank for reagents.

Nitrogen concentration in plant tissue (N %) was determined as follows;

$$\text{Percent N} = \frac{1.401\ [\ (V1\ M1 - V2M2) - (\ V3M1 - V4M2)]}{W} \times df$$ where:

*V*1 – millilitres of HCL acid put in receiving flask for samples;

*V*2 – millilitres of NaOH used in titration;

*V*3 – millilitres of HCL acid put in receiving flask for blank;

*V*4 – millilitres of NaOH used in titrating blank;

*M*1 – molarity of HCL acid;

*M*2 – molarity of NaOH;

W – weight of sample taken (0.5 g);

df – dilution factor of sample (if 1 g was taken for estimation, the dilution factor was 100.

3.3.2.3 Phosphorous concentration

Plant sample of 0.5g at harvest for each pot was wet-digested in di-acid and then made up to 100 ml volume. Five millilitres out of this 100 ml was put in a 50 ml volumetric flask, then standard phosphate solution added. Standard solution was formed by dissolving 0.2195 g of analytical-grade KH_2PO_4 and further diluted to 1 litre for the solution to contain 50 μg P/ml. Ten millilitres of vanadomolybdate reagent added to the volumetric flask. The contents in the flask were made up to 50 ml with deionized water, shook thoroughly, and kept for 10 minutes. A yellow coloured solution developed which was read on spectrophotometer. Vanadomolybdate was formed by dissolving 22.5 g of $(NH_4)\ 6MO_7O_2.4H_2O$ in 400 ml of distilled water, 1.25 g of ammonium vanadate in 300 ml of boiling deionized water. Vanadate solution was then added to the molybdate solution and cooled to room temperature. Two hundred and fifty millilitres of concentrated HNO_3 was added and diluted the contents to 1 litre with distilled water. Measuring of the concentrations of P was then done on a spectrophotometer. This procedure was adopted from Plank (1992) and Motsara and Roy (2008). The absorbance read was used to determine the P concentration from the standard curve (Appendix 5) and calculation done as follows;

P content (µg) in 1 g of sample = $C \times df$;

where:

C = concentration of P (µg/ml) as read from the standard curve;

df = dilution factor, which is 1 000.

3.3.2.4 Potassium concentration

Potassium content was measured at plant harvest for each pot using an atomic emission spectrophotometer based on the procedure of Motsara and Roy (2008) (model 969; UNICAM, Cambridge, UK). Plant sample of 0.5 g was made up to 100 ml volume after it was digested in di-acid. Five millilitres of this volume was put in a 50 ml volumetric flask and 10 ml and KCl (AR-grade) solution reagent was added, KCl (AR-grade) was prepared by dissolving 1.908 g of KCl in 1 litre of distilled water for it to contain 1 mg K/M. The contents in the flask were made up to volume with deionized water, shook thoroughly, and kept for 10 minutes. The solution developed which was read on spectrophotometer. The absorbance was read then used to determine the K content from the standard curve (Appendix 5). Content of K for the particular absorbance observed for the sample was determined as follows;

K content (µg) in 1 g of sample = $C \times df$; where:

C = concentration of K (µg/ml) as read from the standard curve;

df = dilution factor, which is 100 × 20 = 2 000.

3.3.2.5 Magnesium concentration

Motsara and Roy (2008) procedure was used to determine Mg content at plant harvest for each pot. Plant sample of 0.5 g was wet-digested in di-acid, and the volume was made up to 100 ml with distilled water. Five millilitres of this volume was put in a 50 ml volumetric flask and 10 ml of Mg standard solution reagent was added, which had been formed by dissolving 10.141 g of $MgSO_4.7H_2O$ in 250 ml of distilled water, and made it to 1 litre volume to give 1000 µg Mg/ml solution. Lastly ten millilitres of this solution was added to 100 ml of distilled water to obtain 10 µg Mg/ml. The final solution developed was read on a spectrophotometer. The absorbance was used to determine the Mg content from the standard curve. Content of Mg for the particular absorbance observed for the sample was determined as follows;

Mg content (µg) in 1 g of sample = $C \times df$: where:

C = concentration of Mg (µg/ml) as read from the standard curve;

df = dilution factor, which is 100 × 20 = 2 000,

3.3.2.6 Calcium concentration

Plant sample of 0.5 g at harvest for each plot was wet-digested in di-acid, and the volume was made up to 100 ml. Five millilitres of this volume was put in a 50 ml volumetric flask and 10 ml of calcium standard solution reagent was added (Calcium standard solution was prepared by adding 0.2247 g of standard $CaCO_3$ into 5 ml of deionized water then 10 ml of HCl was added to ensure complete dissolution of $CaCO_3$. This was then diluted to 1 litre with deionized water to give Ca solution of 100 µg Ca/ml. Ten millilitres of this solution was add to 100 ml of distilled water to obtain 10 µg Ca/ml). The contents in the volumetric flask were made up to volume with deionized water. The final solution developed was read on a spectrophotometer. The absorbance was used to determine the Ca content from the standard curve. Content of Ca for the particular absorbance observed for the sample was determined as follows;

Calcium content (µg) in 1 g of sample as per Motsara and Roy (2008) formula = $C \times df$: where:

C = content of Ca (µg/ml) as read from the standard curve;

df = dilution factor, which is $100 \times 20 = 2\,000$.

3.3.3 Determination of physiological parameters

The following physiological parameters were measured on DAT 5, DAT 25 and DAT 38.

3.3.3.1 Chlorophyll fluorescence

Leaf chlorophyll fluorescence was determined using a portable pulse-amplitude modulated fluorometer (FMS 2; Hansatech Instruments, King's Lynn, UK). The Roháček and Barták (1999) and Marjiorie *et al*. (2009) and (2010) protocol was used. Minimal fluorescence (Fo) was determined in dark-adapted (15 minutes) third youngest 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 (900 nmol-m^{-2} s^{-1}). After 10 second, actinic light (120 nmol-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 (Marjorie *et al*., 2009 and 2010). Non-photochemical quenching was manually calculated using the formula, NPQ = (Fm – Fm')/Fm' (Kate and Giles, 2000; Maxwell and Johnson, 2000).

3.3.3.2 Photosynthetic pigment concentration

Chlorophyll content was determined according to Coombs *et al.* (1985) as described by Netondo (1999) where the third youngest leaf was sampled for all treatments. In the laboratory 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 at room temperatures for the chlorophyll to be extracted by the acetone. Absorbance of the chlorophyll of the solution measured using a spectrophotometer (Novaspec II, Pharmacia Biotech, Cambridge, England) at 480, 645 and 663nm to determine the carotenoids, chlorophyll content of chlorophyll *a* and *b*. 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.

3.4 Statistical data analysis

The data were subjected to Factorial analysis of variance (ANOVA) using SAS statistical computer package (Steel *et al*., 2006). The factors were five levels of aluminium chloride treatments, three replicates, four varieties and time interval that differed as per the parameters collected. Measurements for parameters were repeated for one factor, that is cultivars (Quinn and Keough, 2006). Tukey's HSD test at 5% level was used to separate the treatment means.

CHAPTER FOUR
RESULTS

4.1 Plant growth parameters

4.1.1 Number of leaves

The number of leaves per plant were less for aluminium chloride treated plants as compared to the control plants apart from SB 20 at 100 mg/litre on 43 DAT and at 75 mg/litre on 56 DAT. On 16 DAT SB 123 had more leaves at 187 µmol/litre and on 56 DAT at 750 µmol/litre compared to the control. This was also observed for SB 97 at 75 mg/litre on 56 DAT (Fig.4.1.1). Varieties were significantly different ($p \leq 0.05$) in response to aluminium treatments (Appendix 1, Table 6). Treatments were also found to be significantly different. Tukey`s HSD showed a significant difference ($p \leq 0.05$) for Variety SB 19 as well as SB 20 when each was compared to SB 123 and SB 97 (Appendix 3, Table 14). Variety SB 123 was significantly different to SB 97. Control had a significant difference ($p \leq 0.05$) compared to each Al-treatments of 25, 50, 75 and 100 mg/litre AlC_3 solution (Appendix 3, Table 15).

4.1.2 Shoot height

Varieties SB 20 and SB 97 were shorter on 43 DAT and 56 DAT compared to SB 123 and SB 19 under Al treatments (Fig. 4.1.2). There was a significant difference ($p \leq 0.05$) in varieties and for treatments too (Appendix 1, Table 6). Tukey`s HSD (Appendix 3, Table 14) indicated a significant difference ($p \leq 0.05$) from one of any variety whenever compared to any of the other with the highest mean for SB 123 (Appendix 3, Table 14). The tallest plants were found at control compared to Al-treated plants (Appendix 3, Table 15).

4.1 3 Stem diameter

The stem diameter reduced with increasing Al-treatments (Fig. 4.1.3). There was a significant difference ($p \leq 0.05$) in varieties, treatments as well as for the interaction between varieties and treatments (Appendix 1, Table 6). Tukey`s HSD (Appendix 3, Table 14) indicated a significant difference ($p \leq 0.05$) for variety SB 20 compared to SB 19 as well as to SB 97 (Appendix 3, Table 14). Control had a significant difference ($p \leq 0.05$) compared to each of Al-treatments of 25, 50, 75 and 100 mg/litre AlC_3 solution (Appendix 3, Table 15).

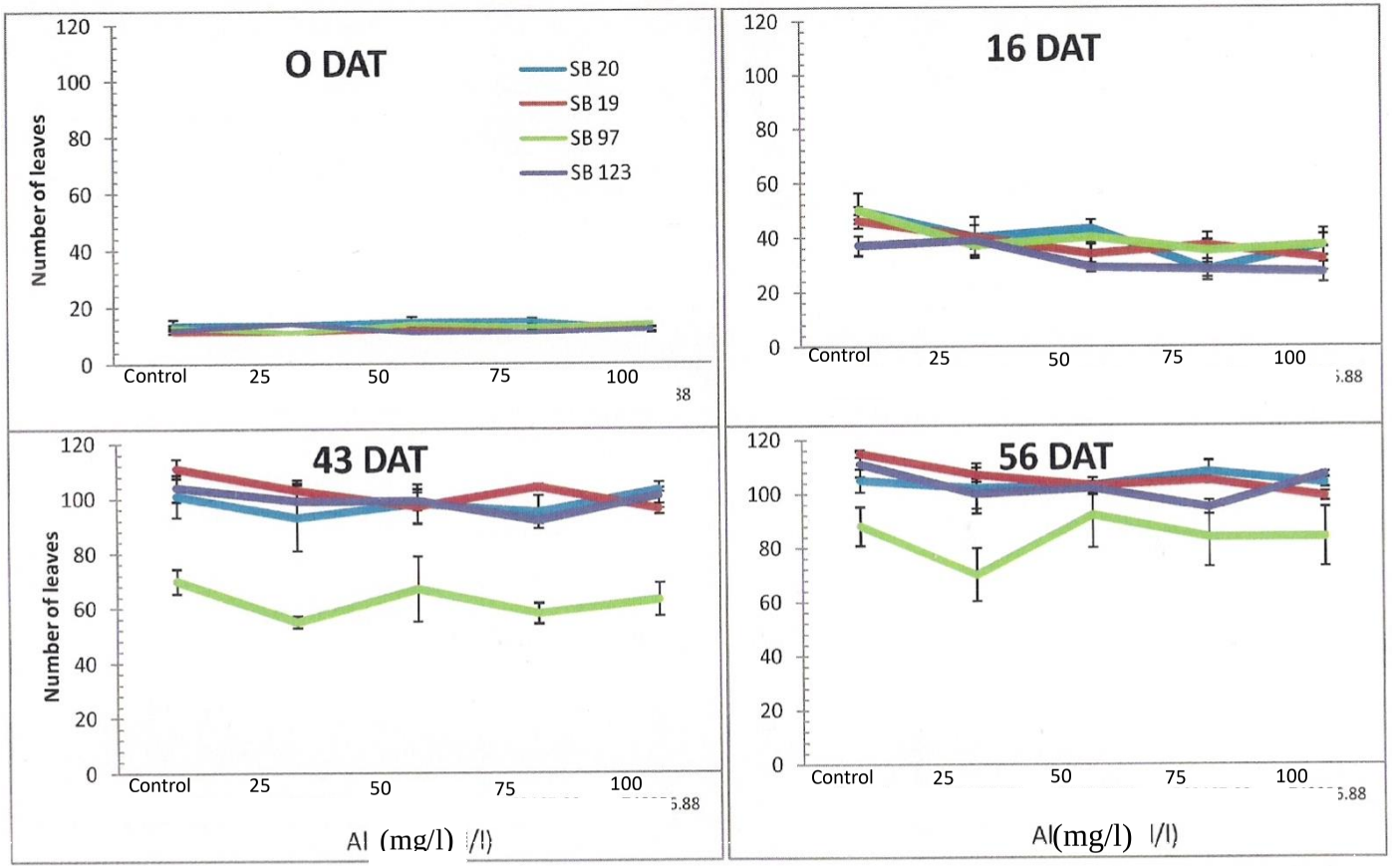

Fig. 4.1.1. Number of leaves per plant of soy bean varieties at 0 DAT, 16 DAT, 43 DAT and 56 DAT subjected to various concentrations of $AlCl_3$ solution (mg/l). Values are means of three replicates ±SEs

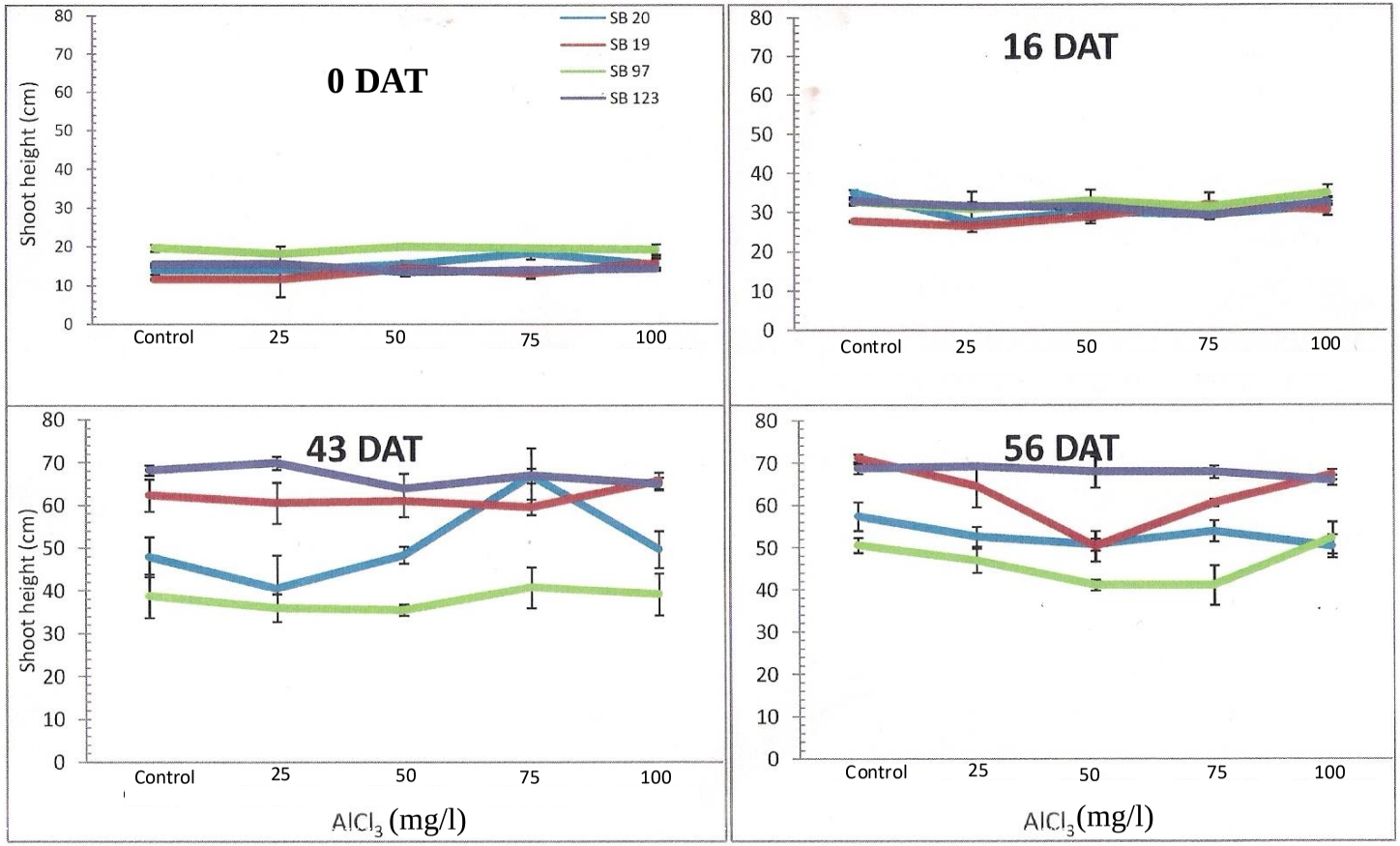

Fig. 4.1.2. Shoot height (cm) per plant of soy bean varieties at O DAT, 16 DAT, 43 DAT and 56 DAT subjected to various concentrations of $AlCl_3$ solution (mg/l). Values are means of three replicates ± SEs

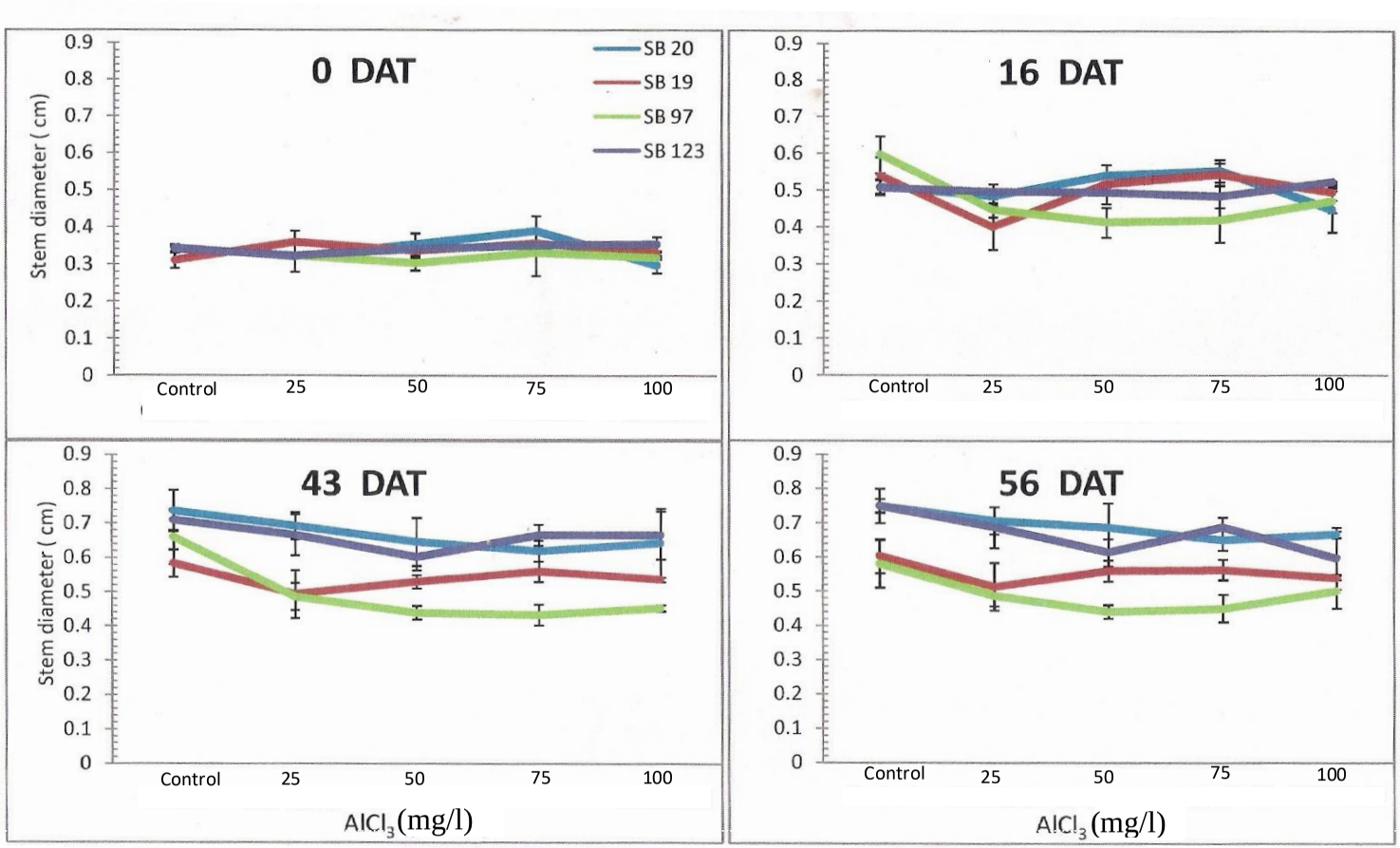

Fig. 4.1.3. Stem diameter (cm) per plant of soy bean varieties at O DAT, 16 DAT, 43 DAT and 56 DAT subjected to various concentrations of $AlCl_3$ solution (mg/l). Values are means of three replicates ± SEs.

4.1.4 Leaf area

Leaf area generally reduced with Al treatments (Fig.4.1.4). Variety SB 97 had significantly a large leaf area mean at the control than the other treatments at 16, 43 and 56 DAT. There was a significant difference ($p \leq 0.05$) within varieties and within Al treatment levels (Appendix 1, Table 6). Tukey`s HSD indicated a significant difference ($p \leq 0.05$) for varieties SB 97, SB 20 and SB 123 when each was compared to SB 19 (Appendix 3, Table 14) with the highest mean leaf area in SB 97. Control had a significant difference ($p \leq 0.05$) compared to each of Al-treatments of 25, 50, 75 and 100 mg/litre AlC_3 solution (Appendix 3, Table 15).

4.1.5 Dry weight

On 16 DAT and 56 DAT, the dry weight of soy bean varieties per plant decreased for Al-treated plants apart from SB 123 on 16 DAT (Fig. 4.1.5). On 56 DAT, at Al-treatments of 25, 50 mg/litre $AlCl_3$ solution; variety SB 123 was significantly different ($p \leq 0.05$) from other varieties (Fig. 4.1.5). There was a significant difference ($p \leq 0.05$) among varieties and treatments as well (Appendix 1, Table 6). Tukey`s HSD showed a significant difference ($p \leq 0.05$) for variety SB 123 compared to each of either SB 20, SB 97 and SB 19 (Appendix 3, Table 14). Control had a significant difference ($p \leq 0.05$) compared to each of Al-treatments of 25, 50, 75 and 100 mg/litre AlC_3 solution (Appendix 3, Table 15).

4.1.6 Relative growth rate

The relative growth rate generally decreased with increasing $AlCl_3$ concentration as observed in SB 19, SB 97 and SB 123 (Table 4.1.6). There was a significant variation ($p \leq 0.05$) among varieties as well as treatments (Appendix 1, Table 6). Tukey`s HSD showed variety SB 123 and SB 20 were both each significantly different (Table 4.1.6) as compared to either SB 19 as well to SB 97. Control had a significant difference ($p \leq 0.05$) compared to each of Al-treatments of 25, 50, 75 and 100 mg/litre $AlCl_3$ solution (Appendix 3, Table 15).

4.1.7 Root: shoot ratios

There was a significant difference ($p \leq 0.05$) for variety SB 20 compared to each of the rest varieties at the control as well as at 75 mg/litre $AlCl_3$ treatment solution (Fig. 4.1.7). There was no significant difference ($p \geq 0.05$) in varieties, and in treatments (Appendix 1, Table 6). Tukey`s HSD (Appendix 3, Table14) showed varieties to have had no significant

difference ($p \geq 0.05$). Treatments too did not show a significant difference (Appendix 3, Table 15)

4.2 Aluminium and Plant mineral nutrients

4.2.1 Aluminium concentration in plants

Aluminium concentration increased in Al-treated plants (Fig. 4.2.1). There were no significant differences in aluminium concentration among varieties ($p \geq 0.05$) (Appendix 1, Table 7). Treatments varied significantly ($p \leq 0.05$) for aluminium concentration in plant leaves (Appendix 1, Table 7). Tukey`s HSD showed that treatments of 25, 50, 75 and 100 mg/litre $AlCl_3$ solution varied significantly ($p \leq 0.05$) when each was compared to the control (Appendix 3, Table 15).

4.2.2 Nitrogen concentration in plants

Nitrogen concentration in $\mu g.\ g^{-1}$ DW was low in aluminium treated soy bean plant`s leaves at all levels of treatments compared to the control (Fig. 4.2.2) for every variety. In the low aluminium chloride treatments SB 20 had more nitrogen content, but was later overshadowed by other varieties at 75 and 100 mg/litre (high aluminium concentration) as shown in Fig. 4.2.2. In all the varieties nitrogen content was low at 75 and 100 mg/litre. Varieties just like treatments had no significant ($p \geq 0.05$) differences (Appendix1, Table 7). Nitrogen was found to be highly concentrated in varieties SB 19 and SB 20 (Appendix 3, Table 14).

4.2.3 Phosphorous concentration in plants

Plant phosphorous concentration in $\mu g.\ g^{-1}$ DW was lower in aluminium treated soy bean plant leaves at all levels of treatments compared to the control (Fig. 4.2.3). Varieties had no significant difference ($p \geq 0.05$). There was no significant difference ($p \geq 0.05$) for treatments (Appendix 1, Table 7).

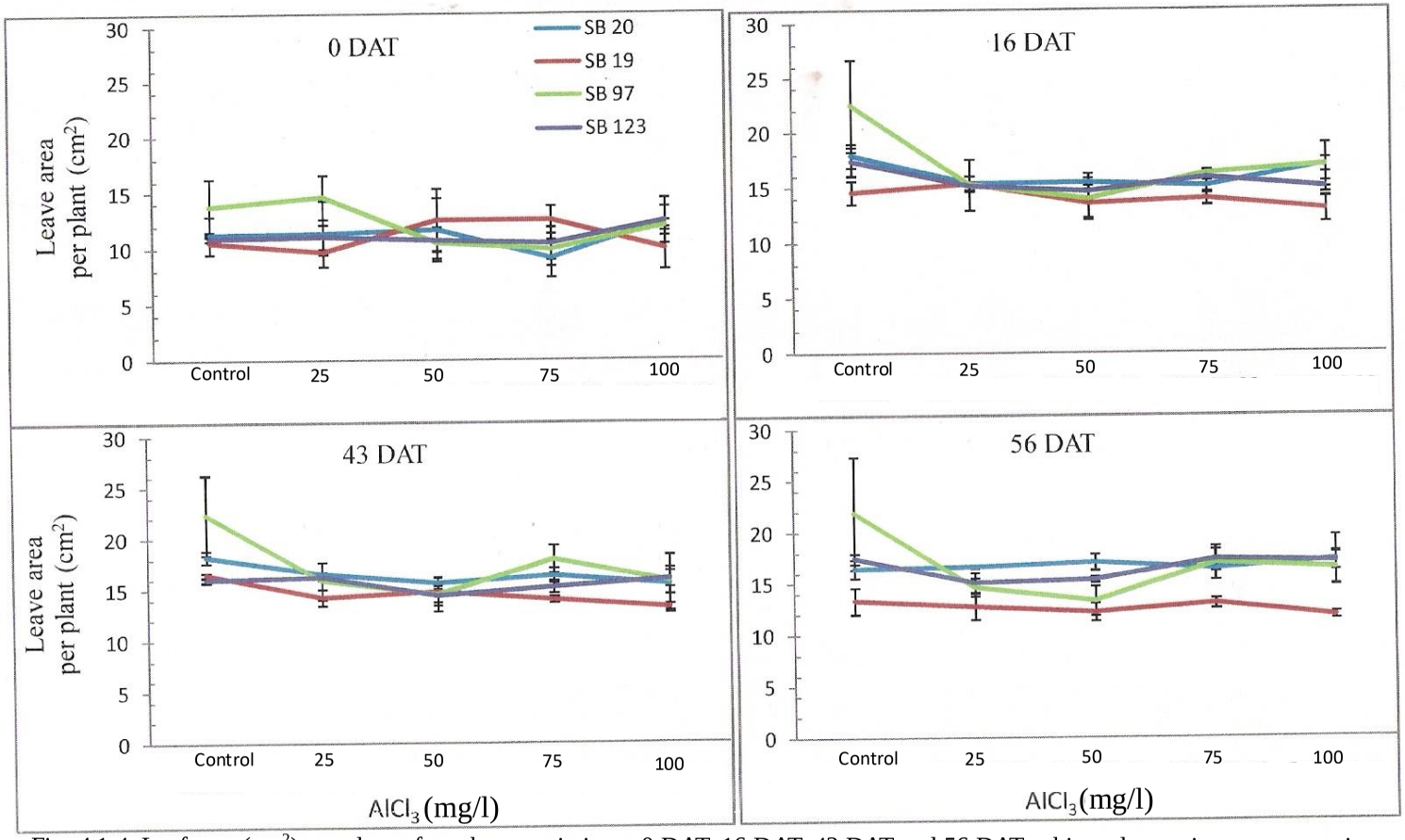

Fig. 4.1.4. Leaf area (cm^2) per plant of soy bean varieties at 0 DAT, 16 DAT, 43 DAT and 56 DAT subjected to various concentrations of $AlCl_3$ solution (mg/l). Values are means of three replicates ± SEs.

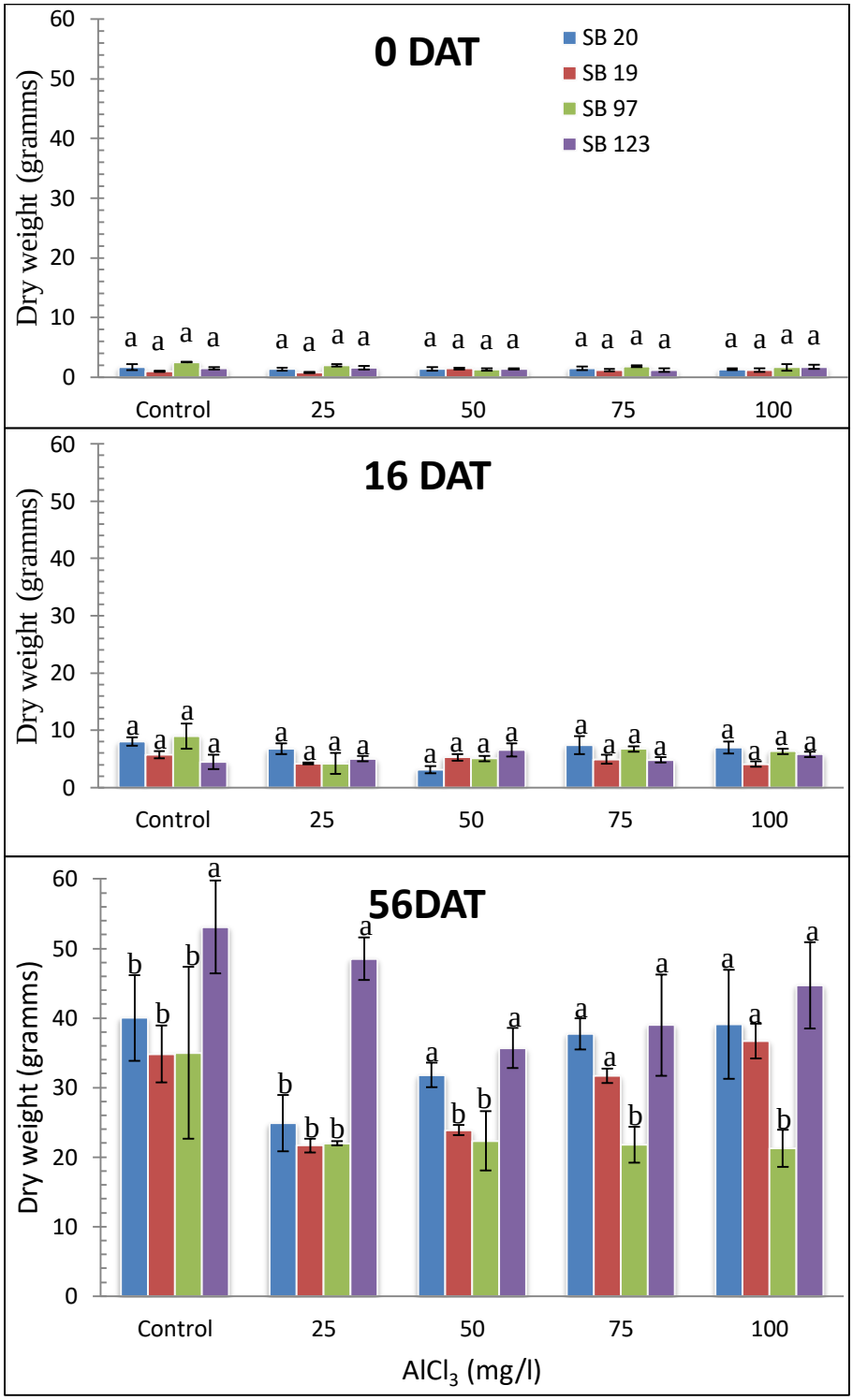

Fig. 4.1.5. Total dry weight (grams) of soy bean varieties subjected to various concentrations of $AlCl_3$ solution (mg/l) at different DAT. Values are means of three replicates ± SEs. Means with the same latter are not significantly different.

Table 4.1.6. Relative Growth Rate ($g.g^{-1}$ day^{-1}) in four soy bean varieties subjected to increasing $AlCl_3$ concentrations (mg/l). Values are means of three replicates for 16, 43 and 56 DAT. Means with the same latter are not significantly different within varieties.

$AlCl_3$ Treatment (mg/l)	RGR ($g.g^{-1}$ day^{-1}) for varieties				
	SB 20	SB 19	SB 97	SB 123	**Tukey`s grouping means for Treatments**
Control	0.49a	0.45a	0.54a	0.58a	**0.52 a**
25	0.33b	0.34b	0.30b	0.64a	**0.39 b**
50	0.31b	0.31b	0.34b	0.41a	**0.34 c**
75	0.48a	0.38a	0.35b	0.46a	**0.42 b**
100	0.50a	0.33b	0.39a	0.52a	**0.43 b**
Tukey`s grouping means for Varieties	**0.52 a**	**0.36 c**	**0.38 b**	**0.52 a**	

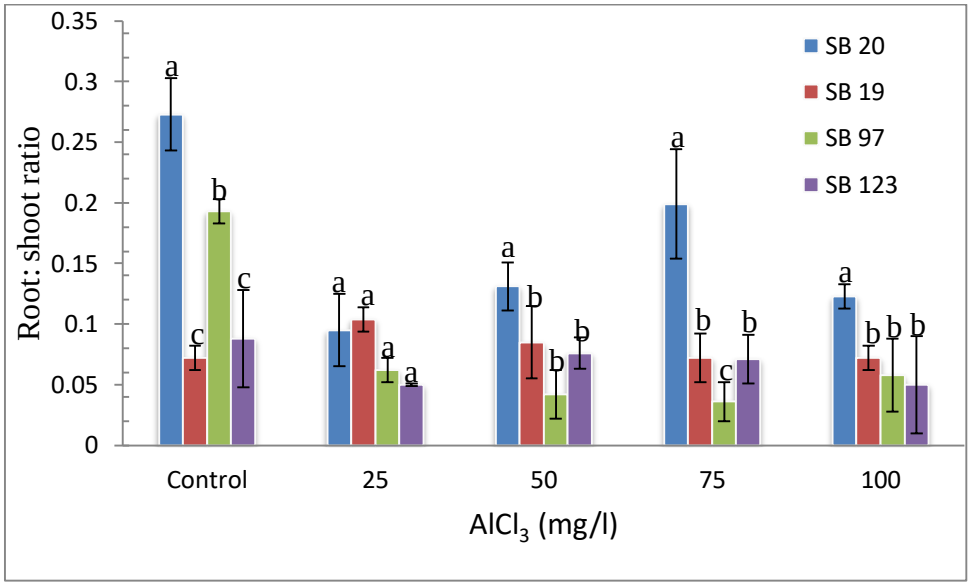

Fig. 4.1.7. Root: Shoot ratio of soy bean varieties in relation to the $AlCl_3$ solution (mg/l) treatments on 56 DAT. Values are means of three replicates ± SEs. Means with the same latter are not significantly different.

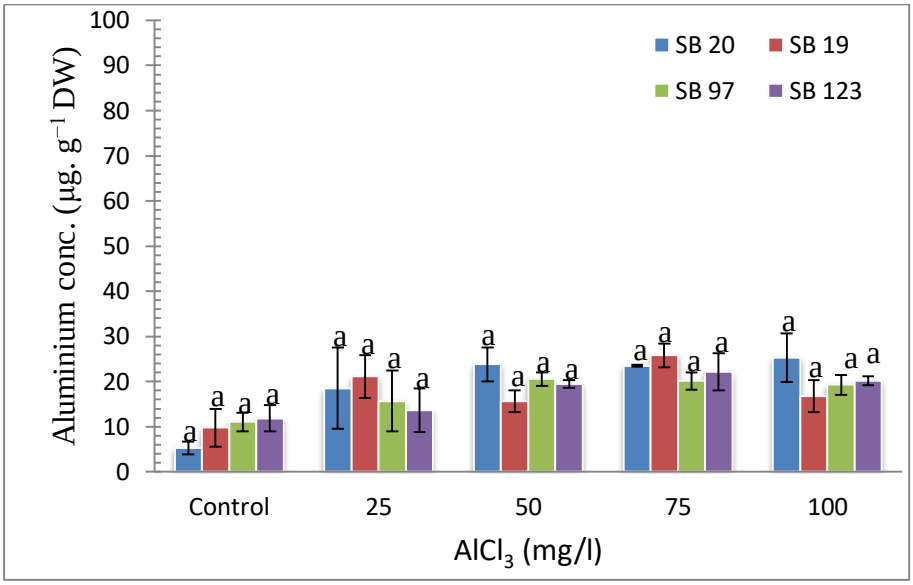

Fig. 4.2.1. Aluminium concentration (µg. g^{-1} DW) in the shoots of four soy bean varieties on 56 DAT subjected to various $AlCl_3$ (mg/l) treatments. Values are means of three replicates ± SEs. Means with the same latter are not significantly different.

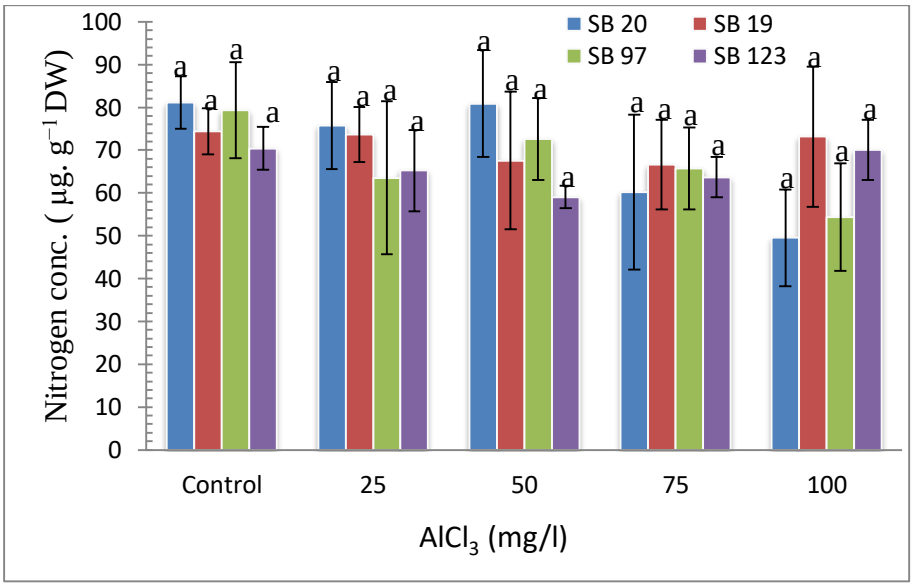

Fig. 4.2.2. Nitrogen concentration (µg. g^{-1} DW) in the shoots of four soy bean varieties at maturity subjected to various $AlCl_3$ (mg/l) treatments. Values are means of three replicates ± SEs. Means with the same latter are not significantly different.

4.2.4 Potassium concentration in plants

Potassium concentration in µg. g^{-1} DW decreased with $AlCl_3$ concentration treatments (Fig. 4.2.4). There were no significant differences ($p \geq 0.05$) between aluminium treatments and among varieties (Appendix 2, Table 7).

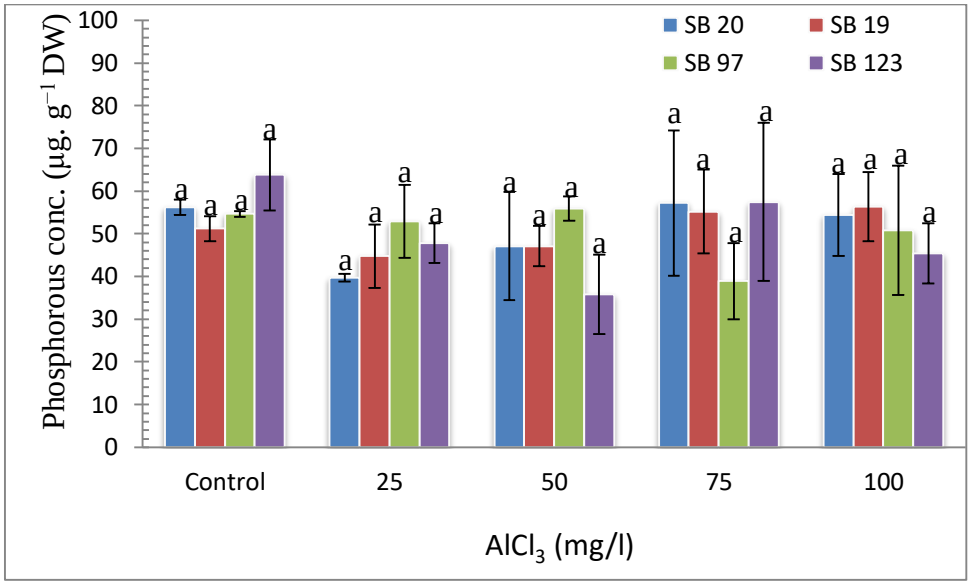

Fig.4.2.3. Phosphorous concentration (µg. g^{-1} DW) in leaves of four soy bean varieties at maturity subjected to various $AlCl_3$ (mg/l) treatments. Values are means of three replicates ± SEs. Means with the same latter are not significantly different.

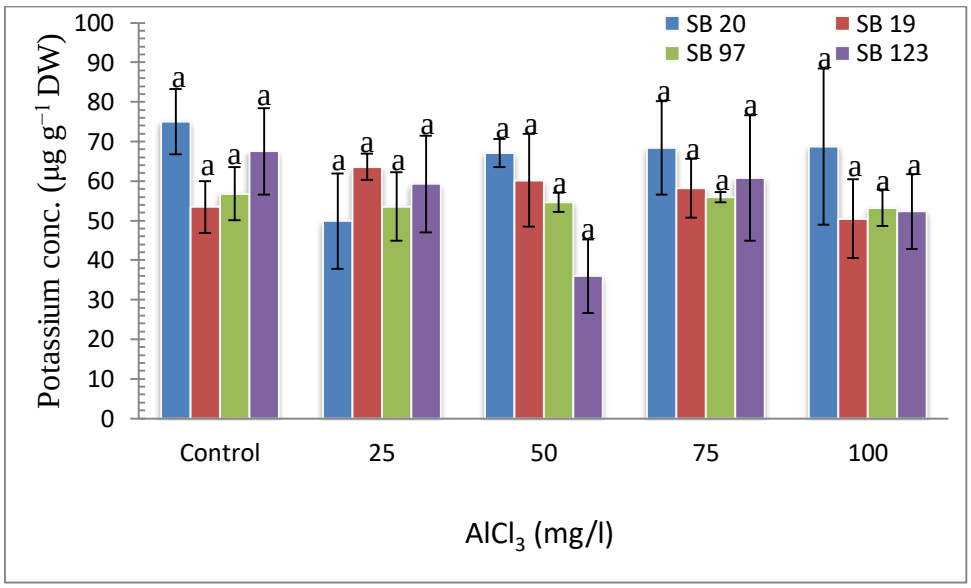

Fig.4.2.4. Potassium concentration (µg. g^{-1} DW) in the leaves of four soy bean varieties at maturity subjected to various $AlCl_3$ (mg/l) treatments. Values are means of three replicates ± SEs. Means with the same latter are not significantly different.

4.2.5 Magnesium concentration in plants

Magnesium concentration in µg. g^{-1} DW did not show a clear trend in consideration to aluminium chloride concentration (Fig. 4.2.5). Varieties did not show significant difference ($p \geq 0.05$) in Mg content in leaves. Similarly, treatments did not also show significant difference (Appendix 2, Table 7).

4.2.6 Calcium concentration in plants

Calcium concentration in µg. g^{-1} DW was low at Al-treatments apart from at 25 and 75 mg/litre $AlCl_3$ solution for SB 123 when compared to the control (Fig.4.2.6). Varieties were not significantly different ($p \geq 0.05$), this was similar to treatments (Appendix 2, Table 7).

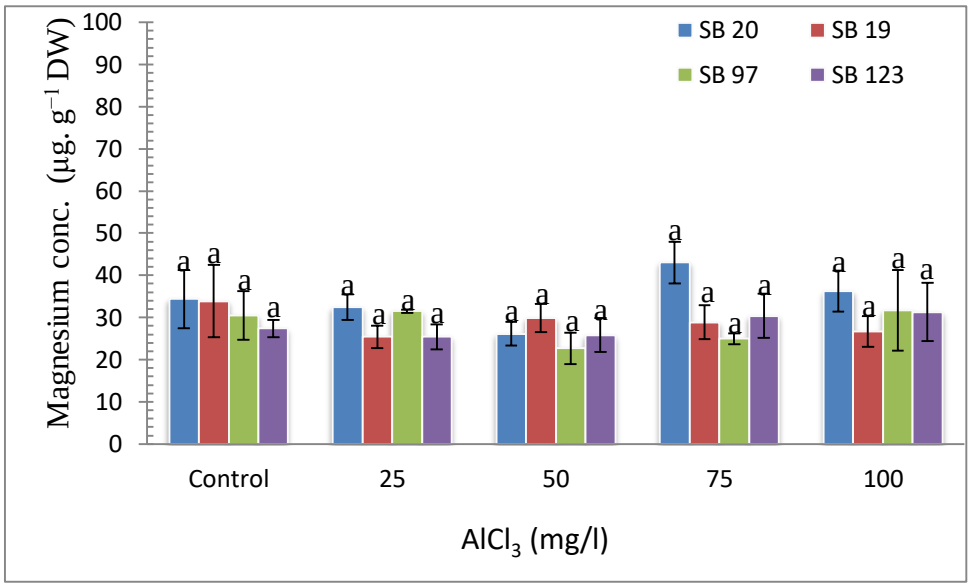

Fig. 4.2.5. Magnesium concentration (µg. g^{-1} DW) in the leaves of four soy bean varieties at maturity subjected to various $AlCl_3$ (mg/l) treatments. Values are means of three replicates ± SEs. Means with the same latter are not significantly different.

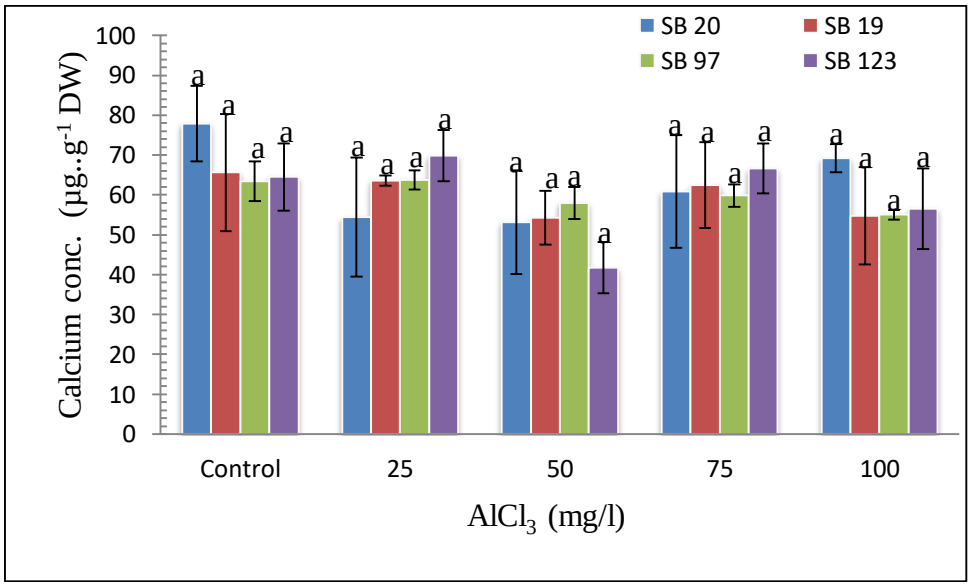

Fig. 4.2.6. Calcium concentration (µg. g^{-1} DW) in the leaves of four soy bean varieties at at maturity subjected to various $AlCl_3$ (mg/l) treatments. Values are means of three replicates ± SEs. Means with the same latter are not significantly different.

4.3 Chlorophyll fluorescence

4.3.1 Maximum quantum yield (Fv/Fm)

The Fv/Fm ratio was high at the control compared to the Al-treated soy bean plant leaves apart from SB 123 at 100 and SB 19 at 100 m/l (Table 4.3.1). There was a significant difference ($p \leq 0.05$) in DAT (Appendix 2, Table 8). There was no significantly difference ($p \geq 0.05$) in the Fv/Fm ratio for varieties and treatments (Appendix 2, Table 8). Tukey`s HSD showed a significant difference ($p \leq 0.05$) when 5 DAT was compared to each of 25 DAT and 38 DAT (Appendix 3, Table 16).

4.3.2 Effective quantum yield (ϕPSII)

Effective quantum yield reduced with increased aluminium chloride concentration in varieties (Table 4.3.2). However the increase was not clear for SB 19. SB 97 had a high ϕPSII at 100 mg/litre compared to the control (Table 4.3.2). There was no significant difference ($p \geq 0.05$) among varieties and for treatments (Appendix 1, Table 8) but a significant difference ($p \leq 0.05$) was observed for DAT. Tukey`s HSD showed a significant difference ($p \leq 0.05$) when 5 DAT and 38 DAT was each compared to 25 DAT (Appendix 3, Table 16).

4.3.3 Non-photochemical quenching (NPQ)

Heat dissipated for all varieties was generally more in $AlCl_3$ treated leaves compared to the control in both days apart from SB 20 and SB 19 where the control had high NPQ (Table 4.3.3). There was no significant difference ($p \geq 0.05$) among varieties and for treatments (Appendix 1, Table 8). However, there was a significant difference ($p \leq 0.05$) in DAT. Tukey`s HSD showed a significant difference ($p \leq 0.05$) when 5 DAT and 38 DAT was each compared to 25 DAT (Appendix 3, Table 16).

Table 4.3.1. Leaf Maximum quantum yield (Fv/Fm) in four soy bean varieties subjected to increasing $AlCl_3$ concentrations (mg/l). Values are means of three replicates for 5, 25 and 38 DAT. Means with the same latter for varieties are not significantly different.

$AlCl_3$ Treatment (mg/l)	Fv/Fm (Relative Units) for varieties				
	SB 20	SB 19	SB 97	SB 123	**Tukey`s grouping means for Treatments**
Control	0.58a	0.55a	0.53a	0.51a	**0.55a**
25	0.58a	0.52a	0.50a	0.51a	**0.53a**
50	0.53a	0.51a	0.46a	0.46a	**0.49a**
75	0.58a	0.48a	0.53a	0.49a	**0.52a**
100	0.54a	0.65a	0.46a	0.52a	**0.54a**
Tukey`s grouping means for Varieties	**0.57a**	**0.54a**	**0.50a**	**0.51a**	

Table 4.3.2. Leaf Effective quantum yield (ϕPSII) in four soy bean varieties subjected to increasing $AlCl_3$ concentrations (mg/l). Values are means of three replicates for 5, 25 and 38 DAT. Means with the same latter for varieties are not significantly different.

$AlCl_3$ Treatment (mg/l)	ϕPSII (Relative Units) for varieties				
	SB 20	SB 19	SB 97	SB 123	**Tukey`s grouping means for Treatments**
Control	0.5 a	0.47a	0.54a	0.58a	**0.54a**
25	0.58a	0.55a	0.45a	0.44a	**0.51a**
50	0.47a	0.50a	0.45a	0.48a	**0.48a**
75	0.57a	0.48a	0.52a	0.42a	**0.49a**
100	0.54a	0.57a	0.55a	0.47a	**0.53a**
Tukey`s grouping means for Varieties	**0.55a**	**0.52a**	**0.50a**	**0.48a**	

Table 4.3.3. Leaf Non-photochemical quenching (NPQ) in four soy bean varieties subjected to increasing $AlCl_3$ concentrations (mg/l). Values are means of three replicates for 5, 25 and 38 DAT. Means with the same latter for varieties are not significantly different.

$AlCl_3$ Treatment (mg/l)	NPQ (Relative Units) for varieties				
	SB 20	SB 19	SB 97	SB 123	**Tukey`s grouping means for Treatments**
Control	0.70a	0.75a	0.69a	0.50a	**0.66a**
25	0.52a	0.44a	0.79a	1.65a	**0.85a**
50	0.41a	0.43a	1.15a	0.37a	**0.59a**
75	0.63a	0.86a	0.86a	1.05a	**0.85a**
100	0.46a	0.69a	0.69a	0.60a	**0.61a**
Tukey`s grouping means for Varieties	**0.54a**	**0.63a**	**0.83a**	**0.84a**	

4.4 Plant photosynthetic pigment concentrati*on*

4.4.1 Chlorophyll *a* concentration

There was low chlorophyll *a* concentration ($mg.g^{-1}$) in the varieties as the $AlCl_3$ concentration in the growth medium was increased apart from SB 19 at 75 mg/litre, and for SB 97 at 100 mg/litre (Table 4.4.1). Varieties did not show a significant difference ($p \geq 0.05$) in chlorophyll *a* concentration when subjected to $AlCl_3$ treatments (Appendix 1, Table 9). There was however a significant difference ($p \leq 0.05$) in this parameter among treatments and DAT. Tukey`s HSD showed that treatments of 25, 50, 75 and 100 mg/litre $AlCl_3$ solution varied significantly ($p \leq 0.05$) when each was compared to the control (Table 4.4.1). A significant difference ($p \leq 0.05$) was observed when 5 DAT was compared to each of 25 DAT and 38 DAT (Appendix 3, Table 16).

4.4.2 Chlorophyll *b* concentration

Chlorophyll *b* concentration ($mg.g^{-1}$) in leaf per plant was low at control compared to each of Al-treatments in the growth medium for varieties except SB 19 at 75 mg/litre $AlCl_3$ solution and SB 97 at 25 and 75 mg/litre $AlCl_3$ solution (Fig. 4.4.2). There was no significant difference ($p \geq 0.05$) among varieties and among treatments (Appendix 1, Table 9) but a significant difference ($p \leq 0.05$) was observed within DAT. Tukey`s HSD indicated a significant difference ($p \leq 0.05$) when 25 DAT and 38 DAT was each compared to 5 DAT (Appendix 3, Table 16).

4.4.3 Total chlorophyll concentration

Total chlorophyll concentration ($mg.g^{-1}$) was higher at the control plants compared to Al-treated plants for SB 20 (Fig.4.4.3). There was no significance difference ($p \geq 0.05$) among varieties and among treatments but a significance difference ($p \leq 0.05$) among DAT (Appendix 1, Table 9). Tukey`s HSD indicated a significant difference ($p \leq 0.05$) when 5 DAT was compared to each of 25 DAT and 38 DAT (Appendix 3, Table 16).

Table. 4.4.1. Chlorophyll *a* concentration ($mg\ g^{-1}$) of soy bean varieties at 5 DAT, 25 DAT and 38 DAT subjected to various concentrations of $AlCl_3$ (mg/l) treatments. Values are means of three replicates. Means with the same latter are not significantly different.

$AlCl_3$ Treatment (mg/l)	Chlorophyll *a* Concentration for varieties ($mg\ g^{-1}$)				
	SB 20	SB 19	SB 97	SB 123	**Tukey`s grouping means for Treatments**
Control	2.33a	1.29a	1.31a	1.60a	**1.64a**
25	1.13a	1.14a	1.27a	1.17a	**1.25b**
50	1.36a	0.84a	1.21a	1.26a	**1.17b**
75	1.15a	1.39a	1.21a	1.11a	**1.21b**
100	1.43a	1.09a	1.39a	0.99a	**1.23b**
Tukey`s grouping means for Varieties	**1.18a**	**1.21a**	**1.28a**	**1.23a**	

Table.4.4.2**.** Chlorophyll *b* concentration (mg g^{-1}) of soy bean varieties at 5 DAT, 25 DAT and 38 DAT subjected to various concentrations of $AlCl_3$ (mg/l) treatments. Values are means of three replicates. Means with the same latter are not significantly different.

$AlCl_3$ Treatment **(**µmol/l**)**	Chlorophyll *b* Concentration (mg g^{-1}) for varieties				
	SB 20	SB 19	SB 97	SB 123	**Tukey`s grouping means for Treatments**
Control	1.13a	0.84a	0.79a	0.96a	**0.93a**
25	0.51b	0.80a	0.82a	0.67a	**0.70a**
50	0.90a	0.62a	0.90a	0.68a	**0.73a**
75	0.70a	1.01a	0.55a	0.57a	**0.71a**
100	0.84a	0.67a	0.77a	0.60a	**0.72a**
Tukey`s grouping means for Varieties	**0.78a**	**0.79a**	**0.77a**	**0.70a**	

Table.4.4.3. Total chlorophyll concentration (mg g^{-1}) of soy bean varieties at 5 DAT, 25 DAT and 38 DAT subjected to various concentrations of $AlCl_3$ (mg/l) treatments. Values are means of three replicates. Means with the same latter are not significantly different.

$AlCl_3$ Treatment **(**mgl/l**)**	Total chlorophyll concentration (mg g^{-1}) for varieties				
	SB 20	SB 19	SB 97	SB 123	**Tukey`s grouping means for Treatments**
Control	3.39a	1.89a	2.02a	2.02a	**2.33a**
25	1.56a	2.09a	2.05a	2.19a	**1.97a**
50	2.23a	1.74a	2.35a	1.61a	**1.98a**
75	1.84a	2.40a	1.49a	2.01a	**1.94a**
100	2.28a	1.72a	2.06a	1.65a	**1.93a**
Tukey`s grouping means for Varieties	**2.26a**	**1.98a**	**1.99a**	**1.90a**	

4.4.4 Chlorophyll *a*/*b* ratio

Chlorophyll *a*/*b* ratio (mg.g^{-1}) in leaf per plant was higher for control plants compared to Al-treated plants for SB 20 and SB 19 (Fig. 4.4.4). There were no significant difference ($p \geq 0.05$) among varieties and among treatments (Appendix 1, Table 9). There was a significant difference ($p \leq 0.05$) among DAT. Tukey`s HSD indicated a significant difference ($p \leq 0.05$) when 5 DAT was compared to each of 25 DAT and 38 DAT (Appendix 3, Table 16).

Table.4.4.4. Chlorophyll *a/b* concentration (mg g^{-1}) of soy bean varieties at 5 DAT, 25 DAT and 38 DAT subjected to various concentrations of $AlCl_3$ (mg/l) treatments. Values are means of three replicates. Means with the same latter are not significantly different.

$AlCl_3$ Treatment (µmol/l)	Chlorophyll *a/b* concentration (mg g^{-1}) for varieties				
	SB 20	SB 19	SB 97	SB 123	**Tukey`s grouping means for Treatments**
Control	2.07a	2.00a	1.99a	1.94a	**2.00a**
25	2.06a	1.64a	2.31a	2.00a	**2.00a**
50	1.65a	1.33a	1.44a	2.01a	**1.61a**
75	2.06a	1.60a	1.83a	1.77a	**1.82a**
100	1.68a	1.83a	1.81a	1.78a	**1.77a**
Tukey`s grouping means for Varieties	**1.90a**	**1.68a**	**1.88a**	**1.90a**	

4.4.5 Carotenoid concentration

Carotenoids concentration (mg.g^{-1}) in leaf per plant was low at the control when compared to any of Al-treated plants apart from SB 20 (Fig. 4.4.5). There was no significant difference ($p \geq 0.05$) among DAT, among varieties and among treatments (Appendix 1, Table 9).

Fig. 4.4.5. Carotenoids concentration (mg g^{-1}) of soy bean varieties at 5 DAT, 25 DAT and 38 DAT subjected to various concentrations of $AlCl_3$ (mg/l) treatments. Values are means of three replicates. Means with the same latter are not significantly different.

$AlCl_3$ Treatment (µmol/l)	Carotenoids Concentration (mg g^{-1}) for varieties				
	SB 20	SB 19	SB 97	SB 123	**Tukey`s grouping means for Treatments**
Control	0.17a	0.28a	0.23a	0.23a	**0.23a**
25	0.21a	0.31a	0.31a	0.18a	**0.25a**
50	0.32a	0.25a	0.33a	0.32a	**0.31a**
75	0.18a	0.29a	0.36a	0.27a	**0.28a**
100	0.28a	0.38a	0.40a	0.32a	**0.35a**
Tukey`s grouping means for Varieties	**0.23a**	**0.30a**	**0.33a**	**0.26a**	

CHAPTER FIVE

DISCUSSION

5.1 Effects of aluminium chloride stress on growth parameters

Aluminium had adverse effects on dry weight and morphological parameters such as number of leaves, plant height, and shoot: root ratio. Increasing concentration of Al in the soil had a reduction on leaf area, stem height and number of leaves (Gary *et al*., 1998). Similar results were reported by Ketan *et al*. (2005) in *Butea monosperma* and Nidhi and Gauray (2014) when studying wheat. This study has shown a reduction on growth of the four Soy bean varieties subjected to increasing concentration of $AlCl_3$. The soy bean varieties showed different growth habits following $AlCl_3$ solution treatments. Variety SB 19 had more leaves but with a smaller leaf area. This was also observed in variety SB 97, which is an adaptation to increase leaf area for the plants to carry out its physiological functions such as photosynthesis (Mahasi *et al*., 2010). The two varieties (SB 97 and SB 20) showed higher growth rates. They had high leaf number, as well as shoot height and stem diameter in earlier days of measurement compared to the rest. This faster growth might have contributed to a larger dry weight in SB 20,which is a late maturing variety. Controversies have previously emerged about phytotoxicity effects of aluminium on plant dry weight. For example, Marjorie *et al.* (2010) found a decrease in the general dry weight with aluminium stress in blueberry genotypes while Sivaguru and Horst (1993) found no clear effects in rice genotypes. Cordovilla *et al*. (1999) found that roots were more sensitive than shoots to aluminium stress in *Phaseolus vulgaris*. In *Phaseolus vulgaris*, Wignarajah (1992) demonstrated that aluminium affected shoot growth more than root growth. 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. However in this study relative growth rate generally decreased with aluminium chloride treatment apart from DAT 43 and DAT 56.

Dry weight of varieties studied had a significant difference ($p \leq 0.05$) in response to increasing concentration of $AlCl_3$ (Appendix 1; Table 6), as earlier found by Ketan *et al*. (2005) in *Butea monosperma*. According to Ketan *et al*. (2005), rapid dry weight reduction in tap roots might have contributed to a major share to total root mass (Frantzius *et al*., 2000), and cause reduction in root/shoot ratio with increasing aluminium chloride stress. Growth

reduction in roots and shoots have been used as a marker of aluminium stress in *Oryza sativa* cultivars (Marjorie *et al.*, 2010). In this study, the results suggested that increase in aluminium chloride in most cases had significant effects. A general decrease in growth with increasing aluminium chloride concentration might have been caused by aluminium induced reduction in photosynthates and stomatal factors as suggested by Marjorie *et al*. (2010). Dry weights of the varieties were reduced with aluminium chloride solution concentration since aluminium treated plants had smaller mean value compared to the control. Aluminium in the soil affected water uptake by plants (Hong *et al*., 2006). Under Al stress the plant might have spend more photosynthetic energy on root production in search of water and /or reducing water loss (Fathy *et al*., 2015). In this study, SB 123 gained more dry weight compared to the other varieties. Intensive root development to avoid $AlCl_3$ is dependent on the genotypes (Kuo and Kao 2003), an adaptation that may have been employed by variety SB 20 which showed a higher root: shoot ratio.

Aluminium negatively affected the growth of the soy bean plants. This is reflected in significantly reduced number of leaves, stem diameter, leaf area, dry weight and relative growth rate (Appendix 3; Table 14). Al reduced water absorption hence decreasing productivity. Out of the four varieties studied, SB 20 showed more tolerance to Al stress.

5.2 Aluminium concentration of the leaf

The variety SB 20 in this study accumulated more aluminium in leaves compared to other varieties. On the overall, aluminium treated plants accumulated more aluminium in leaves than the control plants. Therefore, Inclusion mechanisms may be used by soy bean plants to tolerate Al toxicity (Delhaize *et al*., 1995; Marjorie *et al*., 2009). The results of this study however are in agreement with those of Marjorie *et al*. (2009) where they found out that the tolerant 'Brigitta' genotype of blueberry accumulated more aluminium in its roots and leaves than the other cultivars and that more aluminium was found accumulated in aluminium chloride treated plants. The accumulation of high amounts of aluminium in tolerant varieties have also been reported by Goncxalves *et al*. (1996) while studying its effects in *Sorghum bicolor* cultivars. However, contradicting results have been found in the accumulation of Al in root compared to leaves in other plants (Rout and Das, 2001; Godbold *et al*., 2003, Vanguelova *et al*., 2005; Yasuhiro *et al*; 2006; Marjorie *et al*., 2010). For incidence, Marjorie *et al*. (2010) found out that 'Legacy' variety of blueberry accumulated a lower aluminium

content in the roots than 'Bluegold' variety, showing typical behaviour for aluminium tolerant and aluminium sensitive cultivars, respectively.

5.3 Effects of aluminium on levels of mineral nutrients in leaf tissues

There could be possibly higher nitrate reductase activity in SB 20 and SB 19 soy bean plant varieties thus increasing nitrogen levels in these plants compared to the rest as suggested by Rout and Das (2001). Xiao *et al*. (2003a and 2003b) and Chen (2006) indicate that contents of nitrogen in maize shoots and longan leaves decreases significantly with increasing aluminium concentration. Nitrogen content of longan stems increases when aluminium concentration in nutrient solution increases, but up to a point where further aluminium increase, leads to a decrease (Xiao *et al*., 2003a). Aluminium has also been found to have no effect on N in some plants for example in citrus and peach leaves (Graham, 2001). The soy bean varieties under this study did not show significant difference to the levels of aluminium treatments. Means of nitrogen decreased in plants subjected to aluminium because aluminium enhances efflux of the anions of nitrates by 44% (Cheng *et al.,* 2000; Jonathan *et al*., 2004).

SB 20 and SB 97 had the highest phosphorous (P) content accumulated in leaves compared to other varieties although no significant difference was observed in varieties of soy beans under study under aluminium treatments. Aluminium interferes with the uptake and transport of phosphorous (Luciane *et al.*, 2007). Low phosphorous as found in varieties SB 123 and SB 97 decrease root respiration, interfere with enzymes governing the deposition of polysaccharides in cell walls and increase in cross-linking pectins in cell wall (Andreas and Heinz, 2011). This will consequently lead to less production in these varieties (Ho *et al*., 2004). Aluminium increase P content of roots and decrease P content of shoots (Liang *et al*., 2001; Quartin *et al*., 2001). In this case there may be formation of P and aluminium complexes in root, which inhibits transport of P from root to shoot leading to this phenomenon (Lynch and Brown, 2001). As postulated by Liang *et al*. (2001) this further indicates that aluminium induced decrease in the activity of ATP-dependent H^+ transport system since phosphorous is a component in ATP molecule. Xiao *et al.* (2003a) reported that aluminium decreases P content of longan leaves, which is in agreement with this study where the higher levels of aluminium concentration caused a decreased mean of P in leaves (Appendix 3; Table 15). When studying the effects of aluminium and P interaction on soy

bean root growth and root organic acid exudation, Hong *et al.* (2006) established that adding P significantly increases Al tolerance in genotypes that differ in P efficiency.

Aluminium caused a decrease in accumulation of K when the mean was considered in soy bean varieties. Aluminium may have competed with K for root uptake sites and depressed K uptake in shoots of this varieties under study as earlier found by Graham (2001) and Liang *et al*. (2001). There is a net K^+ efflux and H^+ influx at the root apex, whereas in the rest of the root these fluxes were reversed (Yang *et al*, 2012). Lin and Myhre (1991) in Mundayatan (2007) found that K content of both roots and shoots of 5 citrus rootstocks increased when aluminium concentration in nutrient solution increased up to a point, but then decreased as Al concentration increased further. The cation K was found to be highly concentrated in SB 20 and SB 19, which shows the varieties to have had better cell expansion and osmoregulation (Schachtman *et al*., 1997). High stomatal K requirement is reported for photosynthesis (Chow *et al*.,1990). Plants utilize two systems for K acquisition, low–and high–affinity uptake mechanisms (Meriño-Gergichevich *et al*., 2010). Therefore low-affinity uptake systems may have been utilized for Al-treated soy bean as it accumulated less K.

Aluminium treatment levels applied did not have a significant effect on the varieties under study. This was also found in *Quercus glauca* leaves, where 14 weeks of aluminium treatment did not influence the Mg content (Chen, 2006). In another study magnesium content of maize shoots did not change significantly after 21 mg/l of aluminium treatment, but decreased remarkably under 81 mg/l aluminium treatment (Lidon *et al*., 1999). Besides the role of Mg in chlorophyll structure and as an enzyme cofactor, another important role of Mg in plants is in the export of photosynthates, therefore, might have had a non significant enhanced degradation of chlorophyll in Mg deficient source leave (Xiao *et al*., 2003b). A similar decrease based mean of Mg content due to aluminium was observed in leaves of peach 'Nemaguard'(Simon *et al*., 1994a), 3 triticale cultivars (Graham, 2001) and tomato (Quartin *et al*., 2001), both leaves and stems of longan (Chen *et al*., 2006) and in the shoots of 2 maize cultivars (Ambrosio *et al*., 2003).

Aluminium chloride treatment reduced Ca content in leaves of the soy bean varieties studied as control had a higher mean whenever compared to any treatment. The reduction may be attributed to reduction in Ca uptake (Cronan and Grigal *et al*., 1995; Xiao *et al*., 2003b). Plant roots might have responded to external low pH and Al by a sustained elevation in cytosolic free calcium ion (Ca^{2+}) concentration (Plieth *et al.*, 1999). It was probably for

this reason that more Ca was found concentrated in SB 20 and SB 19. Aluminium interfered with the binding of the Calcium ions in the cell walls by the same order of magnitude as their respective influx (Luciane, *et al*., 2007). Uptake of Ca^{2+} from the soil solution for SB 123 and SB 97 might have decreased because of ion interactions, precipitation and increases in ionic strength that reduce the activity of Ca^{2+} (Ketan *et al*., 2005). Calcium was applied in adequate amounts in form of calcium superphosphate fertilizer, therefore it might have mitigated aluminium stress (especially leaf necrosis) to the seedlings stage of these soy bean varieties (Ketan *et al*., 2005). In particular Ca^{2+} plays an important role in mechanisms of Al^{3+} toxicity because it binds cations or screens negative charges on the plasma membrane. This might have reduced the activity of Al^{3+} close to the cell surface and to the sensitive meristems (Miguel *et al*., 2013) in SB 20 and SB 19.Calcium is important during aluminium chloride stress in preserving membrane integrity (Chen *et al*., 2010), signalling in osmoregulation and influencing K/Na selectivity (Ketan *et al*., 2005).

SB 20 had high concentration of Al in the leaves. Generally plants under Al were highly concentrated with Al in the leaves. Aluminium did not interfere with N concentration in the varieties based on the non significant difference in the means. Under higher Al concentration; the soy beans are able to concentrate normal N in the leaves. Al treatments did not significantly affect the concentration of nutrients; P, K, Mg and Ca. However, some varietal differences which were not significant were observed, indicating that the varieties could be behaving differentially in the absorption and accumulation of nutrients.

5.4 Influence of aluminium chloride on chlorophyll fluorescence

The treatment of aluminium chloride did not affect the photochemical efficiency of PSII (Fv/Fm), ϕPSII and NPQ of the varieties of soy beans investigated differently. In citrus leaves, aluminium chloride caused a significant decrease of the 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 (Li-Song *et al*., 2005). They gave the potential to estimate photosynthetic performance and, thereby, plant productivity under different environmental conditions (Maxwell and Johnson, 2000; Sikuku *et al*., 2010).

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. This was in agreement with the results of Ambrosio *et al.* (2003) in maize. Mean values for maximum quantum yield were high at the control treatment compared to aluminium treated plants. This shows that photosynthetic apparatus of the plants were 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. In comparison, 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 have been found to be in the normal (0.7–0.8) ranges for healthy blueberry Al treated plants (Marjorie *et al*., 2009; 2010). According to Bjoŕkman and Demmig (1987) and Kate and Giles (2000), Fv/Fm ratio for normal plants have an optimal value of 0.83. This means soy bean plants in this study exhibited normal photosynthesis despite being subjected to $AlCl_3$ treatment.

Effective quantum yield (ϕPSII) had a low mean value at the control compared to aluminium chloride treated soy bean plants under investigation. These two photochemical parameters in SB 123 were lower even under the control condition compared with the other varieties. The variety SB 123 may be intrinsically less efficient at managing its energy for photochemical processes than the other varieties (Giannakoula *et al*., 2008). This cultivar-specific behaviour indicate that it might have lower productivity with respect to the other varieties. Seedlings of a non-tolerant *Z. mays* hybrid grown in $AlCl_3$ medium accumulated a greater quantity of carbohydrates in the apex of seminal roots in the presence of aluminium with respect to the tolerant genotype (Hoshino *et al*., 2000). The high values of ΦPSII in SB 20 showed that the photochemical activity was the main way to dissipate safely the excess energy of excitation. This was an indication that electron transport rate was never saturated showing that other sinks, different from the assimilatory process, were likely to accept these electrons (Erwin *et al.* 2014). In this way 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 a role in the production of extra ATP for the pseudocyclic photophosphorylation. Even if evidence for significant rates in leaves is lacking in this study,

Watt (2003) suggested that the Mehler reaction is an important sink for electrons in C4 plants but they estimated rates too low to account for the extra ATP demand.

Thermal energy dissipation measured as NPQ in the four soy bean cultivars did not have a clear pattern with increasing aluminium chloride concentration. In most cases NPQ was high in aluminium treated plants compared to control, more so the highest mean value was found at 25 and 75 mg/l compared to the control. 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 water-water cycle (Mehler reaction) and photorespiration in aluminium treated leaves may have been upregulated to cope with the increased excess of excitation (Osmond and Grace, 1995; Savitch *et al*., 2003; Marjiorie *et al*., 2010) in SB 123 variety that had a high NPQ. Bilger and Bjoŕkman (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). 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. The main role of NPQ is to indicate dissipation of the excess energy of excitation The low non-photochemical quenching (Chen. and Cheng, 2003) in control plants, indicated therefore less thermal energy dissipation. A higher mean value in aluminium chloride treated soy bean leaves contributed to excess of thermal energy of dissipation (NPQ). This explains the fact that apart from photochemistry, fluorescence strategy was adopted to dissipate excess energy to some extent. SB 123 soy bean variety appears to have been strongly affected by Al stress since it exhibited high fluorescence and was found to have dissipated more energy.

Chen *et al*. (2005a and 2005b) observed that, Al-stressed 'Cleopatra' tangerine leaves only used a smaller fraction of the absorbed light in electron transport, since CO_2 assimilation decreased to a greater degree than leaf chlorophyll concentration or leaf light absorption in response to aluminium (Wan, 2007). As a result, more excess excitation energy may have existed in aluminium stressed soy bean plant leaves when compared with controls under high photon flux at midday (Chen *et al*. , 2005a and 2005b). 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). Closure of PSII reaction centres will result in formation of reactive oxygen species. 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 (Chen *et al*., 2005a and 2005b, Li-Song *et al*., 2010).

Generally plants under Al had low values of Fv/Fm and ϕPSII showing that photosynthetic apparatus were affected by Al. Aluminium did not interfere with chlorophyll fluorescence parameters in the varieties based on the non significant difference in the means. Some varietal differences which were not significant were observed as SB 20 and SB 19 had high mean values of Fv/Fm and ϕPSII. SB 123 behaved different to the rest as it dissipated excess excitation energy as indicated by NPQ compared to the rest.

5.5 Effects of aluminium chloride on photosynthetic pigment concentration

Aluminium induced a decrease in chlorophyll a concentration in soy beans. This has been reported in other plant species, such as citrus (Chen *et al*., 2005a; Jiang *et al*., 2008; 2009a; 2009b), soy bean (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). It should, however be noted that a decrease in chlorophyll concentration means (Appendix 3; Table 14) of soy bean plants in response to aluminium was probably not the primary factor to limit CO_2 assimilation (Chen *et al*., 2005b; Jiang *et al*., 2008; 2009a and 2009b; Yang *et al*, 2008; Li-Song *et al*., 2010; Ridolfi and Garrec, 2000). A study by Chen *et al*. (2005b) support this postulate since chlorophyll concentration means were lower in Al-treated than in control leaves. Peixoto *et al*. (2002) found that a combination of factors such as reduced pigment concentration, impaired PSII photochemistry and the distribution of enzymatic machinery accounted for the aluminium induced decrease in CO_2 assimilation in sorghum.

Aluminium might have caused a decrease in chlorophyll synthesis in aluminium treated soy bean plant leaves when compared to the control by inhibiting the activity of -aminolevulinic acid (-ALA) dehydratase responsible for the formation of monopyrrole

porphobilonogen, which is a part of the Chlorophyll molecule as well as the cytochromes (Pereira *et al*., 2006). Mihailovic *et al*. (2008) found that in aluminium sensitive maize inbred line, chlorophyll reduction coincided with 5-ALA synthesis inhibition, chlorophyllase activation and leaf deprivation of Fe and Mg. Therefore decrease in chlorophyll *a* with increasing aluminium concentration for the varieties in this study may be attributed to the inhibition of the activity of - aminolevulinic acid (-ALA) dehydratase. Chlorophyll *a* concentration was highest in SB 20 while SB 19 had higher chlorophyll *b* concentration compared to varieties SB 97 and SB 123. Reduction in both chlorophyll *a* concentration means under aluminium toxicity stress may been due to decreased Mg concentrations found in this study, which may result in a correspondingly decreased PAR utility efficiency and affect the photosynthetic capacity (Xiao-Bin *et al*., 2007; Marjiorie *et al*., 2009). The decrease in total chlorophyll in varieties SB 19, SB 123 and SB 97 with increase in aluminium chloride concentration suggests the possibility of chlorophyll photobleaching within PSI and PSII as also observed by Li-Song *et al.* (2005), resulting in a smaller fraction of absorbed light energy for electron transport. Peixoto *et al.* (2002) found results that were similar in *S. bicolor* cultivars where total chlorophyll was substantially decreased after 48 h of aluminium exposure.

Chlorophyll a/b ratio means decreased markedly under aluminium treatment in these varieties unlike in the studies reported by Milivojevi *et al*. (2000). 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). There are different views about the effects of aluminium on chlorophyll a/b ratio. The ratio was increased by aluminium in 'Cleopatra' tangerine (Chen *et al*., 2005b) and in *Eucalyptus grandin* × *E. urophylla* (Yang *et al.*, 1996), decreased in rice (Li-Song *et al.*,2005) and soy bean (Ying and Liu, 2005) and unaffected in beech (Ridolfi and Garrec, 2000). The aluminium stressed soy bean varieties SB 20, SB 19, SB 123 and SB 97 had a higher chlorophyll a/b ratio on 5 DAT. However, In 'Sour pummelo' (*Citrus grandis*) it was found that chlorophyll a/b ratio remained unchanged or decreased in response to aluminium depending on the concentration of boron and P applied in the nutrient solutions as observed by Jiang *et al.* (2008). This change in chlorophyll antenna size is probably a strategy to reduce light absorption and avoid possible damage to the photosystems

of these varieties due to aluminium stress (Azmat and Hasan, 2008). This Photoprotective strategy has been reported for evergreen species under other types of stresses (Adams and Barker, 1998; Adams *et al*., 2004; Savitch *et al*., 2002). Marjiorie *et al*. (2010) also found that, at the beginning of aluminium treatment application, the chlorophyll antenna size was reduced in less quantities and that, chlorophyll antenna size in aluminium chloride stress was to maintain ϕPSII. This suggests that there is a slow acclimation of the photosynthetic apparatus to aluminium stress (Dong *et al*., 2008).

The photoprotective carotenoids means increased within varieties under investigation with increasing aluminium concentration. This concept has also been stated by Marjorie *et al*. (2010). It has been found that carotene functions as a passive light protecting filter and have got the role of accessory pigments transferring energy and oxygen (Adams *et al*., 1998). This increase in carotenoids played 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; Demmig-Adams and Adams, 1996; Niyogi *et al*., 1998; Marjorie *et al*., 2010). 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 also suggested by Demmig-Adams and Adams (1996) and Marjorie *et al*. (2009). In this study therefore carotenoids pigments may have prevented chlorophyll and thylakoid membrane from the damage of absorbed energy by photo oxidation (Sükran *et a*., 1998; Martin *et al*., 1996). It has been suggested that NPQ development in Al-treated 'Cleopatra' tangerine leaves may be impaired with antheraxanthin + zeaxanthin (Ying *et al*., 2006). These pigments have photoprotective functions against thermal energy dissipation just as carotenoids (Chen *et al*., 2005a; Ali *et al*., 2008). The mechanisms underlying these phenomena are yet to resolved and therefore need to be addressed in future.

SB 20 had high concentration of chlorophyll *a* in the leaves. Generally plants under Al were lowly concentrated with chl *a*, chl *b* and chl *a+b*. Al treatments significantly affected the concentration of chl *a*. Al treatments did not significantly affected the concentration of photosynthetic pigments; chl *b*, chl *a+b*, chl *a/b* and carotenoids. Under even the higher Al concentration the soy beans are able to concentrate normal amounts of this pigments.

However, some varietal differences which were not significant were observed in the means, indicating that the varieties could be behaving differentially in PSII impairment activity.

CHAPTER SIX

CONCLUSIONS, RECOMMENDATIONS AND SUGGESTIONS FOR FUTURE STUDIES

6.1. Conclusion

i. Aluminium negatively affected the growth of the soy bean plants. This is reflected in reduced number of leaves, stem diameter, leaf area, dry weight and relative growth rate. Al reduced water absorption hence decreasing productivity. Out of the four varieties studied, SB 20 showed more tolerance to Al stress.

ii. SB 20 had high concentration of Al in the leaves. Generally plants under Al were highly concentrated with Al in the leaves. Aluminium did not interfere with N concentration in the varieties based on the non significant difference in the means. Under even the higher Al concentration the soy beans are able to concentrate normal N in the leaves. Al treatments did not significantly affect the concentration of nutrients; P, K, Mg and Ca. However, some varietal differences which were not significant were observed, indicating that the varieties could be behaving differentially in the absorption and accumulation of nutrients.

iii. Generally plants under Al had low values of Fv/Fm and ϕPSII showing that photosynthetic apparatus were affected by Al. Aluminium did not interfere with chlorophyll fluorescence parameters in the varieties based on the non significant difference in the means. Some varietal differences which were not significant were observed as SB 20 and SB 19 had high mean values of Fv/Fm and ϕPSII. SB 123 behaved different to the rest as it dissipated excess excitation energy as indicated by NPQ compared to the rest.

iv. SB 20 had high concentration of chlorophyll *a* in the leaves. Generally plants under Al were lowly concentrated with chl *a*, chl *b* and chl *a*+*b*. Al treatments significantly affected the concentration of chl *a*. Al treatments did not significantly affected the concentration of photosynthetic pigments; chl *b*, chl *a*+*b*, chl *a/b* and carotenoids. Under higher Al concentration the soy beans are able to concentrate normal amounts of this pigments. However, some varietal differences which were not significant were

observed in the means, indicating that the varieties could be behaving differentially in PSII impairment activity.

6.2 Recommendations

i. Plant growth parameters are recommended to be used for determining effect of Al stress to soy bean since significant differences were observed. This indicate that stressed plants were negatively affected by Al.

ii. Nutrient concentration should be part of routine determination in soy bean plants subjected to Al stress although the results from this study show that they were strongly not affected. SB 20 and SB 19 showed higher concentration of N, Mg and Ca and the two varieties are therefore good candidates for cultivation in Al affected soils.

iii. Despite the lack of significant difference in chlorophyll fluorescence parameters, there was some consistence results where Fv/Fm and ϕPSII showed that variety SB 20 stood out as showing higher tolerance to Al stress. Based on this results the variety may be recommended for cultivation in Al affected soils**.**

iv. Plants with higher carotenoid concentration suggest that they can offer protection to the photosynthetic apparatus under higher light. Although there was no significant difference but the general trend was that high Al concentration in growth medium lead to more carotenoid in the leaves.

6.3 Suggestion for future studies

i. In this study, root length was not studied but Al affect root growth, thus further studies should be carried out on root length since Al affect cell division and cell elongation.

ii. It is not clear on the mechanisms involved in absorption of mineral nutrients and partitioning of these mineral nutrients in different part of the plants and these needs to be studied in future.

iii. Chlorophyll fluorescence parameters were not conclusive and should combine with gas exchange parameters for instance measurements of photosynthetic rate, stomatal conductance and transpiration rate among others, because this would

indicate the overall rate of photosynthesis since chlorophyll fluorescence concentrated on the activities of photosynthetic apparatus.

iv. The functions of carotenoids in protecting photosynthetic apparatus should also be studied when plants are subjected to Al stress. Future studies should look into reasons why chlorophyll *a* is often affected by Al stress.

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Appendices.

Appendix 1: Analysis of Variance (ANOVA) for parameters

Table 6. ANOVA for plant growth parameters.

Number of leaves

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	79	336287.1625	4256.7995	64.23	<.0001
Error	160	10603.3333	66.2708		
Corrected Total	239	346890.4958			

R-Square	Coeff Var	Root MSE	Number of leaves Mean
0.969433	13.62364	8.140690	59.75417

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Varieties	3	8558.3458	2852.7819	43.05	<.0001
Treatments	4	1520.0583	380.0146	5.73	0.0002
DAT	3	309956.3458	103318.7819	1559.04	<.0001
Treatments*Varieties	12	1210.3417	100.8618	1.52	0.1211
DAT*treatments* Varieties	36	1241.4583	34.4850	0.52	0.9884

Shoot height

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	79	85005.59296	1076.02016	48.87	<.0001
Error	160	3522.58593	22.01616		
Corrected Total	239	88528.17890			

R-Square	Coeff Var	Root MSE	Shoot height Mean
0.960209	11.90894	4.692138	39.40013

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Varieties	3	5120.45618	1706.81873	77.53	<.0001
Treatments	4	304.72426	76.18107	3.46	0.0097
DAT	3	69613.31371	23204.43790	1053.97	<.0001
Treatments*Varieties	12	261.36729	21.78061	0.99	0.4615
DAT*treatments* Varieties	36	755.92012	20.99778	0.95	0.5497

Stem diameter

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	79	3.91909566	0.04960881	9.03	<.0001
Error	160	0.87928733	0.00549555		
Corrected Total	239	4.79838300			

R-Square	Coeff Var	Root MSE	Stem diameter Mean
0.816753	14.66012	0.074132	0.505671

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Varieties	3	0.46480101	0.15493367	28.19	<.0001
Treatments	4	0.14577702	0.03644425	6.63	<.0001
DAT	3	2.72575568	0.90858523	165.33	<.0001
Treatments*Varieties	12	0.10849072	0.00904089	1.65	0.0841
DAT*Treatments* Varieties	36	0.09618848	0.00267190	0.49	0.9937

leaf area

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	79	1805.991319	22.860650	3.13	<.0001
Error	160	1170.264967	7.314156		
Corrected Total	239	2976.256286			

R-Square	Coeff Var	Root MSE	leave area Mean
0.606800	18.45413	2.704470	14.65509

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Varieties	3	234.3399125	78.1133042	10.68	<.0001
Treatments	4	182.6383868	45.6595967	6.24	0.0001
DAT	3	908.6248491	302.8749497	41.41	<.0001
Treatments*Varieties	12	205.3956620	17.1163052	2.34	0.0086
DAT*treatments* Varieties	36	111.9386265	3.1094063	0.43	0.9983

Table 6. ANOVA for plant growth parameters "Cont"

Dry weight

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	59	38197.71541	647.41891	25.42	<.0001
Error	120	3056.20733	25.46839		
Corrected Total	179	41253.92274			

R-Square	Coeff Var	Root MSE	Dry weight Mean
0.925917	38.94883	5.046622	12.95706

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Varieties	3	1455.17954	485.05985	19.05	<.0001
Treatments	4	357.42854	89.35713	3.51	0.0096
DAT	2	32130.52682	16065.26341	630.79	<.0001
treatments*Varieties	12	351.95887	29.32991	1.15	0.3260
DAT*treatments* Varieties	24	626.86010	26.11917	1.03	0.4403

Relative growth rate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	59	4.17827991	0.07081830	3.52	<.0001
Error	120	2.41378400	0.02011487		
Corrected Total	179	6.59206391			

R-Square	Coeff Var	Root MSE	Relative growth rate Mean
0.633835	33.73439	0.141827	0.420422

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Varieties	3	0.64683760	0.21561253	10.72	<.0001
Treatments	4	0.58941391	0.14735348	7.33	<.0001
DAT	2	1.54577618	0.77288809	38.42	<.0001
Treatments*Varieties	12	0.42539818	0.03544985	1.76	0.0621
DAT*treatment*Soy bean	24	0.30672302	0.01278013	0.64	0.9008

Root: Shoot ratio

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	19	0.37167392	0.01956179	1.47	0.1519
Error	40	0.53380867	0.01334522		
Corrected Total	59	0.90548258			

R-Square	Coeff Var	Root MSE	Root: Shoot ratio Mean
0.410471	102.1561	0.115521	0.113083

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Varieties	3	0.10271218	0.03423739	2.57	0.0680
treatments	4	0.04856150	0.01214037	0.91	0.4676
treatments*Varieties	12	0.22040023	0.01836669	1.38	0.2173

Table 7; ANOVA for Aluminium and plant mineral nutrients

Al

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	19	1709.126667	89.954035	1.98	0.0343
Error	40	1817.166667	45.429167		
Corrected Total	59	3526.293333			

R-Square	Coeff Var	Root MSE	Al Mean
0.484681	37.51456	6.740116	17.96667

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Varieties	3	36.478667	12.159556	0.27	0.8483
treatments	4	1270.306667	317.576667	6.99	0.0002
Varieties*treatment	12	402.341333	33.528444	0.74	0.7065

N

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	19	4131.88400	217.46758	0.59	0.8890
Error	40	14663.16000	366.57900		
Corrected Total	59	18795.04400			

R-Square	Coeff Var	Root MSE	N Mean
0.219839	28.00798	19.14625	68.36000

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Varieties	3	262.793333	87.597778	0.24	0.8687
Treatments	4	1549.727333	387.431833	1.06	0.3904
Varieties*treatments	12	2319.363333	193.280278	0.53	0.8841

Table 7; ANOVA for plant mineral nutrients "Cont"

P

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	19	3853.97067	202.84056	0.77	0.7265
Error	40	10548.78667	263.71967		
Corrected Total	59	14402.75733			

R-Square	Coeff Var	Root MSE	P Mean
0.267586	32.20409	16.23945	50.42667

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Varieties	3	8.385333	2.795111	0.01	0.9985
Treatments	4	790.945667	197.736417	0.75	0.5640
Varieties*treatments	12	3054.639667	254.553306	0.97	0.4963

K

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	19	4276.09917	225.05785	0.77	0.7256
Error	40	11691.24667	292.28117		
Corrected Total	59	15967.34583			

R-Square	Coeff Var	Root MSE	K Mean
0.267803	29.37918	17.09623	58.19167

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Varieties	3	1138.385833	379.461944	1.30	0.2883
Treatments	4	609.365000	152.341250	0.52	0.7206
Varieties*treatments	12	2528.348333	210.695694	0.72	0.7226

Mg

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	19	1285.879167	67.677851	0.94	0.5408
Error	40	2873.333333	71.833333		
Corrected Total	59	4159.212500			

R-Square	Coeff Var	Root MSE	Mg Mean
0.309164	28.32232	8.475455	29.92500

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Varieties	3	415.4085000	138.4695000	1.93	0.1406
Treatments	4	295.7933333	73.9483333	1.03	0.4039
Varieties*treatment	12	574.6773333	47.8897778	0.67	0.7719

Ca

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	19	3425.83917	180.30732	0.77	0.7296
Error	40	9412.91333	235.32283		
Corrected Total	59	12838.75250			

R-Square	Coeff Var	Root MSE	Ca Mean
0.266836	25.24103	15.34024	60.77500

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Varieties	3	108.112500	36.037500	0.15	0.9271
treatments	4	1703.803333	425.950833	1.81	0.1459
Varieties*treatments	12	1613.923333	134.493611	0.57	0.8516

Table 8; ANOVA for chlorophyll fluorescence parameters

Fv/Fm

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	59	1.41923406	0.02405481	0.58	0.9896
Error	120	4.98285200	0.04152377		
Corrected Total	179	6.40208606			

R-Square	Coeff Var	Root MSE	FV/FM Mean
0.221683	38.55174	0.203774	0.528572

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Varieties	3	0.13002713	0.04334238	1.04	0.3759
Treatments	4	0.09545448	0.02386362	0.57	0.6815
DAT	2	0.44078541	0.22039271	5.31	0.0062
Treatments*Varieties	12	0.18815601	0.01567967	0.38	0.9692
DAT*treatments*Varieties	24	0.36422219	0.01517592	0.37	0.9971

Table 8; ANOVA for chlorophyll fluorescence parameters “Cont”

ϕPSII

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	59	1.72102880	0.02916998	0.68	0.9527
Error	120	5.18164800	0.04318040		
Corrected Total	179	6.90267680			

R-Square	Coeff Var	Root MSE	ϕPSII Mean
0.249328	40.60159	0.207799	0.511800

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Varieties	3	0.12053267	0.04017756	0.93	0.4283
Treatments	4	0.11231724	0.02807931	0.65	0.6278
DAT	2	0.28052770	0.14026385	3.25	0.0423
Treatments*Varieties	12	0.23975422	0.01997952	0.46	0.9326
DAT*treatments*Varieties	24	0.46779841	0.01949160	0.45	0.9867

NPQ

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	59	57.4270286	0.9733395	1.28	0.1304
Error	120	91.4460871	0.7620507		
Corrected Total	179	148.8731158			

R-Square	Coeff Var	Root MSE	NPQ Mean
0.385745	122.6944	0.872955	0.711487

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Varieties	3	2.92516661	0.97505554	1.28	0.2846
Treatments	4	2.33267776	0.58316944	0.77	0.5499
DAT	2	9.82232278	4.91116139	6.44	0.0022
Treatments*Varieties	12	10.67017561	0.88918130	1.17	0.3146
DAT*treatments* Varieties	24	23.46262182	0.97760924	1.28	0.1905

Table 9; ANOVA for Photosynthetic pigment

Chl a

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	59	69.4792869	1.1776150	3.60	<.0001
Error	120	39.2338924	0.3269491		
Corrected Total	179	108.7131793			

R-Square	Coeff Var	Root MSE	Chl a Mean
0.639106	44.05001	0.571795	1.298058

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Varieties	3	2.09434906	0.69811635	2.14	0.0993
Treatment	4	5.23039919	1.30759980	4.00	0.0044
DAT	2	47.17048255	23.58524128	72.14	<.0001
Treatments*Varieties	12	7.73531425	0.64460952	1.97	0.0326
DAT*treatments* Varieties	24	5.32867642	0.22202818	0.68	0.8634

chl b

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	59	10.39977148	0.17626731	1.19	0.2052
Error	120	17.70065701	0.14750548		
Corrected Total	179	28.10042849			

R-Square	Coeff Var	Root MSE	chl b Mean
0.370093	50.63554	0.384064	0.758488

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Varieties	3	0.23498478	0.07832826	0.53	0.6619
Treatments	4	1.36437956	0.34109489	2.31	0.0615
DAT	2	1.26233422	0.63116711	4.28	0.0160
Treatments*Varieties	12	2.83547808	0.23628984	1.60	0.0998
DAT*treatments* Varieties	24	2.68371503	0.11182146	0.76	0.7807

Chl a+b

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	59	83.6431990	1.4176813	1.98	0.0008
Error	120	85.9959773	0.7166331		
Corrected Total	179	169.6391763			

R-Square	Coeff Var	Root MSE	Chl a+b Mean
0.493065	41.70488	0.846542	2.029839

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Varieties	3	3.47810255	1.15936752	1.62	0.1888
Treatments	4	4.12768952	1.03192238	1.44	0.2250
DAT	2	37.11153868	18.55576934	25.89	<.0001
Treatments*Varieties	12	22.16603959	1.84716997	2.58	0.0045
DAT*treatments* Varieties	24	12.78463751	0.53269323	0.74	0.7975

Table 9; ANOVA for Photosynthetic pigment `` cont``

Chl a/b

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	59	231.1501565	3.9177993	5.12	<.0001
Error	120	91.8696093	0.7655801		
Corrected Total	179	323.0197658			

R-Square	Coeff Var	Root MSE	Chl a/b Mean
0.715591	47.56071	0.874974	1.839700

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Varieties	3	1.5818349	0.5272783	0.69	0.5606
Treatments	4	3.9701281	0.9925320	1.30	0.2753
DAT	2	198.0021660	99.0010830	129.32	<.0001
Treatments*Varieties	12	4.1157603	0.3429800	0.45	0.9402
DAT*treatments* Varieties	24	17.5423200	0.7309300	0.95	0.5297

C x+c

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	59	2.08496164	0.03533833	1.03	0.4358
Error	120	4.11276933	0.03427308		
Corrected Total	179	6.19773098			

R-Square	Coeff Var	Root MSE	Cx+c Mean
0.336407	65.76291	0.185130	0.281511

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Varieties	3	0.23882049	0.07960683	2.32	0.0785
Treatments	4	0.31191148	0.07797787	2.28	0.0651
DAT	2	0.17477658	0.08738829	2.55	0.0823
Treatments*Varieties	12	0.21014879	0.01751240	0.51	0.9043
DAT*treatments* Varieties	24	0.82094388	0.03420599	1.00	0.4744

Appendix 2: Means breakdown

Table 10; Means breakdown for plant growth parameters

Soy bean**;** 1,2,3,4 are SB 97, SB 19, SB 20, SB 123 respectively. Treatments; 1, 2, 3, 4, 5 are 0 mg/l, 25 mg/l, 50 mg/l, 75 mg/l, 100 mg/l.

Effect=VARIETIES

Time (day)	treatment	Glycine max	Mean of NUMBER OF LEAVES	Std. Error of NUMBER OF LEAVES	Mean of SHOOT HEIGHT	Std. Error of SHOOT HEIGHT	Mean of STEM DIAMETER
.	.	1	49.5833	3.67833	33.8783	1.42424	0.44450
.	.	2	64.3500	5.34144	42.2422	2.92642	0.48350
.	.	3	63.6667	5.06286	36.0933	2.00525	0.55183
.	.	4	61.4167	5.28019	45.3867	2.99133	0.54285

Time (day)	treatment	Glycine max	Std. Error of STEM DIAMETER	Mean of LEAVE AREA	Std. Error of LEAVE AREA
.	.	1	0.014258	15.7438	0.61748
.	.	2	0.013662	13.0581	0.30016
.	.	3	0.021459	15.0524	0.43638
.	.	4	0.019332	14.7660	0.33578

Effect=TIME IN DAY

Time (day)	treatment	Glycine max	Mean of NUMBER OF LEAVES	Std. Error of NUMBER OF LEAVES	Mean of SHOOT HEIGHT	Std. Error of SHOOT HEIGHT	Mean of STEM DIAMETER
0	.	.	12.7333	0.22658	15.6622	0.44535	0.33617
16	.	.	37.3167	1.13483	31.0417	0.48854	0.49352

Time (day)	treatment	Glycine max	Std. Error of STEM DIAMETER	Mean of LEAVE AREA	Std. Error of LEAVE AREA
0	.	.	.005208054	11.2926	0.36752
16	.	.	.009042883	15.7572	0.37463

Effect=TIME IN DAY

Time (day)	treatment	Glycine max	Mean of NUMBER OF LEAVES	Std. Error of NUMBER OF LEAVES	Mean of SHOOT HEIGHT	Std. Error of SHOOT HEIGHT	Mean of STEM DIAMETER
43	.	.	90.0500	2.44064	53.0217	1.73405	0.5915
56	.	.	98.9167	1.77129	57.8750	1.37814	0.6015

Time (day)	treatment	Glycine max	Std. Error of STEM DIAMETER	Mean of LEAVE AREA	Std. Error of LEAVE AREA
43	.	.	0.015321	15.9701	0.35421
56	.	.	0.015552	15.6005	0.42801

Effect=TREATMENT

Time day)	treatment	Glycine max	Mean of NUMBER OF LEAVES	Std. Error of NUMBER OF LEAVES	Mean of SHOOT HEIGHT	Std. Error of SHOOT HEIGHT	Mean of STEM DIAMETER
.	1	.	64.5625	5.70748	40.9167	2.94835	0.55377
.	2	.	58.2917	5.39026	38.4506	2.94444	0.49333
.	3	.	59.7708	5.51411	38.3938	2.62052	0.48813
.	4	.	57.4583	5.48870	38.6104	2.67578	0.50354
.	5	.	58.6875	5.56132	40.6292	2.77790	0.48958

Time (day)	treatment	Glycine max	Std. Error of STEM DIAMETER	Mean of LEAVE AREA	Std. Error of LEAVE AREA
.	1	.	0.023233	16.3345	0.71345
.	2	.	0.020892	14.4415	0.40281
.	3	.	0.018257	13.7811	0.39833
.	4	.	0.018273	14.3507	0.44219
.	5	.	0.020467	14.3677	0.46134

Table 10; Means breakdown for plant growth parameters ``cont``

Effect=VARIETIES

time (Day)	treatment	Glycine max	Mean of DRY WEIGHT	Std. Error of DRY WEIGHT	Mean of RELATIVE GROWTH RATE	Std. Error of RELATIVE GROWTH RATE
.	.	1	10.8431	1.73372	0.38333	0.027720
.	.	2	9.7931	1.51982	0.35933	0.017031
.	.	3	14.1942	2.35423	0.42236	0.026213
.	.	4	16.9978	3.04012	0.51667	0.035455

Effect=TIME IN DAY

time (Day)	treatment	Glycine max	Mean of DRY WEIGHT	Std. Error of DRY WEIGHT	Mean of RELATIVE GROWTH RATE	Std. Error of RELATIVE GROWTH RATE
0	.	.	1.4517	0.06700	0.29200	0.014940
16	.	.	5.7300	0.26140	0.50727	0.027229
56	.	.	31.6895	1.58253	0.46200	0.021468

Effect=TREATMENT

time (Day)	treatment	Glycine max	Mean of DRY WEIGHT	Std. Error of DRY WEIGHT	Mean of RELATIVE GROWTH_ RATE	Std. Error of RELATIVE_ GROWTH RATE
.	1	.	15.5075	3.02607	0.51667	0.039160
.	2	.	12.2650	2.56447	0.39472	0.033843
.	3	.	11.2572	2.09183	0.34128	0.023696
.	4	.	12.7314	2.37871	0.41611	0.024450
.	5	.	13.0242	2.58649	0.43333	0.030428

Effect=Treatment

Rep.	Time (Day)	Trt	*Glycine max*	Mean of Dry weight	Mean of Relative Growth Rate
.	.	1	.	15.5075	0.51667
.	.	2	.	12.2650	0.39472
.	.	3	.	11.2572	0.34128
.	.	4	.	12.7314	0.41611
.	.	5	.	13.0242	0.43333

Effect=*Soy bean*

Replication	Time (day)	Treatment	*Glycine max*	Mean of Root: Shoot Ratio
.	.	.	1	0.07833
.	.	.	2	0.14293
.	.	.	3	0.16420
.	.	.	4	0.06687

Effect=Replication

Replication	Time (day)	Treatment	*Glycine max*	Mean of Root: Shoot Ratio
1	.	.	.	0.08220
2	.	.	.	0.12800
3	.	.	.	0.12905

Effect=TREATMENT

Replication	Time (day)	Treatment	*Glycine max*	Mean of Root: Shoot .. Ratio
.	.	1	.	0.11300
.	.	2	.	0.07758
.	.	3	.	0.14075
.	.	4	.	0.14858
.	.	5	.	0.08550

Table 11; Means breakdown for aluminium and plant mineral nutrients; 1,2,3,4 are SB 97, SB 19, SB 20, SB 123 respectively. Treatments; 1, 2, 3, 4, 5 are 0 mg/l, 25 mg/l, 50 mg/l, 75 mg/l, 100 mg/l.

Effect=VARIETIES

Glycine max	treatment	Mean of Al	Error of Al	Std. Mean of N	Error of N	Std. Mean of P	Std. Error of P
1	.	17.3333	1.62890	67.1133	5.25103	50.6267	3.71769
2	.	17.8200	1.98979	71.1067	4.58414	50.0933	3.58677
3	.	19.2800	2.73085	69.5200	5.73198	50.9400	4.32586
4	.	17.4333	1.59627	65.7000	2.60874	50.0467	4.81098

Glycine max	treatment	Mean of K	Error of K	Std. Mean of Mg	Error of Mg	Std. Mean of Ca	Std. Error of Ca
1	.	54.8267	2.09776	28.2667	2.23783	60.0133	1.54728
2	.	57.1867	3.38422	28.9400	2.01607	60.1400	4.08298
3	.	65.5733	5.15683	34.4467	2.31812	63.0933	5.09584
4	.	55.1800	5.30732	28.0467	1.83889	59.8533	3.94838

Table 11; Means breakdown for aluminium and plant mineral nutrients ``cont``

Effect=TREATMENT

Glycine max	treatment	Mean of AL	Std. Error of AL	Mean of N	Std. Error of N	Mean of P	Std. Error of P
.	1	9.4833	1.42753	76.3333	3.40183	57.4250	2.23438
.	2	17.2583	2.92467	69.5583	5.27029	48.1417	3.05548
.	3	19.8583	1.35263	70.0250	5.39245	50.5167	4.71066
.	4	22.8750	1.27998	64.0917	5.06071	48.3167	6.64533
.	5	20.3583	1.74106	61.7917	6.05050	47.7333	4.73716

Glycine max	treatment	Mean of K	Std. Error of K	Mean of Mg	Std. Error of Mg	Mean of Ca	Std. Error of Ca
.	1	63.1917	4.39894	31.5250	2.81990	67.8500	4.61602
.	2	56.5667	4.44371	28.6917	1.45854	62.9333	3.87571
.	3	54.5000	4.81003	26.1083	1.67008	51.7833	3.95537
.	4	60.5250	4.68974	31.8083	2.70612	62.4417	4.13845
.	5	56.1750	5.66755	31.4917	3.01279	58.8667	3.90579

Table 12; Means breakdown for Chlorophyll flouresence parameters

Soy bean; 1,2,3,4 are SB 97, SB 19, SB 20, SB 123 respectively. Treatments; 1, 2, 3, 4, 5 are 0 mg/l, 25 mg/l, 50 mg/l, 75 mg/l, 100 mg/l.

Effect=VARIETIES

Time day)	treatment	Glycine max	Mean of Fv/Fm	Std. Error of Fv/Fm	Mean of ΦPSII	Std. Error of ΦPSII	Mean of NPQ	Std. Error of NPQ
.	.	1	0.49667	0.027098	0.50276	0.031273	0.83389	0.12781
.	.	2	0.54336	0.027782	0.51622	0.030436	0.63280	0.10696
.	.	3	0.56458	0.027770	0.54993	0.027246	0.54322	0.09723
.	.	4	0.50969	0.029839	0.47829	0.027904	0.83604	0.19100

Effect=TIME IN DAY

Time day)	treatment	Glycine max	Mean of Fv/Fm	Std. Error of Fv/Fm	Mean of ΦPSII	Std. Error of ΦPSII	Mean of NPQ	Std. Error of NPQ
5	.	.	0.59843	0.017709	0.56632	0.023686	0.98510	0.15922
25	.	.	0.49722	0.024956	0.47412	0.026910	0.41435	0.07903
38	.	.	0.49007	0.027342	0.49497	0.024197	0.73502	0.08766

Effect=TREATMENT

Time (day)	treatment	Glycine max	Mean of Fv/Fm	Std. Error of Fv/Fm	Mean of ΦPSII	Std. Error of ΦPSII	Mean of NPQ	Std. Error of NPQ
.	1	.	0.55775	0.033246	0.54358	0.032789	0.65911	0.10301
.	2	.	0.53053	0.030095	0.50661	0.032876	0.84822	0.20773
.	3	.	0.48967	0.028508	0.47586	0.029705	0.59069	0.12700
.	4	.	0.52122	0.031406	0.49714	0.036850	0.84824	0.17873
.	5	.	0.54369	0.034572	0.53581	0.031510	0.61117	0.12028

Table 13; Means breakdown for plant pigment

Varieties; 1,2,3,4 are SB 97, SB 19, SB 20, SB 123 respectively. Treatments; 1, 2, 3, 4, 5 are 0 mg/l, 25 mg/l, 50 mg/l, 75mg/l, 100 mg/l.

Effect=VARIETIES

Time (day)	treatment	Glycine max	Mean of CHL a	Std. Error of CHL a	Mean of CHL b	Std. Error of CHL b	Mean of CHL a+b	Std. Error of CHL a+b
.	.	1	1.27764	0.08989	0.76673	0.042064	1.99471	0.10657
.	.	2	1.20649	0.11277	0.78716	0.069766	1.96716	0.15180
.	.	3	1.47949	0.13533	0.78276	0.053275	2.26224	0.15906
.	.	4	1.22862	0.12139	0.69731	0.067813	1.89524	0.15562

Time (day)	treatment	Glycine max	Mean of CHL a/b	Std. Error of CHL a/b	Mean of C_{x+C}	Std. Error of C_{x+C}
.	.	1	1.87689	0.20946	0.32640	0.025223
.	.	2	1.67831	0.16761	0.30482	0.027710
.	.	3	1.90400	0.19380	0.23287	0.016381
.	.	4	1.89960	0.22982	0.26196	0.036568

Table 13; Means breakdown for plant pigment ``Cont``

Effect=TIME IN DAY

Time (day)	treatment	Glycine max	Mean of CHL a	Std. Error of CHL a	Mean of CHL b	Std. Error of CHL b	Mean of CHL a+b	Std. Error of CHL a+b
5	.	.	2.02202	0.080138	0.64788	0.033692	2.66938	0.10263
25	.	.	0.93608	0.073646	0.85046	0.063744	1.66007	0.10338
38	.	.	0.93608	0.074426	0.77712	0.048816	1.76007	0.12735

Time (day)	treatment	Glycine max	Mean of CHL a/b	Std. Error of CHL a/b	Mean of C x+c	Std. Error of C x+c
5	.	.	3.32262	0.16066	0.32553	0.015129
25	.	.	1.12538	0.08218	0.26123	0.027746
38	.	.	1.07110	0.05245	0.25777	0.026508

Effect=TREATMENT

Time (day)	treatment	Glycine max	Mean of CHL a	Std. Error of CHL a	Mean of CHL b	Std. Error of CHL b	Mean of CHL a+b	Std. Error of CHL a+b
.	1	.	1.63500	0.15398	0.93117	0.083049	2.32969	0.17149
.	2	.	1.24561	0.11461	0.69994	0.039247	1.97453	0.17309
.	3	.	1.16719	0.12760	0.73264	0.063787	1.98192	0.13761
.	4	.	1.21490	0.12561	0.70769	0.071147	1.93694	0.17863
.	5	.	1.22759	0.11499	0.72100	0.060423	1.92611	0.14542

Time (day)	treatment	Glycine max	Mean of CHL a/b	Std. Error of CHL a/b	Mean of C x+c	Std. Error of C x+c
.	1	.	1.99908	0.21309	0.22800	0.019693
.	2	.	2.00175	0.24345	0.25072	0.029180
.	3	.	1.60822	0.22502	0.30750	0.032653
.	4	.	1.81697	0.24237	0.27547	0.025898
.	5	.	1.77247	0.19789	0.34586	0.041171

Appendix 3: Tukey`s Studentized Range (HSD) tests

1, 2, 3 and 4 are varieties SB 97, SB 19, SB 20 and SB 123 respectively.

1,2 3, 4 and 5 are treatments 0 mg/l, 25 mg/l, 50 mg/l, 75 mg/l, 100 mg/ll.

Table 14; HSD tests for varieties

Test for Number of leaves

Alpha	0.05
Error Degrees of Freedom	160
Error Mean Square	66.27083
Critical Value of Studentized Range	3.67165
Minimum Significant Difference	3.8588

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Varieties
A	64.350	60	2
A	63.667	60	3
A	61.417	60	4
B	49.583	60	1

Test for Shoot height

Alpha	0.05
Error Degrees of Freedom	160
Error Mean Square	22.01616
Critical Value of Studentized Range	3.67165
Minimum Significant Difference	2.2241

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Varieties
A	45.3867	60	4
B	42.2422	60	2
C	36.0933	60	3
C	33.8783	60	1

Test Stem diameter

Alpha	0.05
Error Degrees of Freedom	160
Error Mean Square	0.005496
Critical Value of Studentized Range	3.67165
Minimum Significant Difference	0.0351

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Varieties
A	0.55183	60	3
A	0.54285	60	4
B	0.48350	60	2
C	0.44450	60	1

Table 14; HSD tests for varieties ``cont``

Test for leaf area

Alpha	0.05
Error Degrees of Freedom	160
Error Mean Square	7.314156
Critical Value of Studentized Range	3.67165
Minimum Significant Difference	1.2819

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Varieties
A	15.7438	60	1
A	15.0524	60	3
A	14.7660	60	4
B	13.0581	60	2

Test for Dry weight

Alpha	0.05
Error Degrees of Freedom	120
Error Mean Square	25.46839
Critical Value of Studentized Range	3.68460
Minimum Significant Difference	2.7719

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Varieties
A	16.998	45	4
B	14.194	45	3
C	10.843	45	1
C	9.793	45	2

Test for Relative growth rate

Alpha	0.05
Error Degrees of Freedom	120
Error Mean Square	0.020115
Critical Value of Studentized Range	3.68460
Minimum Significant Difference	0.0779

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Varieties
A	0.51667	45	4
B	0.42236	45	3
B	0.38333	45	1
B	0.35933	45	2

Test for Root: Shoot ratio

Alpha	0.05
Error Degrees of Freedom	40
Error Mean Square	0.013345
Critical Value of Studentized Range	3.79069
Minimum Significant Difference	0.1131

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Varieties
A	0.16420	15	3
A	0.14293	15	2
A	0.07833	15	1
A	0.06687	15	4

Test for Al

Alpha	0.05
Error Degrees of Freedom	40
Error Mean Square	45.42917
Critical Value of Studentized Range	3.79069
Minimum Significant Difference	6.5969

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Varieties
A	19.280	15	3
A	17.820	15	2
A	17.433	15	4
A	17.333	15	1

Test for N

Alpha	0.05
Error Degrees of Freedom	40
Error Mean Square	366.579
Critical Value of Studentized Range	3.79069
Minimum Significant Difference	18.739

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Varieties
A	71.107	15	2
A	69.520	15	3
A	67.113	15	1
A	65.700	15	4

Table 14; HSD tests for varieties ``cont``

Test for P

Alpha	0.05
Error Degrees of Freedom	40
Error Mean Square	263.7197
Critical Value of Studentized Range	3.79069
Minimum Significant Difference	15.894

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Varieties
A	50.940	15	3
A	50.627	15	1
A	50.093	15	2
A	50.047	15	4

Test for K

Alpha	0.05
Error Degrees of Freedom	40
Error Mean Square	292.2812
Critical Value of Studentized Range	3.79069
Minimum Significant Difference	16.733

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Varieties
A	65.573	15	3
A	57.187	15	2
A	55.180	15	4
A	54.827	15	1

Test for Mg

Alpha	0.05
Error Degrees of Freedom	40
Error Mean Square	71.83333
Critical Value of Studentized Range	3.79069
Minimum Significant Difference	8.2954

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Varieties
A	34.447	15	3
A	28.940	15	2
A	28.267	15	1
A	28.047	15	4

Test for Ca

Alpha	0.05
Error Degrees of Freedom	40
Error Mean Square	235.3228
Critical Value of Studentized Range	3.79069
Minimum Significant Difference	15.014

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Varieties
A	63.093	15	3
A	60.140	15	2
A	60.013	15	1
A	59.853	15	4

Test for Fv/Fm

Alpha	0.05
Error Degrees of Freedom	120
Error Mean Square	0.041524
Critical Value of Studentized Range	3.68460
Minimum Significant Difference	0.1119

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Varieties
A	0.56458	45	3
A	0.54336	45	2
A	0.50969	45	4
A	0.49667	45	1

Test for ϕPSII

Alpha	0.05
Error Degrees of Freedom	120
Error Mean Square	0.04318
Critical Value of Studentized Range	3.68460
Minimum Significant Difference	0.1141

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Varieties
A	0.54993	45	3
A	0.51622	45	2
A	0.50276	45	1
A	0.47829	45	4

Table 14; HSD tests for varieties ``cont``

Test for NPQ

Alpha			0.05
Error Degrees of Freedom			120
Error Mean Square			0.762051
Critical Value of Studentized Range			3.68460
Minimum Significant Difference			0.4795

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Varieties
A	0.8360	45	4
A	0.8339	45	1
A	0.6328	45	2
A	0.5432	45	3

Test for Chl a

Alpha			0.05
Error Degrees of Freedom			120
Error Mean Square			0.326949
Critical Value of Studentized Range			3.68460
Minimum Significant Difference			0.3141

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Varieties
A	1.4795	45	3
A	1.2776	45	1
A	1.2286	45	4
A	1.2065	45	2

Test for chl b

Alpha			0.05
Error Degrees of Freedom			120
Error Mean Square			0.147505
Critical Value of Studentized Range			3.68460
Minimum Significant Difference			0.211

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Varieties
A	0.78716	45	2
A	0.78276	45	3
A	0.76673	45	1
A	0.69731	45	4

Test for Chl a+b

Alpha			0.05
Error Degrees of Freedom			120
Error Mean Square			0.716633
Critical Value of Studentized Range			3.68460
Minimum Significant Difference			0.465

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Varieties
A	2.2622	45	3
A	1.9947	45	1
A	1.9672	45	2
A	1.8952	45	4

Test for Chl a/b

Alpha			0.05
Error Degrees of Freedom			120
Error Mean Square			0.76558
Critical Value of Studentized Range			3.68460
Minimum Significant Difference			0.4806

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Varieties
A	1.9040	45	3
A	1.8996	45	4
A	1.8769	45	1
A	1.6783	45	2

Test for Carotenoids

Alpha			0.05
Error Degrees of Freedom			120
Error Mean Square			0.034273
Critical Value of Studentized Range			3.68460
Minimum Significant Difference			0.1017

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Varieties
A	0.32640	45	1
A	0.30482	45	2
A	0.26196	45	4
A	0.23287	45	3

Table 15; HSD tests for treatments

Test for Number of leaves

Alpha	0.05
Error Degrees of Freedom	160
Error Mean Square	66.27083
Critical Value of Studentized Range	3.90201
Minimum Significant Difference	4.5849

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Treatment
A	64.563	48	1
B	59.771	48	3
B	58.688	48	5
B	58.292	48	2
B	57.458	48	4

Test for Shoot height

Alpha	0.05
Error Degrees of Freedom	160
Error Mean Square	22.01616
Critical Value of Studentized Range	3.90201
Minimum Significant Difference	2.6426

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Treatment
A	40.9167	48	1
A	40.6292	48	5
A	38.6104	48	4
A	38.4506	48	2
A	38.3938	48	3

Test Stem diameter

Alpha	0.05
Error Degrees of Freedom	160
Error Mean Square	0.005496
Critical Value of Studentized Range	3.90201
Minimum Significant Difference	0.0418

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Treatment
A	0.55377	48	1
B	0.50354	48	4
B	0.49333	48	2
B	0.48958	48	5
B	0.48813	48	3

Test for leaf area

Alpha	0.05
Error Degrees of Freedom	160
Error Mean Square	7.314156
Critical Value of Studentized Range	3.90201
Minimum Significant Difference	1.5232

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Treatment
A	16.3345	48	1
B	14.4415	48	2
B	14.3677	48	5
B	14.3507	48	4
B	13.7811	48	3

Test for dry weight

Alpha	0.05
Error Degrees of Freedom	120
Error Mean Square	25.46839
Critical Value of Studentized Range	3.91694
Minimum Significant Difference	3.2946

Means with the same letter are not significantly different.

Tukey Grouping		Mean	N	Treatment
	A	15.508	36	1
B	A	13.024	36	5
B	A	12.731	36	4
B	A	12.265	36	2
B		11.257	36	3

Test for Relative growth rate

Alpha	0.05
Error Degrees of Freedom	120
Error Mean Square	0.020115
Critical Value of Studentized Range	3.91694
Minimum Significant Difference	0.0926

Means with the same letter are not significantly different.

Tukey Grouping		Mean	N	Treatment
	A	0.51667	36	1
B	A	0.43333	36	5
B		0.41611	36	4
B		0.39472	36	2
B		0.34128	36	3

Table 15; HSD tests for treatments ``cont``

Test for Root: Shoot ratio

Alpha	0.05
Error Degrees of Freedom	40
Error Mean Square	0.013345
Critical Value of Studentized Range	4.03913
Minimum Significant Difference	0.1347

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Treatment
A	0.14858	12	4
A	0.14075	12	3
A	0.11300	12	1
A	0.08550	12	5
A	0.07758	12	2

Test for Al

Alpha	0.05
Error Degrees of Freedom	40
Error Mean Square	45.42917
Critical Value of Studentized Range	4.03913
Minimum Significant Difference	7.8589

Means with the same letter are not significantly different.

Tukey Grouping		Mean	N	treatment
	A	22.875	12	4
	A	20.358	12	5
	A	19.858	12	3
B	A	17.258	12	2
B		9.483	12	1

Test for N

Alpha	0.05
Error Degrees of Freedom	40
Error Mean Square	366.579
Critical Value of Studentized Range	4.03913
Minimum Significant Difference	22.324

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Treatment
A	76.333	12	1
A	70.025	12	3
A	69.558	12	2
A	64.092	12	4
A	61.792	12	5

Test for P

Alpha	0.05
Error Degrees of Freedom	40
Error Mean Square	263.7197
Critical Value of Studentized Range	4.03913
Minimum Significant Difference	18.935

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	treatment
A	57.425	12	1
A	50.517	12	3
A	48.317	12	4
A	48.142	12	2
A	47.733	12	5

Test for K

Alpha	0.05
Error Degrees of Freedom	40
Error Mean Square	292.2812
Critical Value of Studentized Range	4.03913
Minimum Significant Difference	19.934

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	treatment
A	63.192	12	1
A	60.525	12	4
A	56.567	12	2
A	56.175	12	5
A	54.500	12	3

Test for Mg

Alpha	0.05
Error Degrees of Freedom	40
Error Mean Square	71.83333
Critical Value of Studentized Range	4.03913
Minimum Significant Difference	9.8823

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	treatment
A	31.808	12	4
A	31.525	12	1
A	31.492	12	5
A	28.692	12	2
A	26.108	12	3

Table 15; HSD tests for treatments ``cont``

Test for Ca

Alpha	0.05
Error Degrees of Freedom	40
Error Mean Square	235.3228
Critical Value of Studentized Range	4.03913
Minimum Significant Difference	17.887

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Treatment
A	67.850	12	1
A	62.933	12	2
A	62.442	12	4
A	58.867	12	5
A	51.783	12	3

Test for Fv/Fm

Alpha	0.05
Error Degrees of Freedom	120
Error Mean Square	0.041524
Critical Value of Studentized Range	3.91694
Minimum Significant Difference	0.133

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Treatment
A	0.55775	36	1
A	0.54369	36	5
A	0.53053	36	2
A	0.52122	36	4
A	0.48967	36	3

Test for ϕPSII

Alpha	0.05
Error Degrees of Freedom	120
Error Mean Square	0.04318
Critical Value of Studentized Range	3.91694
Minimum Significant Difference	0.1357

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Treatment
A	0.54358	36	1
A	0.53581	36	5
A	0.50661	36	2
A	0.49714	36	4
A	0.47586	36	3

Test for NPQ

Alpha	0.05
Error Degrees of Freedom	120
Error Mean Square	0.762051
Critical Value of Studentized Range	3.91694
Minimum Significant Difference	0.5699

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Treatment
A	0.8482	36	4
A	0.8482	36	2
A	0.6591	36	1
A	0.6112	36	5
A	0.5907	36	3

Test for Chl a

Alpha	0.05
Error Degrees of Freedom	120
Error Mean Square	0.326949
Critical Value of Studentized Range	3.91694
Minimum Significant Difference	0.3733

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Treatment
A	1.6350	36	1
B	1.2456	36	2
B	1.2276	36	5
B	1.2149	36	4
B	1.1672	36	3

Test for chl b

Alpha	0.05
Error Degrees of Freedom	120
Error Mean Square	0.147505
Critical Value of Studentized Range	3.91694
Minimum Significant Difference	0.2507

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Treatment
A	0.93117	36	1
A	0.73264	36	3
A	0.72100	36	5
A	0.70769	36	4
A	0.69994	36	2

Table 15; HSD tests for treatments ``cont``

Test for Chl a+b

Alpha	0.05
Error Degrees of Freedom	120
Error Mean Square	0.716633
Critical Value of Studentized Range	3.91694
Minimum Significant Difference	0.5526

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Treatment
A	2.3297	36	1
A	1.9819	36	3
A	1.9745	36	2
A	1.9369	36	4
A	1.9261	36	5

Test for Chl a/b

Alpha	0.05
Error Degrees of Freedom	120
Error Mean Square	0.76558
Critical Value of Studentized Range	3.91694
Minimum Significant Difference	0.5712

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Treatment
A	2.0018	36	2
A	1.9991	36	1
A	1.8170	36	4
A	1.7725	36	5
A	1.6082	36	3

Test for Carotenoids

Alpha	0.05
Error Degrees of Freedom	120
Error Mean Square	0.034273
Critical Value of Studentized Range	3.91694
Minimum Significant Difference	0.1209

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Treatment
A	0.34586	36	5
A	0.30750	36	3
A	0.27547	36	4
A	0.25072	36	2
A	0.22800	36	1

Table 16; HSD tests for DAT

Test for Number of leaves

Alpha	0.05
Error Degrees of Freedom	160
Error Mean Square	66.27083
Critical Value of Studentized Range	3.67165
Minimum Significant Difference	3.8588

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	DAT
A	98.917	60	56
B	90.050	60	43
C	37.317	60	16
D	12.733	60	0

Test for Shoot height

Alpha	0.05
Error Degrees of Freedom	160
Error Mean Square	22.01616
Critical Value of Studentized Range	3.67165
Minimum Significant Difference	2.2241

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	DAT
A	57.8750	60	56
B	53.0217	60	43
C	31.0417	60	16
D	15.6622	60	0

Table 16; HSD tests for DAT ``cont``

Test Stem diameter

Alpha			0.05
Error Degrees of Freedom			160
Error Mean Square			0.005496
Critical Value of Studentized Range			3.67165
Minimum Significant Difference			0.0351

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	DAT
A	0.60150	60	56
A	0.59150	60	43
B	0.49352	60	16
C	0.33617	60	0

Test for leaf area

Alpha			0.05
Error Degrees of Freedom			160
Error Mean Square			7.314156
Critical Value of Studentized Range			3.67165
Minimum Significant Difference			1.2819

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	DAT
A	15.9701	60	43
A	15.7572	60	16
A	15.6005	60	56
B	11.2926	60	0

Test for dry weight

Alpha			0.05
Error Degrees of Freedom			120
Error Mean Square			25.46839
Critical Value of Studentized Range			3.35618
Minimum Significant Difference			2.1866

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	DAT
A	31.6895	60	56
B	5.7300	60	16
C	1.4517	60	0

Test for relative growth rate

Alpha			0.05
Error Degrees of Freedom			120
Error Mean Square			0.020115
Critical Value of Studentized Range			3.35618
Minimum Significant Difference			0.0615

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	DAT
A	0.50727	60	16
A	0.46200	60	56
B	0.29200	60	0

Test for Fv/Fm

Alpha			0.05
Error Degrees of Freedom			120
Error Mean Square			0.041524
Critical Value of Studentized Range			3.35618
Minimum Significant Difference			0.0883

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	DAT
A	0.59843	60	5
B	0.49722	60	25
B	0.49007	60	38

Test for ϕPSII

Alpha			0.05
Error Degrees of Freedom			120
Error Mean Square			0.04318
Critical Value of Studentized Range			3.35618
Minimum Significant Difference			0.09

Means with the same letter are not significantly different.

Tukey Grouping		Mean	N	DAT
	A	0.56632	60	5
B	A	0.49497	60	38
B		0.47412	60	25

Table 16; HSD tests for DAT ``cont``

Test for NPQ

Alpha	0.05
Error Degrees of Freedom	120
Error Mean Square	0.762051
Critical Value of Studentized Range	3.35618
Minimum Significant Difference	0.3782

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	DAT
A	0.9851	60	5
B A	0.7350	60	38
B	0.4143	60	25

Test for Chl a

Alpha	0.05
Error Degrees of Freedom	120
Error Mean Square	0.326949
Critical Value of Studentized Range	3.35618
Minimum Significant Difference	0.2477

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	DAT
A	2.0220	60	5
B	0.9361	60	25
B	0.9361	60	38

Test for chl b

Alpha	0.05
Error Degrees of Freedom	120
Error Mean Square	0.147505
Critical Value of Studentized Range	3.35618
Minimum Significant Difference	0.1664

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	DAT
A	0.85046	60	25
B A	0.77712	60	38
B	0.64788	60	5

Test for Chl a+b

Alpha	0.05
Error Degrees of Freedom	120
Error Mean Square	0.716633
Critical Value of Studentized Range	3.35618
Minimum Significant Difference	0.3668

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	DAT
A	2.6694	60	5
B	1.7601	60	38
B	1.6601	60	25

Test for Chl a/b

Alpha	0.05
Error Degrees of Freedom	120
Error Mean Square	0.76558
Critical Value of Studentized Range	3.35618
Minimum Significant Difference	0.3791

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	DAT
A	3.3226	60	5
B	1.1254	60	25
B	1.0711	60	38

Test for Carotenoids

Alpha	0.05
Error Degrees of Freedom	120
Error Mean Square	0.034273
Critical Value of Studentized Range	3.35618
Minimum Significant Difference	0.0802

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	DAT
A	0.32553	60	5
A	0.26123	60	25
A	0.25777	60	38

Appendix 4: Plates

Plate. 1: SB 20 at 100mg/l $AlCl_3$ solution, yellow leaves (pointed) indicate photobleaching photosynthetic pigments.

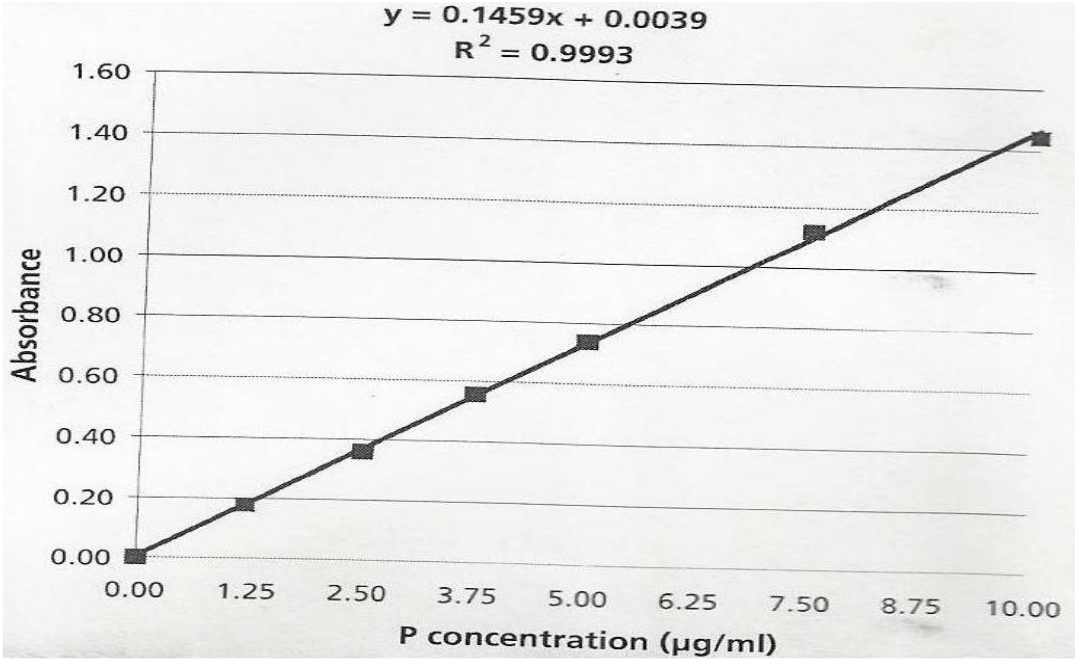
Plate. 2: Early maturing SB 19 (left) and late maturing SB 20 at 0 mg/l $AlCl_3$ solution

Plate 3: SB 20 (left) subjected to 25 mg/l $AlCl_3$ and SB 123 subjected to 100 mg/l $AlCl_3$ solution. The plant`s leaves pointed on are affected are affected

Plate 4: Shows SB 20 subjected to 50 mg/l $AlCl_3$ solution. Note the leaves affected in the circle pointed by an arrow.

Appendix 5. Sample of standard curves used

Standard curve for P



Standard curve for K