RESPONSES OF TEN SOYBEAN [*GLYCINE MAX* (L.) MERRILL] GENOTYPES FOR YIELD AND NODULATIONTO *TRICHODERMA* AND SILICON APPLICATIONS

SHIKSHA JADOO

RESPONSES OF TEN SOYBEAN [*GLYCINE MAX* (L.) MERRILL] GENOTYPES FOR YIELD AND NODULATIONTO *TRICHODERMA* AND SILICON APPLICATIONS

by

Shiksha Jadoo

Submitted in fulfilment of the requirements for the degree

M.Sc. Plant Breeding

School of Agriculture, Earth and Environmental Sciences

College of Agriculture, Engineering and Science

University of KwaZulu-Natal,

Pietermaritzburg,

South Africa

Supervisor: Prof H. Shimelis

Co-supervisor: Prof M. D. Laing

July 2012

ABSTRACT

A study was conducted to determine the responses of 10 selected soybean (*Glycine max* L.) genotypes to potassium silicate (KSi) and *Trichoderma harzianum* (Eco-T®) applications. Preliminary studies involving two independent experiments were conducted under controlled conditions at the University of KwaZulu-Natal during 2010. Potassium silicate at three concentrations (0, 200 and 250ppm) were applied twice weekly over a period of four months to the genotypes laid out in a randomized complete block design. Subsequently, a field experiment was conducted at Ukulinga Research Farm of the University of KwaZulu-Natal, Pietermaritzburg during 2010/2011 to investigate the responses of the genotypes to KSi at 0 and 200ppm, with and without (Eco-T®) seed treatment. This experiment was set out in a randomized complete block design with three replications. Data collected included number of days to 50% flowering, number of days to 50% maturity, plant height, number of pods per plant, number of seeds per pod, 100 seed weight, root mass, shoot mass, seed yield and harvest index. The total number of root nodules formed and the number of active nodules were determined at end of the field experiment. In most cases a decrease was noted in total nodule formation as well as a decrease in the number of active nodules that formed. In the controlled environments there was a significant interaction between genotype and KSi concentrations for all measured traits. In most cases KSi applied at 200ppm was more successful in enhancing growth, improving seed yield and resulted in high harvest indices. The genotypes that produced the highest seed yield and harvest index in these environments were Williams and Barc-2 at 200ppm KSi. Results from correlation analysis revealed that harvest index and seed yields were generally positively associated with plant height, number of pods per plant and 100 seed weight, which in turn were the traits that contributed to most of the variation to seed yield and harvest index as revealed in the principle component analysis (PCA). The field experiment revealed a significant interaction between genotype x KSi x Eco-T®. Potassium silicate applied at 200ppm with Eco-T® usually promoted growth, seed yield and high harvest indices for all the genotypes. The PCA showed seed yield and harvest index were the traits that contributed to most of the variation. Genotypes Williams, LS6161R, Magoye and Barc-2 were the best seed yielders with the highest harvest

indices that responded strongly to the combined use of KSi and Eco-T® under field conditions. Genetic comparison of the ten soybean genotypes with eight microsatellite markers revealed the close genetic relationship between Williams, LS6161 R and Magoye. A link between Barc-2 and Williams was noted by the common parent Clark. Therefore, for these genotypes, the application of KSi at 200 ppm with Eco-T® under field conditions effectively increased seed yield, ranging from 0.45 to 65.26% for some genotypes when compared to the control. An increase was also noted for other agronomic traits and harvest index.

DECLARATION

I Shiksha Jadoo declare that

(i) The research reported in this dissertation, except where otherwise indicated, is my original work.

(ii) This dissertation has not been submitted for any degree or examination at any other university.

(iii) This dissertation does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.

(iv) This dissertation does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:

a) their words have been re-written but the general information attributed to them has been referenced;

b) where their exact words have been used, their writing has been placed inside quotation marks, and referenced.

(v) Where I have reproduced a publication of which I am an author, co-author or editor, I have indicated in detail which part of the publication was actually written by myself alone and have fully referenced such publications.

(vi) This dissertation does not contain text, graphics or tables copied and pasted from the internet, unless specifically acknowledged, and the source being detailed in the dissertation and in the References sections.

Signed:…….. Date……

Shiksha Jadoo (Student)

Signed:…….. Date……

Prof. Hussein A. Shimelis (Principal supervisor)

Signed:…….. Date……

Prof. Mark D. Laing (Co-supervisor)

ACKNOWLEDGEMENTS

I gratefully acknowledge:

My supervisor Prof H. Shimelis for all his guidance, support and advice, directing the progress and development of my dissertation as well as the design of my research experiments.

My co-supervisor Prof M.D Laing for all his contributions of the Potassium Silicate and *Trichoderma harzianum* (Eco-T®) and editing the dissertation.

Mrs S. van der Merwe, the Greenhouse, Research and Maintenance Technician at the University of KwaZulu-Natal, for all her support, direction and help.

Dr. K.S. Yobo and Miss D. Visser for all their assistance in the preparation of my trials.

All the technical and administrative staff in the African Center for Crop Improvement for their time, assistance and advice, whenever needed.

To the National Research Foundation for financial support that made my research possible.

Link Seeds for the supply of soybean seeds and Cedara Research Institute for conducting my soil tests.

To INCOTEC®, South Africa for the SSR analytical services provided during the genetic diversity study and varietal comparison.

To my friends Ameesha Hanuman, Marylyn Christian and Nokulunga Mzimela, Nokwazi Mbili, Vince Ndou, Sandile Hadeba, Fernando Pienaar and Yasheena Chonoolal, for their support.

To my fiancé Uveer Goordheen for all his help, support and advice.

I would like to give special thanks to my parents, Mr Arvind Jadoo and Mrs Urvashni Jadoo, as well as my grandparents, sisters, uncles, aunts and cousins for their financial and emotional support.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

GENERAL INTRODUCTION

Research background

Soybean [*Glycine max* (L.) Merrill] is a legume crop that grows in tropical, subtropical, and temperate climates. Cultivation of soybean dates back thousands of years in areas of East Asia, mainly China. Soybean is an annual crop providing human food and animal feed in the livestock and poultry industries (Crawford, 2006).

Soybeans, like most legume crops, have the ability to produce and provide most of the nitrogen requirements of the plant through a symbiotic relationship with a nitrogen fixing bacterium via nitrogen fixation (McNeil, 2010). A studies conducted by Herridge *et al.* (2008) and Salvagiotti*et al.* (2008) indicated that the percentage of nitrogen fixed by soybean ranged from 50-60% of the total nitrogen that is fixed globally.

Soybeans are relatively a cheap and rich source of plant-based protein. Among food legumes soybean has the highest protein content of 38% and an oil content of 18%. Approximately 95% of the oil produced from soybean is used for human consumption while the rest is used in the manufacturing of commercial products such as cosmetic and sanitation supplies. The low gross margin production cost favors the production of soybean over other crops such as maize (Liu, 2008). Recently soybean has also been used as renewable raw material for diesel oil, which is more environmentally friendly than fossil fuels (Ganduglia, 2009).

Soybean was consumed in the continental Asia for centuries, but over the years it has fast found a place in the global production system. The United States is the top producer of soybean followed by Brazil, Argentina and China. The total soybean production in the world for 2010 was estimated at 258.4 million tons (Soystats, 2011) than reported in 2006 at 221.5 million tons (Baohui *et al*., 2007).

South Africa is fast becoming one of the major soybean producing countries. In 1979 South Africa produced 32000 tons, but by 1990 the country had 120 000 tons of produce (Duxburg *et al*., 1990). In 2009 soybean production increased in South Africa

by approximate ranges of 30,000 tons – 150 080 tons. The major soybean production areas in South Africa are Mpumalanga, the Free State and KwaZulu-Natal where 239 250, 147 250 and 73 250 tons were produced respectively for the period 2009/2010 which collectively represented 82% of the total soybean production. Some 10% of the domestically grown soybeans are used for the production of animal feed as soybean meal (NAMC, 2011). In comparison about 7% of the average soybeans produced are used for human consumption during the past six years (NAMC, 2011).

Soybean was introduced to some parts of Africa such as Egypt, Zimbabwe and Rwanda during the 1920s. By the1960s a gradual interest developed for soybean production in the continental Africa (Chianu *et al*., 2008).Soybean improvements in Africa started in 1974by the International Institute for Tropical Agriculture (IITA) owing to its nutritional, economic as well as agricultural benefits. However various production constraints limited the potential growth and development of the soybean industry in Africa (Tefera, 2011).

Production and productivity of soybean are inhibited by biotic factors (lack of improved varieties, diseases and pests) and abiotic factors (low soil fertility, soil degradation, and drought). Development of improved soybean genotypes with better tolerance to abiotic and biotic stresses is one of the strategies to enhance soybean yield and improve economic return (Hartman *et al*., 2011). The development and use of locally adapted varieties are important to increase yield (Panthee, 2010). Soybeans growth and flowering patterns are dependent on the photoperiod of an environment (Zhang *et al*., 2001). Thus, breeding for maturity period is an important trait. Cultivars that mature best during either long or short photoperiods are most preferred. Cultivars with a short photoperiod requirement may not flower when exposed to long photoperiods but may continue to grow in a vegetative state (Orf, 2008). Developments of soybean varieties are also based on the level of biological nitrogen fixation by the symbiotic relationship between the nitrogen fixing bacteria (*Rhizobium*) and the variety, resulting in the formation of nodules on plant roots. Varieties that are able to fix atmospheric nitrogen with the indigenous *Rhizobium* are termed as promiscuous while other varieties require specific *Rhizobium* strains (Kueneman *et al*., 1984).

Candidate soybean genotypes should be tested for their yield, adaptability and comparative yield stability in the target environments to make specific recommendations based on the goal of farmers. However the development of soybeans through inbreeding, selective breeding and domestication has led to a decrease in genetic diversity (Carter *et al*., 2004). This loss in genetic diversity can result in genotypes becoming more susceptible to adverse growth conditions, pests and pathogens and may show reduced adaptability in a new environment which can lead to decreased soybean productivity. The uses of SSR (simple sequence repeat) DNA markers or microsatellites have shown to be a success on various crops to determine genetic variation (Varshney *et al*., 2005). With this tool breeders will be able to identify the presence of genetic variation in a range of genetic material including in wild, landrace and cultivated soybean types. Research conducted by Wang *et al.* (2010) using 40 SSR markers on 40 soybean genotypes showed greater allele diversity in wild and landrace types than the cultivated genotypes which increases the possibilities for conservation of soybean genetic resources. This in turn can aid in the improvement of soybean production and yield though designed breeding.

Intensive farming practices using agro-chemicals is being practiced widely to meet the increasingly growing food demand. This resulted in increased crop production but also resulted in increased environmental pollution with negative impacts on both humans and animals (Rasul and Thapa, 2004). Also farmers received a smaller financial return from the sale of their produce due to the rising costs of agro-chemicals. The growing environmental concern, as well as the financial constraints, has led to the development of naturally occurring microorganisms as biological agents in crop production (Javaid, 2010).

Biological control of pests and plant pathogens is a fast growing technology that provides as potent substitute to synthetic chemicals because of the apparent endless resource supply (Howarth, 1991). This practice can involve the use of a mixture of naturally occurring beneficial microorganisms to bring about microbial diversity in the soil and plant, which resulted in increased soil quality and an increase in plant health and yield (Higa and Parr, 1994).

The advantage of developing a biological control agent, to suppress biotic and abiotic stresses as well as to promote plant growth, is considered to be more sustainable and environmentally friendly approach (Benhamou and Chet, 1993). The developments of bio-fertilizers are also on the rise in an effort to be eco-friendly. Bio-fertilizers are self sustaining and may cost less (Singh *et al*., 2011).

Trichoderma is a fungus that is abundantly present in most soils which is able to grow rapidly and colonize the soil. This fungus can act in different ways to protect the plant from the pathogens and to promote plant growth (Kleifeld and Chet, 1992). The species, *T. harzianum* has proved to be very effective in the biological control of many fungal plant pathogens and in plant growth promotion (Benhamou and Chet, 1993).

Silicon (Si) is an element that belongs to Group IV in the periodic table and is commonly found in the crust of the earth as silicate polymers. This element which is the second most abundant makes up 27% of the earth's crust and can be found as silicon dioxide in the form of quartz, opal, amorphous silica and as complex silicate rocks, sand and clays. Many but not all plants and grasses can take up Si by the roots. In some species it is transported to the foliar parts (van Soest, 1994). In the soil Si is the second most abundant element, after oxygen, in form of silicon dioxide that makes up 50-70% of the soil mass (Ma and Yamaji, 2006).

The beneficial attributes of Si to plants include: 1) it accumulates at the points of infection by pathogens (Blaich and Grundhöfer, 1998) thus acting as a physical barrier , and 2) Si, dissolves in the cytoplasm as silicic acid and has an impact on improved physiology, providing abiotic stress tolerance, and priming for resistance to biotic stresses (Chérif, 1994).

Research conducted by Woolley (1957) and Miyake and Takahashi (1978) on various crops showed that silicon is essential for plant growth and in the absence of silicon the plant growth is negatively affected. Current research on crops such as sugarcane (Savant *et al*., 1999), rice (Hossain, 2002) and cowpea (Mali and Aery, 2008) showed that in the presence of silicon these crops showed significant improvement in growth and productivity.

Research objective

The objective of this study was to determine agronomic performance, yield and nodulation patterns of 10 selected soybean genotypes as affected by the application of silicon (potassium silicate) and/or *Trichoderma harzianum* under controlled environment and field conditions. Further genetic diversity analysis was conducted using simple sequence repeats (SSR) genetic markers to discern the genetic relation of the 10 genotypes. Results of the study may assist in determining the optimum application levels of silicon and *T. harzianum* in soybean production to enhance yield and agronomic traits.

LITERATURE CITED

BAOHUI, S., MARCHANT, M.A., REED, M.R., and XU, S. 2007. Market Power and Competitive Analysis of China's Soybean Import Market. Contributed Paper Presentation for the International Agricultural Trade Research Consortium (IATRC) July 8-9th, 2007, Beijing, China. Pp 2. (http://www-agecon.ag.ohio state.ed.). Accessed: 24 February 2011.

BENHAMOU, N., and CHET, I. 1993. Hyphal interactions between *Trichoderma harzianum* and *Rhizoctonia solani*: Ultrastructure and gold cytochemistry of the mycoparasitic process. Phytopathology 83:1062-1071.

BLAICH, R. and GRUNDHÖFER, H. 1998. Silicate incrusts induced by powdery mildew in cell walls of different plant species. Journal of Plant Disease Protection 105: 114–120.

CARTER, T.E., NELSON, R., SNELLER, C.H., CUI, Z. 2004. In Soybeans: Improvement, Production, and Uses, (Eds) H.R Boerma, H.R. and J.E.Specht, American Society of Agronomy. Madison, Wisconsin. 16, pp 303–416.

CHERIF, M., ASSELIN, A. and BÉLANGER, R.R. 1994. Dense response induced by soluble silicon in cucumber roots infected by *Pythium spp*. Phytopathlogy 84: 236-242.

CHIANU, J.N., VANLAUWE, B., MYAKA, F., KATUNGI, E., AKECH, C., and SANGINGA, N. 2008.Soybean situation and outlook analysis: the case of Tanzania.(http://www.icrisat.org/). Accessed: 5 July 2012

CRAWFORD, G.W. 2006. *East Asian Plant Domestication*. In: M. Stark (Ed). Archaeology of East Asia. Blackwell Publications, Oxford, UK, Pp 77-95.

DUXBURG, M.R., BIRCH, E.B., and PARSONS, M.J. 1990. *Soybeans in Natal. Agricultural production guidelines for Natal*. Department of Agriculture and Development. Natal, RSA.

GANDUGLIA, F. 2009. *Handbook on Biofuels*. ARPEL, Inter-American Institute for Cooperation on Agriculture, Pp 1-30.

HARTMAN, G.L., WEST, D.W. and HERMAN, T.K. 2011. Crops that feed the World 2. Soybean-worldwide production, use, and constraints caused by pathogens and pests. Food Security 3: 5-7.

HERRIDGE, D.F., PEOPLES, M.B., BODDEY, R.M. 2008. Global inputs of biological nitrogen fixation in agricultural systems. Plant Soil 311: 1- 8.

HIGA, T. and PARR, J.F. 1994. Beneficial and effective microorganisms for a sustainable agriculture and environment. International Nature Farming Research Center. Atami, Japan. Pp 2-4.

HOSSAIN, M.T., MORI, R., SOGA, K., WAKABAYASHI, K., KAMISAKA, S., FUJII, S.,YAMAMOTO, R. and HOSON, T. 2002. Growth promotion and an increase in cell wall extensibility by silicon in rice and some other Poaceae seedlings. Journal of Plant Research 115: 23–27.

HOWARTH, F.G. 1991. Environmental impacts of classical biological control. Annual Revision. Journal of Entomology 36: 485–509.

JAVAID, A. 2010. Beneficial microorganisms for sustainable agriculture. Genetic engineering, bio-fertilisation, soil quality and organic farming, sustainable agriculture reviews 4:347-369.

KLEIFELD, O., and CHET, I. 1992. *Trichoderma harzianum* – interactions with plants and effect on growth response. Plant and Soil 144: 267–272.

KUENEMAN, E. A., ROOT, W. R., DASHIELL, K. E. and HOHENBERG, J. 1984. Breeding soybean for the tropics capable of nodulating effectively with indigenous *Rhizobium spp.* Plant and Soil 82: 387-396.

LIU, K. 2008. Food use of whole soybeans. In L., Johnson, P. J., White and R., Galloway (Eds.), *Soybeans: Chemistry, Production, Processing, and Utilization*. AOCS Press, Urbana, USA. Pp. 441–482.

MA, J.F., and YAMAJI, N., 2006. Silicon uptake and accumulation in higher plants. Trends in plant sciences. Journal of Plant Science 11: 392-397.

MALI, M., and AERY, N.C. 2008. Silicon effect on nodule growth, dry-matter production, and mineral nutrition of cowpea (*Vigna unguiculata*). Journal of Plant Nutrition Soil Science 171: 835-840.

McNEIL, D. L. 2010. In: *The Soybean*, Chapter 11: *Biological Nitrogen Fixation in Soybean*. (Ed) G. Singh. CAB International. Wallingford. Oxfordshire. Pp 227-228.

MIYAKE, Y., and TAKAHASHI, E. 1978. Silicon deficiency of tomato plant. Soil Science and Plant Nutrition 24: 175-189.

NAMC, 2011. The South African Soybean Value Chain. The Markets and Economic Research Centre of the National Agricultural Marketing Council. (http://www.namc.co.za/). Accessed: 25 June 2012.

ORF, J. H. 2008. Breeding, genetics, and production of soybean. In L. Johnson, P. J. White, and R. Galloway (Eds.), Soybeans: Chemistry, Production, Processing, and Utilization. AOCS Press, Urbana. USA. Pp. 33–66.

PANTHEE, D. 2010. *The soybean,* Chapter 5*:Varietal improvement in soybean*. In G. Singh (Ed.), CAB International. Wallingford.Pp. 92–112.

RASUL, G and THAPA, G.B. 2004. Sustainability of ecological and conventional agricultural systems in Bangladesh: an assessment based on environmental, economic and social perspectives. Elsevier Publications. Agricultural systems 79: 327-351.

SALVAGIOTTI, F., CASSMAN, K. G., SPECHT, J.E., WALTERS, T.D. and WEISS, A. 2008. Nitrogen uptake, fixation and response to fertilizer N in soybeans: A review. Agronomy Faculty Publications. Paper 133. (http://digitalcommons.unl.edu/agronomyfacpub/). Accessed: 19 June 2010.

SAVANT, N.K., KORNDORFER, G.H., DATNOFF, L.E., and SNYDER, G.H. 1999. Silicon nutrition and sugarcane production: A Review. Journal of Plant Nutrition 22:1853-1903.

SINGH, J.S., PANDEY, V.C. and SINGH, D.P. 2011. Efficient soil microorganisms: A new dimension for sustainable agriculture and environmental development. Elsevier Publications. Agriculture, Ecosystems and Environment 140: 339-353.

SOYSTATS. 2011. Soystats 2011. The American Soybean Association.

(http://www.soystats.com). Accessed: 12 May 2012.

TEFERA, H. 2011. Soybean - Molecular Aspects of Breeding, Chapter 7:*Breeding for promiscuous soybeans at IITA*. International Institute of Tropical Agriculture (IITA), Chitedze Agricultural Research. Pp 147-162.

van SOEST, P.J. 1994. *Nutritional Ecology of the Ruminant*. (2nd Ed). Cornell University Press, Ithaca, New York, USA, Pp 476.

VARSHNEY, R.K, GRANER, A. and SORRELLS M.E. 2005. Genic microsatellite markers in plants: features and applications. Trends in Biotechnology 23:48–55.

WANG, M. LI, R., YANG, W. and DU, W. 2010. Assessing the genetic diversity of cultivars and wild soybeans using SSR markers. African Journal of Biotechnology 9: 4857-4866.

WOOLLEY, J.T. 1957. Sodium and silicon as nutrients for the tomato plant. Plant Physiology 32: 317-32.

ZHANG, L., WANG, R., and HESKETH, J. D. 2001. Effects of photoperiod on growth and development of soybean floral bud in different maturity. Agronomy Journal 93: 944–948.

CHAPTER ONE

1 LITERATURE REVIEW

1.1 INTRODUCTION

Soybeans [*Glycine max* (L.) Merrill] are native to East Asia. However, the precise origin of the cultivated form of soybean is unknown. Nagata (1960) proposed that although the exact origin of soybeans may be unclear, they probably originated from the north and central parts of China. The author was led to this conclusion by the presence of the species *Glycine ussuriensis* in this region, which is the probable ancestor of *G. max* because other wild types were shown to be the unlikely ancestors (Norman, 1963). China was the leading soybean producer in 1954. However, over the past 50 years soybeans have become an important agricultural commodity and presently the world leaders in soybean production are the United States, Brazil, Argentina and China. This change in top soybean producers since 1954 is due to the growing global demand for soybeans for various uses (Liu, 1997).

Soybean production in South Africa has been very successful. Although the country is not a top producer of soybean, production has increased over the years. This is because soybeans can be grown both under irrigation, usually in the drier seasons, and under rain fed conditions in summer. Soybeans have become popular as a low cost high protein food source for rural populations. This is due to the high protein content in soy meal that is used for both human and animal consumption (Duxburg *et al*., 1990).

However, there are various factors that have negative impacts on soybean production. Some of the challenges faced by growers include biotic (diseases, pests and weeds) and abiotic (weather and soil) factors as well as low yield potential of existing soybean varieties (Strange and Scott, 2005). Losses from these factors can be combated through integrated soybean research. A suitable biological agent could be used to diminish the amount of disease occurrences such as fungi and the application of silicon can act against both biotic and abiotic factors as well as a promote growth. Therefore the purpose of this research was to determine the effect of silicon, and *T. harzianum*

applications on 10 soybean genotypes and to assess the effect on nodulation and yield in the KwaZulu-Natal (Pietermaritzburg) area.

1.2 SOYBEAN

1.2.1 Economic importance of soybean

Soybean is a cheap and excellent source of protein. The bean is made up of 45% protein and 20% oil. The nutritional value of soybean meal is very high and is a value product for livestock and poultry farmers. Because of this high protein content, soybean feeds are sometime favored over conventional feeds. Various food products can be produced from soybean. These products can be used as alternatives to conventional sources of food products such as milk, yoghurt, cheese and burger patties. It was found that these soybean products can help in the prevention of diseases such as various types of cancer, heart disease and osteoporosis (de Kleijn *et al*., 2002). However, whole unprocessed soybeans must be heat treated to destroy the Kunitz protein, which is a trypsin inhibitor for humans and all monogastric animals, before consumption. Ruminants older than five months may eat the untreated soybeans without unfavorable consequence (Duxburg *et al*., 1990).

In low nitrogen soils, soybeans form root nodules that bring about nitrogen fixation and thus decreases the amount of nitrogen fertilizers that are required. In order to decrease the amount of chemicals used in the fertilization of crops including soybean it is essential to use more organic fertilizers (Chouichom and Yamao, 2011). Research conducted in Brazil on the cost involved in soybean production reveals a loss. This is because the amount of produce by farmers is not recovered financially and therefore at present soybean export can be less profitable than expected. This is because soybean products are sold at lower prices than which costs to produce raw soybean and soybean meal (Cavalett and Ortega, 2009).

1.2.2 Taxonomy

Glycine max (L.) Merrill is a cultivated legume that belongs to the kingdom *Plantae*, family *Leguminosae*, the subfamily *Papilionoideae*, the genus *Glycine* (Caldwell, 1973).

1.2.3 Morphology

The soybean plant is an erect annual plant that is usually has lush dense green leaves and covered with short fine hairs. The height of the plants can range for about 0.3 to 3 meters. The first leaves are simple and grow opposite each other on the stem while the leaves that form subsequently are trifoliate, i.e., the leaf consists of three leaflets, and alternate up the stem (Figure 1.1) (Kumudini, 2010)

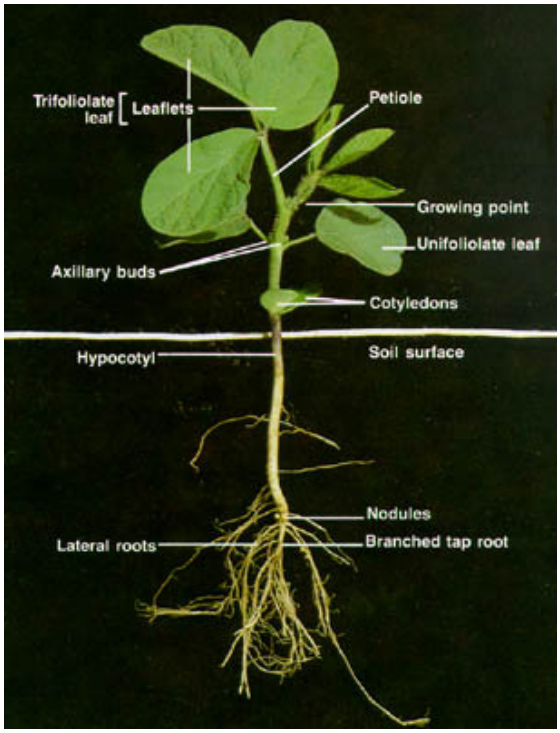

Figure 1.1 The soybean plant showing the auxiliary buds, trifoliolate leaves, cotyledons, lateral roots and branched tap roots (Skow, 1991).

The flowers and lateral branches form at the auxiliary buds at the point of contact between the leaf petiole and the main stem. Soybean flowers are small and consist of five separate; unequal petals that can vary in colour but are usually purple or white

(Figure 1.2). However, the morphology is diverse depending on the cultivar (Johnson and Bernard, 1963).

Figure 1.2 Photo of a soybean flower growing from the auxiliary bud taken at Ukulinga during field trials

The soybean seed can have a variety of colours including yellow, green, black and brown. The yellow and green seeds are more often seen with varying patters of black or brown. The embryo of the seed is covered by a seed coat. The seed is made up of two parts, the seed coat which covers the embryo and two cotyledons which form part of the embryo region. The seed coat is important because it protects the embryo. The bean is attached to the pod at the hilum, (hil) which is better known as the seed scar. On either end of the hilum is the hypocotyls-axis (hyp) and the raphe (raph) and the micropyle (mic) is located above the hypocotyls-axis (Kumudini, 2010) (Figure 1.3).

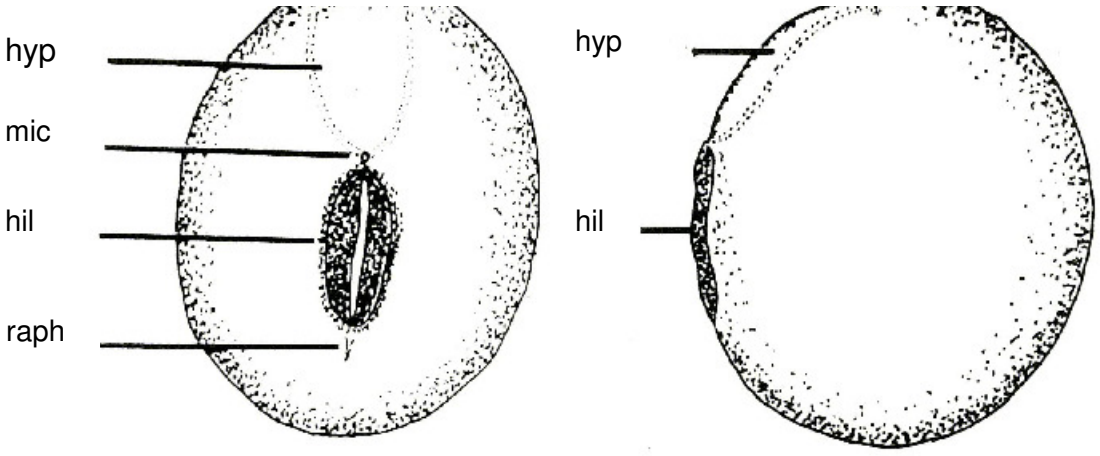

Figure 1.3 Soybean seed showing the hypocotyl-axis (hyp), micropyle (mic), hilum (hil) with a central fissure and the raphe (raph) (Caldwell, 1973).

Three layers can be seen in the seed coat, i.e., the epidermis, hypodermis and the inner parenchyma layer (Figure 1.4). The cells are elongated with their long axis perpendicular to the surface of the seed. The walls are thickened with pits (Kumudini, 2010).

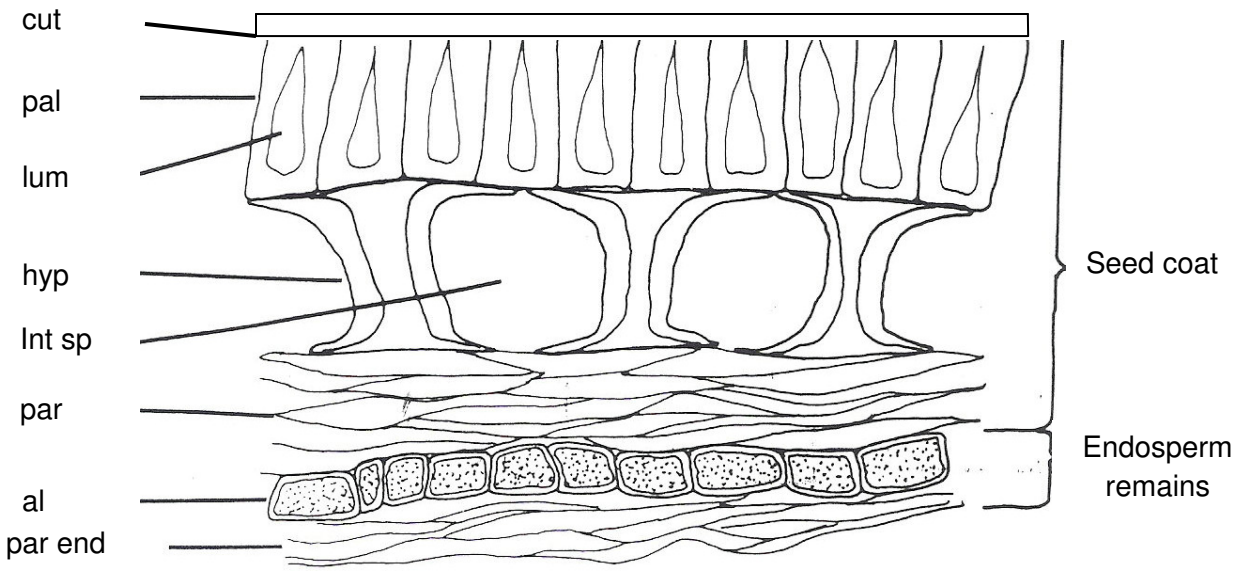

Figure 1.4 The seed coat of a soybean showing the three layers, epidermis, hypodermis and the inner parenchyma layer. Al – aleurene cells of endosperm, cut – cuticle, hyp – hourglass cells of hypodermis, int. sp – intercellular space, lum – lumen, pal – palisade, par – compressed parenchyma cells, par end – remains of parenchyma cells of endosperm (Caldwell, 1973).

The pods of the soybean plant form in clusters of 1-9and reach about 20-70mm long, depending on the growing conditions and the cultivar. Young pods are green in colour and covered in fine transparent hairs (Figure 1.5a). Mature pods are also hairy and range in colours such as brown or tan, black, yellow and grey (Figure 1.5b). This colour change happens as the plant leaves turn yellow and fall off the plant. The pods may be straight but in some instances they are slightly curved. They are made up of two halves that enclose the seeds. The pods may contain 1-4 seeds depending on the cultivar. Pod development is a crucial point in the development of the seeds and sufficient water and nutrients are necessary. The pods reach their full size before the seeds are completely developed (Duxburg *et al*., 1990).

(a) (b)

Figure 1.5 (a) Photo of young green soybean pods, and (b) Photo of mature brown soybean pods taken at UKZN during pot trials.

The root system of a soybean consists of a taproot, which can grow up to 1.2 meters into the soil. The taproot is formed by the radical. In addition; there are a large number of secondary roots that are arranged in four rows along the taproot, together with several orders of branch roots that come from the taproot, as well as many branched adventitious roots that arise from the top 200 mm of the tap root. Most of the effective roots are found in the top 600 mm of soil because the soybean plant is a shallow feeder (Kumudini, 2010).

Nodules formed on the roots of the soybean plant are caused by the interaction of a specific nitrogen-fixing bacterium (*Bradyrhizobium japonicum*) that may be present in the soil or inoculated on the seed. Nodules can be seen after about 10 days after planting and the root system is highly nodulated at maturity. The active nodules are about 3 to 6 mm in size and can provide for the necessary nitrogen requirements of the plant 14 to 21 days after planting (Figure 1.6). However, many nodules may form that do not fix nitrogen (Duxburg *et al*., 1990).

Figure 1.6. Photo of soybean roots showing root nodules taken four weeks after planting at Ukulinga during field trial.

1.2.4 Nodulation

1.2.4.1 Nitrogen

About 80% of the earth's atmosphere is nitrogen gas (N_2) present in an inert or stable state. With a strong stable triple bond between the two nitrogen atoms. Plants require nitrogen for healthy growth. Although there is an abundance of nitrogen available in the atmosphere, this nitrogen is not in an accessible form for plants.

Plants receive most of their nutrients from the soil. These nutrients that are present or put into the soil are usually soluble in water and are then easily absorbed by the roots of the plant and used to manufacture proteins, fats and carbohydrates. Dinitrogen present

in the atmosphere is unable to dissolve in water and therefore needs to be fixed or changed to a soluble form (Tikhonovich *et al.*, 1995).

1.2.4.2 Nitrogen fixation by bacteria

A variety of prokaryotic organisms are able to fix free atmospheric nitrogen into an accessible form. However, eukaryotes lack this ability. Nitrogen fixation is an enzymatic reaction that involves the reduction of dinitrogen from the atmosphere into ammonia, (Sathish Kumar and Bhaskara Rao, 2012).

Soybean plants form a symbiotic relationship with the bacterium *B. japonicum* (Figure 1.7) in order to fix dinitrogen into ammonia which is soluble in water and easily taken up by the plant. In South Africa the natural soil population of this particular bacterium is very low and often inoculation of the bacterium is required (Duxburg *et al*., 1990). *B. japonicum* has the enzyme system, nitrogenase, which provides the biochemical machinery for nitrogen fixation and has the ability to induce nodule formation on the roots of specific leguminous plants (Tikhonovich *et al.*, 1995).

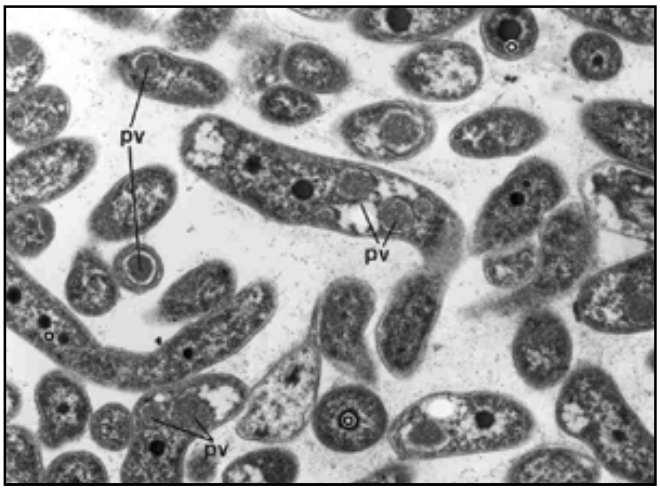

Figure 1.7 View of *Bradyrhizobium* spp. under microscope (Lorquin *et al*., 1997)

1.2.4.3 Formation of root nodules

Nodulation occurs in the roots when the plant secrets a stimulant, usually sugars and amino acids, to attract the nitrogen-fixing bacteria already present in the soil to form nodules on the root hairs. The plant then produces flavonoids and these flavanoids in turn induce the expression of *nod* genes in the bacterium. There are many genes that are involved in nodulation. The main *nod* gene that is involved in nodulation in the bacterium is the *nodD* gene. This gene is subdivided into nodD1 and nodD2. It was found that nodD1 is more important as nodulation cannot take place without it (Figure 1.8). Nod factors are produced from the *nod* genes and these Nod factors then react with the plant and cause the root hairs to curl (Tikhonovich, 1995).

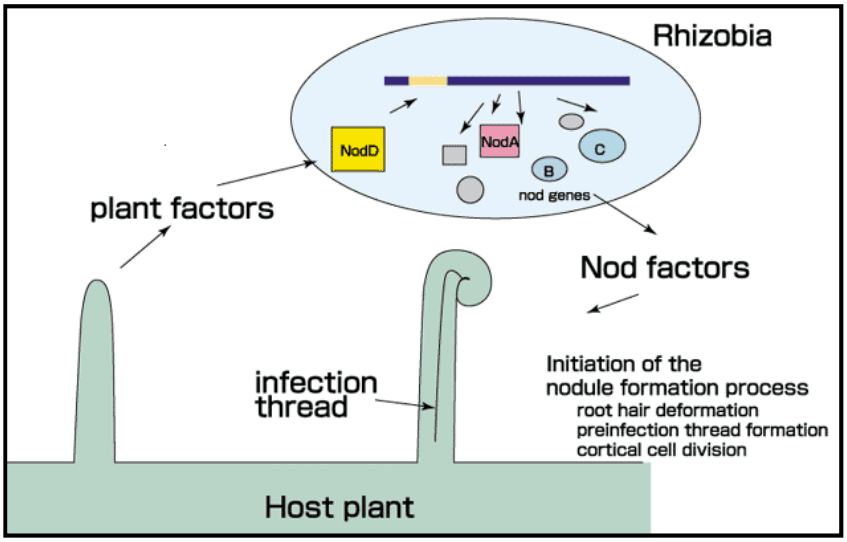

Figure 1.8 Diagram showing the process of root nodule formation and the secretion of flavonoids in the soil that induces the expression of *nod* genes in bacterium and the activation of Nod factors (Long, 1996).

During the curling of the root hair the bacterium attaches itself to the root hair and is taken up (Figure 1.9). Primordium formation occurs when the bacteria induces cell division in the root cortex. The bacteria are released into the plant by an endocytotic process as the infected threads grow towards the centers of mitotic activity and enter the primordium cells. The primordium cells mature and differentiate to form an ideal

environment for the bacteria to fix nitrogen. Thereafter, nitrogen fixation takes place by the bacterium for the plant and the plant provides nutrition for the bacterium. Thus, this is the manifestation for mutualist relationship between soybean plants and nitrogen-fixing bacteria. There may also be inactive nodules that develop on the plant. These nodules do not facilitate nitrogen fixation. When cut the inside of the nodule is white, green or grey whereas active nodules are red or brown in colour (Tikhonovich, 1995).

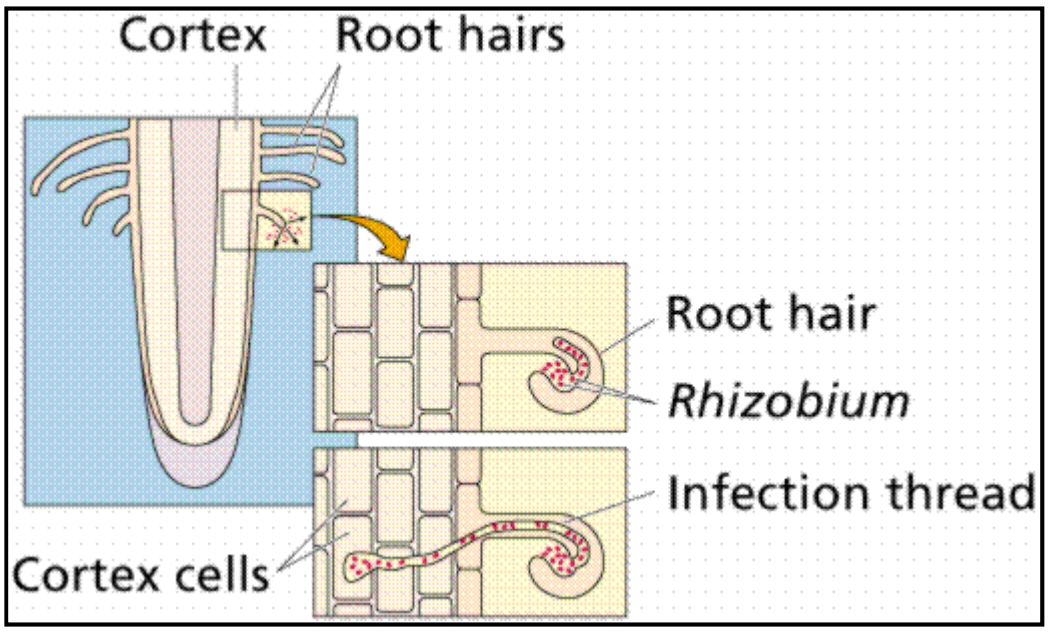

Figure 1.9 Induced nodule formation and uptake of bacteria in legume plants (Farabee, 2010).

1.2.4.4 Successful nodulation practices

In order for nodulation to be successful certain factors have to be present. The bacterium required for nodulation, i.e., *B. japonicum*, does not thrive well in the presence of excessive heat, sunlight, acid soils and particularly dry conditions. These remain some of the reasons for the lack of this bacterium in most South African soils due to the weather conditions. As such it is very important to plant crops in moist soils and have an adequate irrigation system especially during dry hot seasons. Also it is

recommended that for proper nodulation to take place it is vital to inoculate the plant with the specific strain of bacterium for that particular genotype. In some cases the inoculums can be stored in a cool dark place for optimal results (Keyser and Li, 1992).

1.2.5 Production of soybean

1.2.5.1 Land preparation

1.2.5.1.1 Soil type

The soil should be rich in organic matter and the soil moisture should be adequate to support optimum germination rates. Soybean is a hardy crop and can grow in most soils but growth is decreased in soils that are dry and sandy as the beans require sufficient moisture. In soils that contain excess clay content the crop can become water logged (Scott and Aldrich, 1970).

1.2.5.1.2 Tillage

The most common type of tillage used to be the conventional mouldboard-based tillage and is frequently used for the preparation of soybean beds. This form of tillage causes the soil to become granulated and the soil layers are not compacted such that it will increase the penetration of the water into the lower layers to the roots (Allmaras *et al*., 2000). This is because soybeans need to absorb about 50% of their weight in water in order to germinate However in KwaZulu-Natal there have been many different types of tillage used, all aim at being more environmentally conservative to the soil and thus to decrease structural degradation of the soil to lower production costs (Scott and Aldrich, 1970). Research conducted in 1993 on maize and soybean with different tillage techniques revealed similarities in yield of the two crops with no tillage when any crop other than them was previously grown in the area. However, when soybean and maize were grown continuously a decrease of 7 and 8% was noted in the no-tillage area than in the conventional mouldboard-based tillage (Lund *et al*., 1993).

1.2.5.1.3 Weed control

Weeds may reduce crop yields by 35-83%, even if good farming practices are implemented. The most important weeds in soybean include *Abutilon theophrasti* L. (velvetleaf), *Digera arvensis* L. (wild rhubarb), and *Cynodon dactylon* (L.) Pers (couch grass).The space between the planted rows should be covered with residue in order to discourage the growth of weeds. Another method to help eliminate weeds is to allow the weeds to germinate early through early preparation of the seedbed and then spraying a herbicide over the seedbed in order to kill the weeds before planting begins. The control of weeds before planting is usually the most cost effective approach to control weeds. It is also advantageous to select planting areas with less broadleaf weeds. Soybean is a broadleaf plant and application of herbicides for broadleaf weeds may kill the soybeans (Singh *et al.*, 2010).

1.2.5.2 Planting

Once the seedbed is prepared the seed can be planted. The seeds should be planted about 50-100 mm apart and 6-25 mm deep. If seeds are planted deeper or at a depth than recommended, then the seeds may rot or they may not be able to break through the soil. The different rows in which the seeds are planted should be 450-600 mm apart. Soybeans planted in narrower rows have a greater average yield than those planted in wider rows. At time of planting, inoculants i.e., nitrogen-fixing bacteria (rhizobia) should be placed on the seeds. 5g of soybean inoculant should be mixed with about 1 kg of seed (Kumudini, 2010).

1.2.5.3 Seeding rate

The amount of seed that should be planted per hectare depends on the method of planting. In general, it takes approximately 60-100 kg of soybeans per hectare of land (Scott and Aldrich, 1970).

1.2.5.4 Fertilization

In order for soybean to grow vigorous and healthy, it needs various plant nutrients. These include phosphorous and potassium which can be added by the addition of

organic or inorganic fertilizers soil, nitrogen, which the soybean normally obtains from the symbiotic relationship with root nodule nitrogen-fixing bacteria (Higa and Parr, 1994). In soils with low potassium levels, one can add potassium sulphate. The soil acidity can be adjusted by the addition of lime fertilizers. Other nutrients include calcium, which can be added to soils in the form of gypsum, and sulphur, which can be added by adding sulphate-containing fertilizers such as calcium sulphate or potassium sulphate (Scott and Aldrich, 1970). Additional nitrogen fertilizers are not recommended for soils that already have moderate level of nitrogen the addition of the extra nitrogen may inhibit nodulation. Nitrogen-fixing bacteria can be efficient on soybean and thus can supply the full nitrogen requirement for the plant even on nitrogen deficient soils (Duxburg *et al*., 1990).

1.2.5.5 Irrigation

To obtain maximum yields of soybean it is essential that the plants receive from 450-750 mm of water throughout the year. Cultivars that mature faster may require less water. The water requirements in the germination and seedling stages may be higher than in the mature stages as at maturity soybean plants lose most of their leaves and transpiration via leaves decreases. However during germination the soybean seed does require a substantial amount of water to ensure healthy development (Scott and Aldrich, 1970).

1.2.5.6 Harvesting

When using soybeans as a vegetable, the pods should be harvested when they are fully grown but before the pods become yellow in colour. If the soybeans are being used for grains, then when mature, the leaves turn yellow and fall to the ground. The pods can be fully developed and mature from about 80 - 120 days after sowing and the soybean plants should be allowed to completely dry, and or the seeds should have less than 12.5% moisture level before they are harvested (Kumudini, 2010). Some cultivars may reach a height of 1.5m and tend to take 80 - 120 days from sowing until harvesting (Liu, 1997). In KwaZulu-Natal soybeans may take from 100 - 185 day to mature depending on the cultivar (Duxburg *et al*., 1990).

1.2.6 Diseases on soybean

There are over 50 diseases that affect soybean plants. Although rainfall is essential for good soybean growth, in areas with high rainfall and humidity, diseases occur more frequently. Seedling and root diseases are often difficult to diagnose as they can be associated with several causal organisms as well as different symptoms. Despite having good seed, however, seed-borne and soil-borne pathogens can reduce plant stand, size and yield (Forbes *et al*., 1986).

1.2.7 Ecology of soybean

Flowering of soybeans is controlled by short day lengths and warm temperatures. The optimum growing temperatures for growth of soybeans are between 20-30℃ while significant retardation of growth is seen with temperatures below 20℃ and over 40℃. Soybean has the ability to grow in a wide range of soils but it has an optimum growth in moist alluvial soils. The period of flowering in soybeans are from 3-5 weeks and thus soybeans are able to survive short drought periods during the flowering stage where other crops such as maize with a flowering period of one week may fail (Hartman *et al*., 2011). Soybeans are not able to grow well in acidic soils or in soils where there are no nematode infestations. Soybeans are able to reach moisture at considerable soil depths due to the diffuse root system (Hartman *et al*., 2011).

1.2.8 Factors that influence successful soybean production

General factors that affect soybean production are as follows:

1. Selection of suitable land that favour soybean germination, which should be properly treated to decrease or eradicate weeds.
2. Selection of superior cultivars for the target environment.
3. Disease free and vigorous seeds. The seed coat must not be cracked or damaged.
4. An effective irrigation system must be implemented if the area receives rainfall that is less than the prescribed amount and if the area is very hot and dry.

5. A proper crop rotation system.

6. Proper planting time. Soybeans plants should be planted early or late in the growing season to reduce disease on the seedlings as the seedlings are more susceptible to disease than mature plants. However, this technique may be ineffective against certain pathogens such as *Sclerotinia* and rust.

7. Adequate and balanced fertilizers.

8. Proper harvesting time. Harvesting should be done after the plants have dried and pods have turned brown or tan. Plants should be harvested in a way to decrease seed loss i.e. before pod shattering.

9. Effective, environmentally friendly, disease control methods (Duxburg *et al*., 1990).

1.2.9 Selection in soybean

Soybean are principally bred for improved yield, high oil and protein contents, resistance to pod shattering, degree of biological nitrogen fixation, resistance to lodging, drought tolerance, and resistance to diseases and pests (Tefera, 2011). Over the past 30 years the role of soybeans in industry has developed significantly as a source of protein and oil that lead to the production of complex products (Cianzio, 2007). Therefore, yield as well as protein and oil content are important to food and oil industries. Soybean varieties are distinguishable by various characteristics such as flower colour, pubescence colour, pod colour, seed colour, leaf shape, stem type, among others. In the selection process these traits are selected at the target environments. Maturity is one of the important traits when selecting a soybean cultivar (Duxburg *et al*., 1990).

1.2.10 Soybean genetic diversity

Soybeans are one of the most cultivated crops worldwide. This crop is valued mainly for the high oil and protein content and is thus used in many efforts globally to aid in world hunger (FAO, 2004). Over the years various soybean breeding lines have been

developed in the efforts to improve genotypes for increased productivity and yield. However, this has resulted in a bottle neck effect of genetic diversity in soybean (Hyten *et al*., 2006). The decrease in genetic diversity can lead to an increase susceptibility to pests and pathogens and a decrease in adaptability (Wang *et al*., 2010). This can eventually lead to a decrease in soybean production. Therefore there is a need for breeders to identify and detect the presence or absence of useful alleles in the soybean. This helps to determine the genetic diversity, to compare varieties as well as to conserve currently grown germplasm for breeding and production (Priolli *et al*., 2002).

1.3 MOLECULAR MARKERS

Before the advent of molecular markers identification of different crop genotypes were carried out using various phenotypic characteristics such as morphology and pigmentation. However, these methods were very costly and time consuming for plant breeders. The use of molecular markers has resulted in a reduction of time and costs incurred by these methods, by allowing breeders to distinguish between closely related genotypes (Song *et al*., 1999).

1.3.1DNA based molecular markers and their applications in plant breeding

Molecular markers reveal genetic differences in the primary structure of DNA between individuals. Compared to protein markers, DNA based polymorphisms are more stable, and can reveal subtle changes in the genomic DNA (Powell *et al*., 1996; Horacek *et al*., 2009). Different DNA based marker techniques have been successfully used such as restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and simple sequence repeats (SSR) and single nucleotide polymorphisms (SNP) (Powell *et al*., 1996; Lusser *et al*., 2012). In comparison to RFLPs, RADPs, and AFLPs the SSR markers have the ability to identify unique alleles in crop species such as soybeans (Wang *et al*, 2006). Research conducted on genetic diversity of soybean by Tanya *et al*. (2001), using 5 Korean, 8 Thai and 3 wild soybean, and Li *et al*. (2010), on 303 accessions of domestic soybean and its wild progenitor *G. soja*, revealed that SSRs were more useful due to

the high resolving power for the estimation of genetic distances and relationships amongst the genotypes than SNPs.

1.3.2 Simple sequence repeats (SSR) DNA markers

The simple sequence repeats (SSRs)DNA markers or microsatellites are tandem repeats of 1-6 nucleotides of DNA that are used to identify the presence or absence alleles (Zane *et al*., 2002). These markers are able to identify genotypes and to determine genetic distances between genotypes which in turn can be used in varietal comparison. Due to their high polymorphic nature and the ease of marker detection via PCR, SSR markers are highly favoured (Sammour, 2011).

SSR markers were used in various studies for instance Yoon *et al*. (2009) genotyped 2,758 accessions of soybean landraces in Korea using 6 SSR primers. Allele frequency and genetic similarities were shown to be different in 158 Chinese soybean germplasms with 67 SSR loci (Xie *et al*., 2005).

1.4 BIO-FERTILIZERS

The use of chemical fertilizers has improved the yield of crops over the past 100 years. However, this improvement had come with a negative effect to the environment and eventually to humans (Peoples *et al*., 1995).

Bio-fertilizers are natural agents that are found in the soil. Bio-agents do not disturb the natural ecosystem and will enhance the nutrient supply as well as increase soil fertility (Wagner, 1997). The bio-fertilizers can be living such as a microorganism or non-living such as a naturally occurring element. This means that on average bio-fertilizers maybe cheaper as the microbial cultures can be grown. However, costs can be incurred for storage and maintenance of these cultures. And since the microbes are living organisms they are able to grow and be self-sufficient in most cases (Lumpkin and Plucknett, 1982). Phosphate soluble bacterial (PSB) as a bio-fertilizer on fruit crops proved very effective in increasing growth and fruit yield (Maksoud *et al*., 2009).

1.4.1 The use of *Trichoderma* spp. as bio-agent

1.4.1.1. Taxonomy of the *Trichoderma* spp.

The Trichoderma spp. belongs to the kingdom Fungi, division Ascomycota, subdivision Pezizomycotina, class Sordariomycetes, order Hypocreales, and family *Hypocreaceae* (Persoon) (Harman *et al*., 2004).

1.4.1.2. Morphology

Conidia of *Trichoderma* are ellipsoidal and are 3-5 x 2-4 µm in size and are usually smooth. However, tuberculate to finely warted condia are produced by a few species (Harman and Kubicek, 1998). Conidiophores are highly branched with the main branches producing lateral side branches that may be paired or non-paired. The longest branches are distant from the tip and often have phialides arising from the main axis. All primary and secondary branches arise at, or near, 90° with respect to the main axis. The conidiophores that have paired branches assume a pyramidal aspect and the conidiophores terminate in one or two phialides. The main branches are often ended by long, simple or branched, hooked, straight or sinuous, septate, thin-walled elongations. Fungal hyphae are 5-10 µm in diameter (Figure 1.10) (Harman *et al*., 2004).

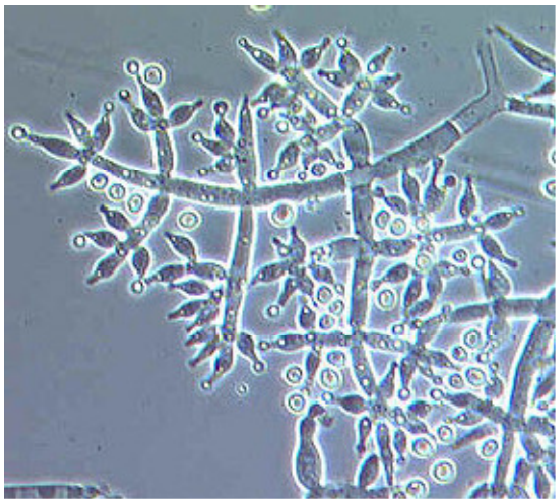

Figure 1.10. Long, branched *Trichoderma* hyphae which produce phialides at the ends of the branches (Harman *et al*., 2004).

1.4.1.3. *Trichoderma* as a biological control agent and growth promoter

There are a number of different mechanisms by which this fungus acts as a biological control agent. Primarily, the mechanisms of biocontrol with *Trichoderma* are mycoparasitism, antibiosis, and competition for resources and space to grow (Harman, 2006). This fungus also produces extracellular enzymes and some of these were found to be associated with biological control of plant disease (Elad, 1982). *Trichoderma* is naturally resistant to many toxic compounds in the soil (Weeden *et al*., 1976). The genes for these enzymes were inserted into the plant genome and have shown to induce resistance to a range of fungi that are pathogenic to plants (Lorito *et al*., 1998). This fungus is able to induce systemic and localized resistance in plants (Bigirimana *et al*., 1997).

Trichoderma can induce also, increase plant growth and uptake of nutrients attributed to the walling off of the *Trichoderma* thallus. This is done by bioactive molecules that are produced by the fungus that colonizes the root epidermis and outer cortical layers (Harman, 2006). This fungus can also increase the rate of seed germination (Benítez *et al*., 1998). Further, *Trichoderma* can increase the number of deep roots in a plant leading to drought resistance (Weeden *et al*., 1976). In soils with an ion imbalance this fungus can secret organic acids that result in the breakdown of carbon sources which results in the solubilization of certain cations. This increases soil fertility and crop productivity (Harman *et al*., 2004).

1.4.2 Silicon

1.4.2.1 How essential is silicon to plants?

An element is essential if: 1) the deficiency of it makes it impossible for the plant to complete its life cycle. This effect is not merely due to the amelioration by the element of some unfavorable chemical or microbiological condition of the substrate; and 2) the element must be part of a molecule of an essential plant constituent or metabolite (Epstein, 1994).

Silicon is an element that is commonly available in the soil and is an essential nutrient that is important for plant growth in trace quantities. The appreciable fractions of dry matter of the essential nutrients of a crop plant are shown in Table 1.1 (Epstein, 1994). Miyake and Takahashi (1978) showed that Si is essential for the growth of plants through tests conducted with various plants grown with and without the addition of Si. Woolley (1957) had conducted nutrient deficiency experiments with and without the presence of Si on tomato plants and not much difference was found between the experiment and control plants. However, the Si content in the plants that were deprived of Si showed that 4.2 ppm of Si was found in the plants and 2.8 ppm was found in the roots and it proposed that these quantities of Si is sufficient for plant growth(Epstein, 1994).

Table 1.1 Fractions of the dry matter content of essential nutrients in a plant (Epstein, 1994)

Element	Range of concentrations (dry weight basis)	Remarks
Nitrogen %	0.5-6.0	Essential macronutrients
Phosphorus %	0.15-0.50	
Potassium %	0.80-8.0	
Calcium %	0.10-6.0	
Iron ppm	20-600	
Manganese ppm	10-600	
Zinc ppm	10-250	Essential micronutrients
Copper ppm	2-50	
Cobalt	0.05-10	Essential in all nitrogen-fixing systems
Sodium %	0.001-8.0	Essential for some plants; often beneficial
Silicon %	0.1-8.0	
Aluminum ppm	0.1-500	Not known to be essential; often toxic to plants on acid soils

1.4.2.2 Effect of silicon on plant growth

Research on different plants treated with and without a Si nutrient solution showed that in its absence plants such as tomato and cucumber show deficiency symptoms (Miyake and Takahashi, 1978) and soybean and strawberry showed a decrease in the plant growth (Miyake and Takahashi, 1985). From these findings it was deduced that Si may in fact be an element that is essential for higher plants (Takahashi *et al*., 1990). Silicon was also successfully used in an experiment to correct a nutrient with phosphorus and zinc in cucumbers (Marschner *et al*., 1990). The uses of Si as a growth promoter made it an essential component in the commercial production of rice and sugarcane (Jones and Handreck, 1967).

1.4.2.3 Uptake and accumulation of silicon in plants

The Si is taken up through the roots in many plants such as rice. The amount of Si taken up will differ for different plants and this will affect the amount of deposition of Si for each different plant. Monocotyledonous plants are usually able to take up and store more Si in the shoots than dicotyledonous plants (Ma and Takahashi, 2002). The Si is taken up as silicic acid at a pH that is below 9.0. Silicic acid is transported to the cortical cells by a transporter in rice. A second transporter then takes the Si to the xylem and Si then accumulates in the xylem which results in xylem loading.

An increased concentration of the two transporters leads to more silicic acid being transported and accumulated in the plant. In one case silicic acid is polymerized to silica gel and in the shoots the silica gel is concentrated by transpiration as water is removed. More Si is found to be deposited in mature cells and is deposited as a double cuticle-Si layer below the cuticle (Ma and Yamaji, 2006).

1.4.2.4 Possible Genes that control silicon uptake in plants

Rice is a crop that can accumulate a substantially large amount of Si up to 10% of the shoot dry weight, and can be more than that of the macronutrients such as nitrogen, phosphorus and potassium. Legume plants do not accumulate Si as effectively as rice

(Raven, 1983). According to the report of Ma and Yamaji (2006) the Lsi1 gene responsible for silicon uptake in rice, also affects levels of silicic acid in *Xenopus laevis* when injected with cRNA coding for the Lsi1 gene (Ma and Yamaji, 2006).

1.4.2.5 Possible mechanisms by which silicon controls plant diseases

Si is taken up the roots of a plant and accumulated in apoplast and this lead to the hypothesis that Si acts as a physical barrier that confers resistance to pathogens (Datnoff, 1997). However, investigations have shown that plants that are Si-amended and infected with a pathogen tend to produce elevated quantities of phenolic materials and chitinases, than plants that are unamended, that readily accumulate in the cell. These phenolics were shown to be toxic to fungi and this reduces the plant's susceptibility to fungi (Cherif *et al*., 1992). Therefore, Si can be used to enhance the natural defenses of the plant.

1.4.2.6 Effect of silicon on biotic stress resistance

Silicon can help reduce the effects of stresses on plants such as diseases, fungi and bacteria. With an increase in the levels of Si in the plant a decrease is noted in the disease symptoms on the plant. There are two mechanisms of disease resistance proposed. In the first mechanism the Si forms a physical barrier that cannot be penetrated by fungi or insects and in the second the Si acts as a modulator of host resistance of the pathogen. The host defense mechanism can be activated by Si i.e. if a plant root becomes infected with a pathogen, such as *Pythium*, Si can enhance the activity of chitinases and polyphenoloxidases (Cherif *et al*., 1994).

1.4.2.7 Effect of silicon on abiotic stress resistance

Silicon has the ability to effectively reduce physical stresses on the plant. Silicon can increase some plants' resistance to gamma radiation and plants that are treated with Si have a much higher recovery rate than those that are not treated with Si. Since the Si forms a double layer under the cuticle, plants undergoing water stress such as drought, can be reduced as the Si layer decreases the transpiration. Typhoons, strong winds,

adverse temperatures and limited sunshine can negatively affect the plant and Si can help to strengthen the plant, prevent sterility in plants in periods of excess transpiration due to strong winds and to increase crop yields in adverse temperatures (Ma, 2004).

1.4.2.8 Effect of silicon on chemical stress

Phosphorus is an element that is essential for plant growth. In soil that has a phosphorus deficiency, the growth of plants is improved by Si addition. Silicic acid does not dissociate at a pH of 9 and therefore it is unlikely that interactions can occur with the anionic phosphorus. Si however is able to affect the uptake of manganese and iron in the soil. Soil that is treated with Si showed a decrease in the amount of manganese and iron taken up. Phosphorus shows a high affinity for manganese and iron and thus if absent there will be more phosphorus that is available internally, in the plant. Thus indirectly Si is able to increase the phosphorus in plants that show a phosphorus deficiency by decreasing the excess amount of manganese and iron that are taken up by the plant (Ma, 2004).

An excess of nitrogen from fertilizers can cause lodging, susceptibility to diseases such as blast diseases and decrease the quality of brown rice grain by increasing the protein content. Silicon can help improve these conditions by strengthening the plant stems and leaf blades, inhibiting diseases and decreasing the protein content in brown rice to increase the quality of the yield (Morimiya, 1996). Silicon can reduce the stresses that are caused by elements such as manganese, iron, zinc and cadium which may become toxic to the plants in incorrect quantities. Injuries to plants by erroneous salt concentrations and growth inhibition can be reduced by the application of Si. Aluminum is an element that can be toxic to plants in large quantities and Si was shown to decrease the toxic effect of aluminum and proved more effective at higher concentrations of Si (Ma, 2004).

1.5 CONCLUSION

This chapter provided a literature review as general framework on soybean production and constraints. In the presence of growth promoting agents such as *Trichoderma* and silicon there could be a significant increase in yield in soybeans. This increase may be different for different cultivars. The response of cultivars would be affected by the target environment (soil fertility, disease and pests). Thus studies should be conducted to recommend the optimum conditions and suitable cultivars for use by local farmers. The use of growth promotion agents (natural agents) can improve the structure and composition of the soil thus acting as soil enhancers i.e. through nitrogen fixation with *Trichoderma* which can be beneficial to local farmers to reduce overall production costs. The ensuing chapter of this study investigates the role of Potassium silicate (KSi) and *Trichoderma* when used singly or in combination in affecting soybean growth and productivity. This chapter also indicates the importance of genetic diversity analysis in crop breeding program and the special application of SSR DNA markers for varietal comparison and associational studies with phenotypic traits.

1.6 LITERATURE CITED

ALLMARAS, R.R., SCHOMBERG, H.H., DOUGLAS, C.L. and DAO, T.H. 2000. Soil organic carbon sequestration potential of adopting conversation tillage in U.S. croplands. Journal of Soil and Water Conservation 55: 365-373.

BENITEZ, T. DELGADO-JARANA, J. RINCON, A. REY, M. and LIMON, C.1998.Biofungicides: *Trichoderma* as a biocontrol agent against Phytopathogenic fungi. Recent Research Developments in Microbiology 2: 129-150.

BIGIRIMANA, J., DE MEYER, G., POOPE, J., ELAD, Y., and HOFTE, M., 1997. Induction of systemic resistance on bean (Phaseolus vulgaris) by *Trichoderma harzianum*. Medical Facuality Landbouww. University of Gent. 62: 1001-1007.

CALDWELL, B.E. 1973. Soybeans: improvement, production and uses. R.W. Howell, R.W. Judd and H.W. Johnson (Eds). Agronomy series 16. American Society of Agronomy. Madison, Wisconsin. Pp 353-390.

CAVALETT, O. and ORTEGA, E. 2009. Emergy, nutrients balance, and economic assessment of soybean production and industrialization in Brazil. Elsevier Publication. Journal of Cleaner Production 17: 762-771.

CHERIF, M., ASSELIN, A. and BÉLANGER, R.R. 1994. Dense response induced by soluble silicon in cucumber roots infected by *Pythium spp*. Phytopathlogy 84: 236-242.

CHERIF, M., BENHAMOU, M.N., MENZIES, J.G., and BELANGER R.R. 1992. Silicon induced resistance in cucumber plants against *Pythium ultimum*. Physiological and Molecular Plant Pathology 41: 411-425.

CHOUICHOM, S. and YAMAO, M. 2011.Sustainable agricultural development. Organic Fertilizer Use in Northeastern Thailand: An Analysis of Some Factors Affecting Farmers' Attitudes. Earth and Environmental Science 3:185-196.

CIANZIO, S.R. 2007. Breeding Major Food Staples. (Eds) M.S. Kang and P.M Priyadarshan, Blackwell Publishing, UK. Pp 245-250.

DATNOFF, L.E., DEREN, C.W., and SNYDER, G.H. 1997. Silicon fertilization for disease management of rice in Florida. Crop Protection 16: 525-531.

de KLEIJN, M.J., VAN DER SCHOUW, Y.T., WILSON, P.W., GROBBEE, D.E., and JACQUES, P.E. 2002. Dietary intake of phytoestrogens is associated with a favourable metabolic cardiovascular risk profile in postmenopausal U.S.women: the Framingham study. Journal of Nutrition 132: 276-82.

DUXBURG, M.R., BIRCH, E.B., and PARSONS, M.J. 1990. *Soybeans in Natal. Agricultural production guidelines for Natal*. Department of Agriculture and Development. Natal, RSA.

ELAD, Y., CHET, I., and HENIS, Y. 1982. Degradation of plant pathogenic fungi by *Trichoderma harzianum*. Canadian Journal of Microbiology 28: 719-725.

EPSTEIN, E. 1994. The anomaly of silicon in plant biology. Proceedings of the National Academy of Science, USA 91: 11–17.

FAO, 2004. The role of soybean in fighting world hunger. (http:// www.fao.org): Accessed 1 July 2012.

FARABEE, M.J. 2010. Plant Hormones, Nutrition and Transport. (http://www.emc.maricopa.edu/): Accessed 15 September 2010.

FORBES, G.A., ODVODY, G.N and TERRY, J.M. 1986. Seedling Diseases. *In : Compendium of Sorghum Diseases*. Ed Frederickson, R.A. American Phytopathology Society. Satin Paul. Minnesota. Pp 7-8.

HARMAN, G.E., 2006. Overview of mechanisms and uses of *Trichoderma spp*. Phytopathology 96:190-194.

HARMAN, G.E., and KUBICEK, C.P. 1998. *Trichoderma and Gliocladium*, *Enzymes, Biological Control and Commercial Applications*, Taylor and Francis, London, UK, Pp 229-265.

HARMAN, G.E., HOWELL, C.R., VITERBO, A., CHET, I., and LORITO, M., 2004. *Trichoderma* species - opportunistic avirulent plant symbionts. Nature Reviews Microbiology2: 43–56.

HARTMAN, G.L., WEST, D.W. and HERMAN, T.K. 2011. Crops that feed the World 2. Soybean-worldwide production, use, and constraints caused by pathogens and pests. Food Security 3: 5-7.

HIGA, T. and PARR, J.F. 1994. Beneficial and effective microorganisms for a sustainable agriculture and environment. International Nature Farming Research Center. Atami, Japan. Pp 2-4.

HORACEK, J., GRIGA, M., SMYKAL, P. and HYBL M .2009. Effect of environmental and genetic factors on the stability of pea (*Pisum sativum* L.) isozyme and DNA markers. Czech Journal of Genetics and Plant 45:57–71.

HYTEN, D.L., SONG, Q., ZHU, Y., CHOI, I.Y., NELSON, R.L., COSTA, J.M., SPECHT, J.E., SHOEMAKER, R.C. and CREGAN, P.B. 2006. Impacts of genetic bottlenecks on soybean genome diversity. Proceeding of the National Academy of Sciences of the United States of America. 45: 16666-16671.

JOHNSON, H.W., and BERNARD, H.W. 1963. Soybean genetics and breeding. In A. G. Norman (Ed). *The Soybean*. Academic Press, New York. Pp 1-73.

JONES, L.H.P. and HANDRECK, K.A. 1967. Silica in soils, plants and animals. Advanced Journal of Agronomy 19: 107-149.

KEYSER, H.H. and LI, F. 1992. Potential for increasing biological nitrogen fixation in soybean. Biological nitrogen fixation for sustainable agriculture. Plant and Soil 141:119-135.

KLEIFELD, O., and CHET, I. 1992. *Trichoderma harzianum* – interactions with plants and effect on growth response. Plant and Soil 144: 267–272.

KUMUDINI, S. 2010. *The Soybean* Chapter 3*: Botany, Production and Uses*. (Ed) G. Singh. CAB International. Wallingford. Oxfordshire. UK. Pp 48-69.

LI, Y.H., LI, W., ZHANG, C., YANG, L., CHANG, R.Z., GAUT, B.S. and QIU, L.J.2010. Genetic diversity in domesticated soybean(*Glycine max*) and its wild progenitor (*Glycine soja*) for simple sequence repeat and single-nucleotide polymorphism loci. New Phytologist 188: 242-253.

LIU, K. 1997. *Soybeans: Chemistry, Technology, and Utilization*. Chapman and Hall, New York. Pp 379-411.

LONG, S.R. 1996. *Rhizobium* symbiosis: Nod factors in perspective. Plant Cell 8: 1885-1898.

LORITO, M., WOO, S.L., GARCIA FERNANDEZ, I., COLUCCI, G. HARMAN, G.E., PINTOR-TORO, J.A., FILIPPONE, E., MUCCIFORA, S., LAWRENCE, C.B., ZOINA, A., TUZUN, S., and SCALA, F. 1998. Genes from mycoparasitic fungi as a source for improving plant resistance to fungal pathogens. Proceedings of the National Academy of Science 95: 7860-7865.

LORQUIN, J., MOLOUBA, F., and DREYFUS, B.L. 1997. Identification of the carotenoid canthaxanthin from photosynthetic *Bradyrhizobium* strains. Applied Environmental Microbiology 63: 1151–1154.

LUMPKIN, T.A., and PLUCKNETT, L.D., 1982. *Azolla* as a green manure: use and management in crop production. Westview Tropical Agriculture Series. Westview Press, Boulder. Colorado. USA, Pp 230.

LUND, M.G, CARTER, P.R. and OPLINGER, E.S. 1993. Tillage and Crop Rotation Affect Corn, Soybean, and Winter Wheat Yields. Journal of Production Agriculture 6: 207-213.

LUSSER, M., PARISI, C., PLAN, D. and RODRÍGUEZ-CEREZO, E. 2012. Deployment of new biotechnologies in plant breeding. National Biotechnology 30:231–239.

MA, J.F. 2004. Role of silicon in enhancing the resistance of plants to biotic and abiotic stresses. Journal of Soil Science and Plant Nutrition 53: 15-22.

MA, J.F., and TAKAHASHI, E. 2002. *Soil, Fertilizer, and Plant Silicon research in Japan. Elsevier Science*, Amsterdam. The Netherlands. Pp 107–180.

MA, J.F., and YAMAJI, N., 2006. Silicon uptake and accumulation in higher plants. Trends in plant sciences. Journal of Plant Science 11: 392-397.

MAKSOUD, M.A., MALAKA, A.S., EL-SHAMMA, M.S., and FOUAD, A.A. 2009. The beneficial effect of bio-fertilizers and antioxidants on olive trees under calcareous soil conditions. World Journal of Agricultural Sciences 5: 350-352.

MARSCHNER, H., OBERLE, H., CAKMAK, I., and ROMHELD, V. 1990. Growth enhancement by silicon in cucumber (*Cucumis sativus*) plants depends on imbalance in phosphorus and zinc supply. Plant Soil 124: 211-219.

MIYAKE, Y., and TAKAHASHI, E. 1978. Silicon deficiency of tomato plant. Soil Science and Plant Nutrition 24: 175-189.

MIYAKE, Y., and TAKAHASHI, E. 1985. Effect of silicon on the growth of soybean plants in a solution culture. Soil Science and Plant Nutrition31: 625–636.

MORIMIYA, Y. 1996. Role of silicon in production of low protein rice and diagnosis parameters. Journal of Soil Science and Plant Nutrition 67: 696-700.

NAGATA, T. 1960. Studies on the differentiation of soybean in Japan and the world. Memoirs of the Hyogo University Agriculture 3: 63-102.

NORMAN, A. 1963. The soybean genetics, breeding, physiology, nutrition management. Academic Press, New York, Pp 3-4.

PEOPLES, M.B., HERRIDGE, D.F., and LADHA, J.K. 1995. Biological nitrogen fixation: an efficient source of nitrogen for sustainable agricultural production. Plant and Soil 174: 3–28.

POWELL, W., MORGANTE, M., ANDRE, C., HANAFEY, M., VOGEL, J., TINGEY, S. and RAFALSKI, A .1996. The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Molecular Breeding 2:225–238.

PRIOLLI, R.H.G., MENDES-JUNIOR, C.T., ARANTES, N.E. and CONTEL, E.P.B. 2002.Characterization of Brazilian Soybean Cultivars Using Microsatellite Markers. Genetics and Molecular Biology 2: 185-193.

RAVEN, J. A. 1983. The transport and function of silicon in plants. Biological Reviews 58: 179–207.

SAMMOUR, R. H. 2011. Soybean - Genetics and Novel Techniques for Yield Enhancement: Genetic Diversity and Allele Mining in Soybean Germplasm. Intech. Pp 10-11.

SATHISH KUMAR, S.R. and BHASKARA RAO, K, V. 2012. Biological Nitrogen Fixation: A Review. International Journal of Advanced Life Sciences 1: 1-9

SCOTT, W.O., and ALDRICH, S.R. 1970. Modern Soybean Production. S & A Publications, Champaign, Illinois.

SINGH, G., RAM, H. and AGGARWAL, N. 2010. The Soybean, Chapter 7: Agro-techniques for soybean production. Edited G. Singh. CAB International, Wallingford. Oxfordshire. Pp 142-223.

SKOW, D.L. 1991. Unlocking the secrets of soils and foliars. Crowley and Communications, Minnesota, USA.

SONG, Q., QUIGLEY, C.V, CARTER, T.E., NELSON, R.L., BOERMA, H.R., STRACHAN, J. and CREGAN, P.B. 1999. A selected set of trinucleotide simple sequence repeat markers for soybean variety identification. Plant Varieties and Seeds 12: 207-220.

STRANGE, R. N. and SCOTT, P. R. 2005. Plant disease: a threat to global food security. Annual Review of Phytopathology, 43, 83–116.

TAKAHASHI, E., MA, J.F., and MIYAKE, Y., 1990. The possibility of silicon as essential element for higher plants. Comments Agricultural and. Food Chemistry 2: 99-122.

TANYA, P., SRINIVES, P., TOOJINDA, T., VANAVICHIT, A., HA, B.K., BAE, J.S., MOON, J.K. and LEE, S.H. 2001. Evaluation of genetic diversity among soybean genotypes using SSR and SNP. Korean Journal of Crop Science 46: 334-340.

TEFERA, H. 2011. Soybean - Molecular Aspects of Breeding, Chapter 7:*Breeding for promiscuous soybeans at IITA*. International Institute of Tropical Agriculture (IITA), Chitedze Agricultural Research. Pp 147-162.

TIKHONOVICH, I.A., PROVOROV, N.A., ROMANOV, V.I. and NEWTON, W.E. 1995. *Nitrogen Fixation: Fundamental and Applications*, Kluwer Academic Publishers Group, Dordrecht, The Netherlands, Pp 260-795.

WAGNER, G.M. 1997.*Azolla*: A Review of its Biology and Utilisation. Botanical Review 63: 1-26.

WANG, L., GUAN, R., ZHANGXIONG, L., CHANG, R. and QIU, L. 2006. Genetic diversity of Chinese cultivated soybean revealed by SSR markers. Crop Science 46: 1032-1038.

WANG, M. LI, R., YANG, W. and DU, W. 2010. Assessing the genetic diversity of cultivars and wild soybeans using SSR markers. African Journal of Biotechnology 9: 4857-4866.

WEEDEN, C.R., SHELTON, A.M., and HOFFMAN, M.P. 1976. Biological Control: A Guide to Natural Enemies in North America. Accessed from http://www.nysaes.cornell.edu. 25/11/ 2010.

WOOLLEY, J.T. 1957. Sodium and silicon as nutrients for the tomato plant. Plant Physiology 32: 317-32.

XIE, H., GUAN, R., CHANG, R. and QIU, L. 2005. Genetic diversity of Chinese summer soybean germplasm revealed by SSR markers. Chinese Science Bulletin50: 526-535.

YOON, M., LEE, J., KIM, C., KANG, J., CHO, E. and BAEK, H. 2009. DNA profiling and genetic diversity of Korean soybean (*Glycine max* (L.). Merrill). landraces by SSR markers. Euphytica 165: 69-77.

ZANE, L., BARGELLONI, L. and PATARNELLO, T. 2002. Strategies for microsatellite isolation: a review. Molecular Ecology 11:1–16.

CHAPTER TWO

2. RESPONSE OF SELECTED SOYBEAN GENOTYPES TO DIFFERENT SILICON CONCENTRATIONS

Abstract

A study was conducted to determine the responses of 10 selected soybean (*Glycine max* L.) genotypes under silicon (potassium silicate KSi) applications. Two independent controlled experiments were conducted at the University of KwaZulu-Natal during 2010. Ten genotypes established in plastic pots were subjected to an application of three silicon concentrations (0, 200 and 250ppm) using the randomized complete block design. Silicon was applied twice weekly over a period of 4 months. Data collected included number of days to 50% flowering, number of days to 50% maturity, plant height, number of pods per plant, number of seeds per pod, 100 seed weight, root mass, shoot mass, seed yield and harvest index. Results showed significant interactions among genotype by silicon concentration for all the measured traits. Silicon applied at 200 ppm was on average more effective in the growth and seed yield of the selected soybean genotypes and resulted in high harvest indices. The genotypes that produced the highest seed yield and harvest index in both experiments were Williams and Barc-2 at 200ppm Si. These genotypes produced a seed yield of 1.42 g/pot and 1.98 g/pot, respectively. Results from principal analysis (PCA) revealed high harvest index and seed yields for the 10 soybean genotypes used were generally associated with high plant height, increased number of pods per plant and 100 seed weight. The use of silicon at 200 ppm may be effective in increasing the harvest index and seed yield as well as other agronomic traits in soybean.

Keywords: *Glycine, max*, harvest index, seed yield, silicon, soybean,

2.1 INTRODUCTION

Soybean (*Glycine max* L.), is one of the major grain legume crops globally. The worlds' top soybean producers are USA, Argentina and Brazil (NAMC, 2011). Over the past 20 years the compound annual growth rate of soybean production worldwide has increased by 4.4% while the harvested area increased by 3.2% (NAMC, 2011). In 2009/10 South Africa produced the largest soybean at 566 000 tons. In South Africa the KwaZulu-Natal province is the third highest soybean producer (73,250 tons) after Mpumalanga (239,250 tons) and Free State (147,250 tons) provinces (NAMC, 2011).

Soybean is a versatile crop that is rich in protein and oil. It has an average protein content of 40%, oil content of 20%, carbohydrate of 30% and 10% fiber (Mpepereki, 2001). The Food and Agriculture Organization (FAO) is interested in the production of soybean to help provide safe and quality nutrition to developing countries in the effort to eradicate world hunger (FAO, 2004). Soybeans are also used in crop rotation to resort soil nitrogen levels, thus reducing the need and costs of nitrogen fertilizers (Duxburg *et al*., 1990). Research on soybeans and other food crops such as corn and sugarcane have lead to the development of biodiesels to aid in the reduction of greenhouse gases in the atmosphere by providing a possible alternative to fossil fuels (Fargione *et al*., 2008).

There are various factors that limit the potential soybean production in South Africa. The major production constraints include lack of improved and drought resistant cultivars, poor soil fertility, diseases and pests such as rust and bacterial blight, poor distribution and production of seeds, pod shattering, among others. In the past there has been reduced interest in soybean production by farmers due to the low local demand and the lack of improved varieties (Amaza *et al*., 2007).

Silicon (Si) is an element that is found naturally in most soils. The silicon content in soils may range from $\leq$1 to 45% on dry weight basis (Sommer *et al*., 2006). The concentration of silicic acid (plant available silicon) in the soil may range from 0.1 to 0.6 mM (Epstein, 1994). In soils that are neutral to acidic silicon can be found in the form of

silicic acid [$Si(OH)_4$], and in soils that have a pH > 9 silicon is found more often in its ionized form $Si(OH)_3O^-$ which will be readily taken up by plants (Epstein, 1994).

Silicon was previously not considered an essential element for plant growth (Arnon and Stout, 1939). However, according to the re-evaluation of the definition of essentiality by Epstein and Bloom (2003), silicon is now an essential element for higher plants. This is because silicon meets the two new criteria that are necessary to qualify silicon as an essential element. The first requirement is that the element is a part of a fundamental molecule for structure or metabolism and this element when deficient causes abnormalities in growth, development and reproduction (Ma, 2004).

Silicon is classified as a micronutrient for plants and this element can accumulate in plants in the same method that macronutrients are accumulated. The micronutrients are also accumulated in similar or comparable amounts. Plants that are grown with a deprivation of silicon have shown to be weaker in structure and abnormalities may occur more often during growth and development stages (Epstein, 1999).

Research performed on the application of silicate compounds in certain soil types has shown an increase in yield in certain crops including sugarcane, rice and barley (Williams and Vlamis, 1957). Plants do have the ability to convert silicon in the soil into a usable form that is then taken up by the roots (Epstein, 1999). The mechanism of action of silicon seems to be as a result of both physical and physiological means. In an experiment with soybean and nutrients solutions with and without silicon added, the results showed that in the absence of silicon plant growth decreased as compared to when silicon was present. This study was conducted using two varieties of soybean (nodulating and non-nodulating) at one silicon concentration (Miyake and Takahashi, 1985).

There have been reports on several beneficial effects of silicon on plants such as in growth promotion, reduced mineral toxicity, improvement of nutrient imbalance and increased insect and disease resistance. Silicon has also been shown to act as a growth stimulator (Epstein, 1994) and can decrease transpiration rates. This decrease in transpiration may lead to an increase in the photosynthesis of the plant (Ma and

Takahashi, 2002). Subsequently, application of silicon in crop cultivation could enhance yield and its components. The use of silicon to enhance crop productivity has been reported in wheat, soybean, rice and maize (Kupfer and Kahnt, 1992; Pandley and Yadav, 1999).

The level of silicon in the soil may be reduced with repeated cropping suggesting planned application of silicon during cultivation. Further, response to silicon application varies among crop genotypes and the rate of silicon application. Crop species that benefit more silicon applications are those that naturally accumulate high levels of silicon such as rice (Ma, 2001).

The objective of this study was to determine the responses of 10 selected soybean genotypes under different silicon applications. Thus, suitable genotypes and optimum silicon level would be identified for large scale production.

2.2 MATERIALS AND METHOD

2.2.1 Study site, plant materials and treatments

Two independent experiments were carried out during 2010. The experiments were conducted in temperature controlled glasshouse (experiment one) and irrigated tunnel (experiment two) at the University of KwaZulu-Natal (UKZN), Pietermaritzburg. The study used 10 selected soybean genotypes. Details of the test genotypes are indicated in Table 2.1

Table 2.1. List, pedigree and seed source of ten soybean genotypes used in this study.

Name	Pedigree	Source*
BARC-4	Clark 63(8)/Hardee.	USDA
L82-1449-II	-	USDA
L76-1988	Williams(6) x (Harosoy(5) x D54-2437)	USDA
BARC-2	Clark 63(8) x (Hill x Clark)	USDA
Clark	Lincoln(2) x Richland	USDA
Williams	Wayne x L57-0034 (Clark x Adams)	USDA
BARC-14 nodulated	D76-8070(4) X Clark rj1	USDA
BARC-17 nodulated	Ripley(4) X Clark rj1	USDA
Magoye	-	Landrace
LS 6161 R	-	Link seeds, South Africa

* USDA=United States Department of Agriculture

Genotypes were subjected to three different silicon concentrations (0, 200 and 250 ppm Si) prepared from potassium silicate (KSi) and water. The silicon that was used in the trials was a soluble silica liquid fortified with potassium (AgriSil^{TM}). This silicon was obtained from PQ Silicas South Africa. The solution of KSi was made up of silicon at 9.8% of the solution. This percentage was used to calculate the concentrations for the treatments.

2.2.2 Preparation of silicon concentrations

The potassium silicate solution contained 9.8% silicon. Therefore in 100 ml of potassium silicate the concentration of silicon is 9.8 ml. The concentration of the three prepared silicon treatments were measured in parts per million (ppm). The silicon treatments were made by diluting the potassium silicate solution in water. The amount of potassium silicate used for each concentration was calculated using the formula: $C_1V_1=C_2V_2$. The initial concentration of silicon (C_1) in the potassium silicate solution was 9.8 x10^4 ppm and the initial volume was (V_1). The concentration of silicon that is required is (C_2) 100 ppm and the volume that is required is (V_2) 10000 ml. This means

that 10.2 ml of KSi when added to 10L of liquid (water) would give a solution that contained silicon in the concentration of 100ppm.

Therefore 20.4 ml of KSi was used to make up the 200ppm treatment and 25.5 ml of KSi was used to make up the 250ppm treatment. These treatments were prepared weekly using only plastic measuring equipment.

2.2.3 Experimental design and planting

The experiments were set out in the randomized complete block design consisting two factors i.e. ten genotypes and three silicon levels replicated three times. The 30 treatment combinations were randomly assigned in 90 plastic pots of 30cm diameter with a volume of 4 liters. Pots were filled with composted pine bark (National Plant Food cc, South Africa) potting medium which consisted fine composted pine bark with a high water holding capacity. Four plants were established per pot (Figures 2.1 and 2.2).

Figure 2.1. Two weeks old soybean plants established in the glasshouse at the University of KwaZulu-Natal in Pietermaritzburg.

Figure 2.2 Two weeks old soybean plants established in the irrigated tunnel at the University of KwaZulu-Natal in Pietermaritzburg, taken two weeks after planting.

2.2.4 Application of silicon and fertilizers

After planting, the three concentrations of silicon was applied twice a week and water applied as required until maturity. Prior to the study the soil was sampled and analyzed by Fertilizer Advisory Service, KwaZulu-Natal Department of Agriculture and Environmental Affairs, Soil Fertility and Analytical Services, Pietermaritzburg (Table 2.2). The details of the sampled soils are presented in Table 2.2 were used as a guide for the application of fertilizer. All soybean pots received 200 ml of fertilizer containing Nitrogen, Phosphorus and Potassium (NPK), in the ratio 3:1:3 once a week subsequent of and 150 ml calcium nitrate as slow releasing fertilizer once a month until maturity subsequent to emergence.

Table 2.2 Nutrient and lime analysis of soil sampled for this study as determined by the Fertilizer Advisory service, KwaZulu-Natal Department of Agriculture and Environmental Affairs, Soil Fertility and Analytical Services, Pietermaritzburg

P (mg/L)	K (mg/L)	Ca (mg/L)	Mg (mg/L)	EA (cmol/L)	TC (cmol/L)	AS %	pH (KCl)	Zn (mg/L)	Mn (mg/L)	Cu (mg/L)	MIR clay %	MIR organic C %	MIR N %
290	1737	1722	490	0.25	17.31	1	5.65	29.5	45	1.9	<5	>6	>0.6

EA=exchange acidity; TC=total cations; AS=acid saturation; MIR=mid-infrared

2.2.5 Data collection and analysis

In both experiments agronomic data were collected. The days to 50% flowering was taken when two of the four plants in each pot for each genotype at each level of silicon produced flowered. Days to 50 % maturity taken when two of the four plants in each pot for each genotype reached maturity i.e. pods were ready for harvesting. The plant height was taken at maturity and was measured in millimeters (mm) taken from soil surface to plant apex for all plants. At maturity the plants were harvested, the number of pods per plant counted for all plants; number of seeds per pod was counted for all plants; seed yield was taken in (g/pot) for all pots; the weight of 100 seeds was obtained by weighing a random sample of 100 seed, the roots (cut at rhizosphere) and aerial parts were harvested and dried for 72 hours at 70^{o}C in a LABOTEC TERM-O-MAT (Labotec Oven, Model number 385, South Africa) oven before weighing to determine the dry root mass (g) and dry shoot mass (g) respectively together making up the dry biomass. The harvest index was then calculated by dividing the seed yield by the sum of the dry biomass and seed yield. The data collected for each experiment was analyzed separately then a combined analysis of variance carried out using GenStat (Genstat, 2009). Correlation analysis was conducted using the Pearson model on SPSS (2001) to test the association of traits. Principal component analysis was conducted using SPSS (2001) for each experiment. Principal component analysis was used to identify the number of influential components and predictor variables represented in each of the component. The method helps to find a linear combination of variables as a component that accounts for more variation than in the original variables. It then finds another subsequent component uncorrelated with the previous one that accounts for as

much of the remaining variation. Consequently, a few uncorrelated components will account for most of the variation that can be used to replace the original variables into manageable subsets of characters. Thus, the PCA analysis is very useful when several correlated traits are present in the study that might adversely affect the response.

2.3 RESULTS

In experiment I the analysis of variance indicated significant interaction ($P<0.05$) among genotype by silicon concentrations for days to 50% maturity, plant height, pods per plant, 100 seed weight, root mass, shoot mass, seed yield and harvest index (Table 2.3). During this experiment the number of seeds produced per pod was omitted from the ANOVA since all genotypes produced only one seed.

Table 2.3 Analysis of variance on eight agronomic traits among 10 soybean genotypes when tested using three silicon concentration and three replications[a].

Source of variation	df	DM		PH		PPP		HSW		DRM		DSM		SY		HI	
		MS	F.Pro	MS	F.Pro	MS	F.Pro	MS	F.Pro	MS	F.Pro	MS	F.Pro	MS	F.Pro	MS	F.Pro.
Replication	2	0.03		510.10		4.01		5.31		0.27		1.08		0.03		0.03	
Genotypes (g)	9	1430.80	*	4729.00	*	14.77	*	40.55	*	1.05	*	5.21	*	0.20	**	0.04	*
Silicon Concentration (SC)	2	862.43	*	6730.80	*	16.88	*	55.03	*	1.38	*	4.14	*	0.42	*	0.28	*
g x SC	18	153.30	*	1274.70	**	2.29	**	18.73	*	0.44	*	1.84	*	0.11	**	0.03	*
Error	58	0.11		928.50		1.62		7.19		0.13		0.71		0.09		0.01	

[a]df=Degrees of freedom, DM=days to 50% maturity, PH=Plant height, PPP=number of pods per plant, HSW=100 seed weight, DRM= dry root mass, DSM= dry shoot mass, SY=seed yield, HI=harvest index, MS=Mean square, F.Pro= F Probability, * and **,=significant difference at 0.05 and 0.10 probability levels, respectively.

Table 2.4 summarizes the mean response of all measurements obtained during experiment I for all treatment combinations.

During experiment I days to flowering was not recorded. For days to 50% maturity genotype L82-1449-II at 250 ppm Si matured the earliest at 54 days (Table 2.4). At 0 and 200 ppm Si this genotype matured at 56 days (Table 2.4) showing significance difference to days to maturity at 250 ppm Si. Genotypes Barc-17, L76-1988 and Williams at 0 ppm Si also matured relatively early at 70 days (Table 2.4) compared to other genotypes that matured later at 95 and 98 days.

Plant height ranged from 96.70-215.90 mm (Table 2.4). The genotype that displayed the lowest plant height was Barc-4 at 200 ppm Si (96.70 mm) (Table 2.4) and the highest height was noted by Williams at 200 ppm Si at (215.90 mm) (Table 2.4). However, several other genotypes produced a high plant height that showed no significant difference to Williams at 200 ppm Si.

In this experiment the number of pods produced per plant ranged from 1-6 (Table 2.4). The genotypes that produced the highest number of pods per plant were Williams at 200 ppm Si and Barc-2 at 200 ppm Si (6 pods per plant) (Table 2.4). While several genotypes produced one pod per plant.

All genotypes produced only one seed per pod.

The 100 seed weight values varied from 5.73-18.59 g (Table 2.4). Barc-17 at 250 ppm Si produced the lowest 100 seed weight (5.73 g) while the highest was recorded by Williams at 200 ppm Si (18.59 g), although the seed weights obtained for genotypes L82-1449-II at 0 ppm Si, Barc-14, Barc-2, L76-1988 and LS 6161 R at 200 ppm Si (15.90, 15.27, 15.87 and 15.15 g/100 seed, respectively) (Table 2.4) showed no significant difference to Williams at 200 ppm Si.

The dry root mass ranged from 0.19-1.61g per plant (Table 2.4). The genotype that produced the lowest dry root mass was Barc-14 at 200 ppm Si (0.19 g/ plant) (Table 2.4) while several other genotypes produced low dry root masses that showed no

significant difference to this genotype. In this experiment the highest dry root mass was obtained from genotype L82-1449-ll at 250 ppm Si (1.61 g/plant) (Table 2.4).

The study showed differences in dry shoot mass ranging from 0.27-3.36 g per plant (Table 2.3). The genotypes with the lowest dry shoot mass were Barc-4 at 0 ppm Si and L76-1988 at 200 ppm (0.27 g) (Table 2.4). The highest dry shoot mass was exhibited by L82-1449-ll at 200 ppm Si (3.36 g) and L82-1449-ll at 250 ppm Si (3.36 g) (Table 2.4). Although Barc-4 at 0 ppm Si and L76-1988 at 200 ppm Si produced the lowest dry shoot mass several other genotypes produced low dry shoot masses without significance differences to these genotypes.

Seed yield of genotypes varied from 0.06-0.98 (g/pot) (Table 2.4). Barc-14 and Barc-17 at 250 ppm Si had the lowest seed yield (0.06 g/pot) (Table 2.4). Whereas Williams at 200 ppm Si had good level of seed yield (0.98 g/ pot) (Table 2.4), with several other genotypes producing high seed yield that were not significantly different to this genotype.

The harvest indices ranged from 0.05-0.58 (Table 2.4). The genotype with the lowest harvest index (0.05) (Table 2.4) was Barc-14 at 250 ppm Si, with several other genotypes producing harvest indices with no significant difference to this genotype. The highest shown by Williams (0.58) (Table 2.4) at 200 ppm Si, with several other genotypes producing harvest indices with no significant difference to this genotype.

The coefficient of variations (CV) in experiment I ranged from 0.40- 23% (Table 2.4). In general low CV values were displayed in this experiment except 100 seed weight at 23%.

Table 2.4 Mean values on eight agronomic traits among 10 soybean genotypes when tested using three silicon concentration[a].

Genotype	SC (ppm)	DM	PH (mm)	PPP	HSW (g/100 seed)	DRM (g/plant)	DSM (g/plant)	SY (g/plant)	HI
Barc-14	0	95 e	167.60 cdefghij	3.00 b	9.49 abcdef	0.40 ab	0.56 abcd	0.37 abc	0.28 cdefghi
Barc-17		70 c	161.20 bcdefgh	1.00 a	8.00 abc	0.25 a	0.83 abcd	0.16 ab	0.14 abcde
Barc-2		94 d	182.90 defghij	2.00 b	12.14 cdefghi	0.26 a	0.80 abcd	0.38 abc	0.28 cdefghi
Barc-4		95 e	135.30 abcd	1.00 a	8.67 abcde	0.30 a	0.27 a	0.22 ab	0.27 bcdefghi
Clark		95 e	168.60 cdefghij	2.00 b	10.50 bcdefgh	0.70 abc	1.58 abcde	0.29 ab	0.19 abcdefgh
L76-1988		70 c	161.80 bcdefgh	1.00 a	8.53 abcd	0.27 a	1.55 abcde	0.38 abc	0.18 abcdefg
L82-1449-ll		56 b	214.60 ij	4.00 c	15.90 ij	0.23 a	0.66 abcd	0.51 abcde	0.37 hij
LS 6161 R		95 e	167.90 cdefghij	1.00 a	10.61 abcdefgh	0.40 ab	0.73 abcd	0.15 ab	0.15 abcdef
Magoye		95 e	170.30 cdefghij	4.00 c	7.84 abc	0.51 abc	0.86 abcd	0.22 ab	0.15 abcdef
Williams		70 c	132.40 abc	2.00 b	9.91 abcdefg	0.25 a	0.43 ab	0.28 ab	0.30 defghi
Barc-14	200	94 d	198.30 ghij	4.00 c	15.27 ij	0.19 a	0.30 ab	0.31 abc	0.36 ghij
Barc-17		94 d	121.80 abc	1.00 a	7.33 ab	0.27 a	0.69 abcd	0.62 bcde	0.30 defghi
Barc-2		95 e	206.70 hij	6.00 e	15.87 ij	0.46 ab	0.75 abcd	0.89 de	0.45 ij
Barc-4		98 g	96.70 a	2.00 b	11.83 cdefghi	0.21 a	0.30 ab	0.25 ab	0.32 efghi
Clark		97 f	142.40 abcdef	1.00 a	13.00 efghi	0.36 ab	0.53 abcd	0.14 ab	0.14 abcde
L76-1988		98 g	191.80 fghij	5.00 d	14.63 hij	0.28 a	0.27 a	0.39 abc	0.35 fghij
L82-1449-ll		56 b	165.90 cdefghi	2.00 b	13.41 fghi	0.23 a	3.36 f	0.46 abcd	0.11 abc
LS 6161 R		95 e	185.30 efghij	3.00 b	15.15 ij	0.28 a	0.36 ab	0.41 abcd	0.36 ghij
Magoye		95 e	155.30 abcdefg	1.00 a	10.16 bcdefg	0.31 a	1.84 cde	0.22 ab	0.30 defghi
Williams		95 e	215.90 j	6.00 e	18.59 j	0.23 a	0.49 abc	0.98 e	0.58 j
Barc-14	250	98 g	152.80 bcdefg	1.00 a	12.10 cdefghi	0.71 abc	1.27 abcde	0.06 a	0.05 a
Barc-17		98 g	125.00 abc	1.00 a	5.73 a	0.40 ab	0.61 abcd	0.06 a	0.07 a
Barc-2		98 g	132.20 abc	1.00 a	13.51 fghi	0.30 a	0.62 abcd	0.08 a	0.09 ab
Barc-4		97 f	184.60 defghij	1.00 a	13.89 ghi	0.94 bcd	0.69 abcd	0.41 abcd	0.13 abcd
Clark		98 g	139.80 abcde	1.00 a	7.28 ab	0.45 ab	0.89 abcd	0.11 a	0.13 abcd
L76-1988		98 g	125.90 abc	2.00 b	12.78 defghi	0.41 ab	0.66 abcd	0.28 ab	0.21 abcdefgh
L82-1449-ll		54 a	154.90 bcdefg	2.00 b	9.80 abcdefg	1.61 e	3.36 f	0.31 abc	0.06 a
LS 6161 R		98 g	112.50 ab	1.00 a	12.92 efghi	0.27 a	1.66 bcde	0.20 ab	0.08 a
Magoye		98 g	171.50 cdefghij	2.00 b	12.44 defghi	0.51 abc	0.86 abcd	0.08 a	0.11 abc
Williams		97 f	144.20 abcdef	1.00 a	12.06 cdefghi	0.36 ab	0.76 abcd	0.16 ab	0.13 abcd
$LSD_{(0.05)}$		0.55	49.80	2.08	4.38	0.60	1.37	0.49	0.19
% CV		0.40	19.10	16.70	23.00	17.50	17.30	9.70	13.70

[a]SC = silicon concentration, DM=days to 50% maturity, PH = plant height, PPP = number of pods per plant, SPP= number of seeds per pod, HSW=100 seed weight, DRM = dry root mass, DSM = dry shoot mass, SY = seed yield, HI = harvest index.

Means in a column followed by the same alphabets are not significantly different at p=0.05.

Table 2.5 presents' the correlations between the observed agronomic traits in experiment one.

In experiment I seed yield had significant positive correlation with plant height, number of pods per plant, hundred seed weight, and harvest index (Table 2.5). While weak negative associations were detected between seed yield with days to maturity, dry root mass and dry shoot mass.

As in seed yield harvest index showed a significant positive correlation with plant height, number of pods per plant, hundred seed weight and seed yield (Table 2.5). HI had a significant negative correlation with dry root mass and dry shoot mass. This suggests that high harvest index is associated with reduced dry root and shoot mass. These associations indicate that increased seed yield and harvest index in the test soybean genotypes could be achieved via selection for increased plant height, number of pods per plant and hundred seed weight.

Table 2.5 Correlation coefficients showing pair wise relationship among seven agronomic traits of soybean during experiment one [a].

Traits	DM	PH	PPP	HSM	DRM	DSM	SY
PH	-0.18						
PPP	-0.02	0.71**					
HSM	0.05	0.58**	0.61**				
DRM	-0.19	0.03	-0.12	-0.14			
DSM	-0.58**	-0.05	-0.21	-0.13	0.50**		
SY	-0.17	0.57**	0.72**	0.52**	-0.11	-0.02	
HI	0.07	0.50**	0.74**	0.48**	-0.37*	-0.44*	0.75**

[a]DM=days to 50% maturity, PH=Plant height, PPP= number of pods per plant, SPP=number of seeds per pod, HSM=100 seed mass, DRM= dry root mass, DSM=dry shoot mass, SY=seed yield, HI=harvest index, MS=Mean square, F.Pro= F Probability, *, **,=significant difference at 0.05 and 0.01 probability levels respectively.

.

Principal component analysis (PCA) was conducted on eight agronomic traits of soybean to reveal the most significant traits in this experiment. Two principal components i.e. PC1 and PC2 (Table 2.6) with Eigenvalues > 1 contributed 69% of the variation in the study. PC1 alone contributed 45% of the variation. This component included five traits i.e. number of pods per plant, seed yield, harvest index, plant height and hundred seed with high values. Whereas PC2 explained 24% of the variation constituting traits such as shoot and root dry matter (Table 2.6). Days to maturity had negative correlation with PC2.

Table 2.6 Principal component analysis with total variances contributed by eight traits in 10 soybean genotypes collected from experiment I.

Trait	Principal components	
	PC1	PC2
Days to flowering	-0.17	-0.76
Plant height	0.82	0.15
Number of pods per plant	0.90	-0.10
Hundred seed weight	0.74	-0.09
Dry root mass	-0.15	0.69
Dry shoot mass	-0.13	0.89
Seed yield	0.87	0.05
Harvest index	0.83	-0.39
Eigenvalues (explained variance)	3.64	1.94
Proportion of total variance (%)	45	24
Cumulative variance (%)	45	69

During experiment II significant interactions were observed ($P<0.05$) between genotype by silicon concentrations for all characters considered in the study (Table 2.7).

Table 2.7 Analysis of variance on 10 agronomic traits among 10 soybean genotypes when tested using three silicon concentration and three replications[a].

Source of variation	df	DF		DM		PH		PPP		SPP		HSM		DRM		DSM		SY		HI	
		MS	F.Pro	MS	F.Pro	MS	F.Pro	MS	F.Pro	MS	F.Pro	MS	F.Pro	MS	F.Pro	MS	F.Pro	MS	F.Pro	MS	F.Pro.
Replication	2	4.30		0.03		2230.00		1.08		0.34		0.20		0.03		0.05		0.40		0.00	
Genotypes (g)	9	226.67	*	1430.80	*	36344.00	*	23.65	*	0.35	*	11.64	*	7.35	*	23.56	*	5.01	*	0.04	*
Silicon Concentration (SC)	2	459.90	*	862.43	*	1142.00	**	18.71	*	7.08	*	98.84	*	6.49	*	23.33	*	10.26	*	0.02	*
g x SC	18	26.26	*	153.30	*	4594.00	*	2.09	*	0.29	**	2.36	**	0.59	*	2.71	*	0.87	*	0.02	*
Error	58	0.92		0.11		2126.00		0.39		0.20		1.69		0.12		0.17		0.13		0.01	

[a]df=Degrees of freedom, DF=days to 50% flowering, DM=days to 50% maturity, PH=Plant height, PPP= number of pods per plant, SPP=number of seeds per pod, HSM=100 seed mass, DRM= dry root mass, DSM=dry shoot mass, SY=seed yield, HI=harvest index, MS=Mean square, F.Pro= F Probability, *, **,=significant difference at 0.05 and 0.10 probability levels respectively.

The mean result obtained during experiment II for each of the selected agronomic traits of 10 soybean genotypes are summarized in Table 2.8.

In experiment ll genotype L82-1449-ll at 0, 200 and 250 ppm Si flowered the earliest at 35 days (Table 2.8). Genotypes Barc-17, L76-1988 and Williams at 0 ppm Si also flowered relatively early at 43 days (Table 2.8) and were significantly different to genotype L82-1449-ll at each level of silicon. Several other genotypes matured late from 49 to 56 days (Table 2.8).

The same trend was noted in days to maturity. Genotype L82-1449-ll at 250 ppm Si matured the earliest at 54 days (Table 2.8). At 0 and 200 ppm Si this genotype matured at 56 days (Table 2.8) which was significantly different from days to maturity at 250 ppm Si. Genotypes Barc-17, L76-1988 and Williams at 0 ppm Si also matured early at 70 days (Table 2.8) which was significantly different from genotype L82-1449-ll at each level of silicon while several genotypes matured later at 95 and 98 days.

In this study the plant height varied from 95.00-440.00 mm (Table 2.8). The genotype with the shortest plant height was Barc-4 at 200 ppm Si (95 mm) (Table 2.8) and the highest was Williams (440 mm) (Table 2.8) at 200 ppm Si, the latter showed no significant difference to genotypes Barc-2 and LS 6161 R at 200 ppm Si (380.00 and 365.00 mm respectively).

Number of pods produced per plant ranged from 1-8 (Table 2.8). The genotypes that produced the highest number of pods per plant were Williams at 200 ppm Si (8 pods per plant) (Table 2.7). With several genotypes producing one pod per plant.

In experiment II the number of seeds produced per pod ranged from 1-3 (Table 2.8). The genotype that produced the most seeds per pod was Williams at 200 ppm Si (3 seeds) (Table 2.8). And several genotypes produced one seed per pod.

The 100 seed weight varied from 4.96-13.25 g (Table 2.8). Although genotype LS 6161 R at 0 ppm Si produced the lowest 100 seed weight value (4.96 g) (Table 2.8) several other genotypes produce low seed weights that showed no significant difference to this genotype. The highest seed weight was exhibited by genotype Williams at 200 ppm Si

(13.25 g) (Table 2.8). However, genotypes Barc-14 and Barc-2 at 200 ppm Si produced high seed weights at 12.05 and 12.30 g, respectively (Table 2.8).

In this experiment the dry root mass varied from 0.17-2.64 g/plant (Table 2.8). Barc-17 at 0 ppm Si, Magoye at 200 ppm Si and Clark at 250 ppm Si produced the lowest dry root masses (0.17g/plant) (Table 2.8) although several other genotypes produced low dry root masses that were not significantly different to these genotypes. And the highest dry root mass was obtained from genotype Magoye at 250 ppm Si (2.64 g/plant) (Table 2.8).

Dry shoot mass ranged from 0.28-4.02 g (Table 2.8). The genotype with the lowest dry shoot mass was LS 6161 R at 200 ppm Si (0.28 g) (Table 2.8) and highest was Magoye at 250 ppm Si (4.02 g) (Table 2.8). LS 6161 R at 200 ppm Si produced the lowest dry shoot mass and several other genotypes produced low dry shoot masses without showing significance differences to this genotype.

The seed yield ranged from 0.05-1.98 g/pot (Table 2.8). The genotype with the lowest seed yield were Barc-14 at 0 ppm Si (0.05 g/pot) and the highest was Barc-2 at 200 ppm Si (1.98 g/pot) (Table 2.8). Although Barc-4 at 0 ppm Si produced the lowest seed yield several other genotypes produced low seed yield that were not significantly different to this genotype and genotype Williams at 200 ppm Si produced a high seed yield (1.98 g/pot) (Table 2.8) that was not significantly different to Barc-2 at 200 ppm Si.

In experiment ll the harvest index varied from 0.06-0.45 (Table 2.8). The highest HI was noted for Williams at 200 ppm Si (0.45) (Table 2.7) while the lowest was found in Barc-14 at 0 ppm Si at (0.06) (Table 2.8). Barc-14 at 250 ppm Si produced the lowest dry shoot similar to several other genotypes (Table 2.8). Genotypes Barc-2, L76-1988 and LS 6161 R at 200 ppm Si produced high harvest indices without showing significant difference to Williams at 200 ppm Si.

The CVs in experiment II for the agronomic traits ranged from 0.40-29.70% (Table 2.8).

Table 2.8 Mean values on 10 agronomic traits among 10 soybean genotypes when tested using three silicon concentration and three replications[a]

Genotype	SC (ppm)	DF	DM	PH (mm)	PPP	SPP	HSM (g/100 seed)	DRM (g/plant)	DSM (g/plant)	SY (g/pot)	HI
Barc-14	0	49 c	95 e	221.20 bcdefg	1.00 a	1.00 a	8.08 cdefghi	0.30 a	0.48 ab	0.05 a	0.06 a
Barc-17		43 c	70 c	189.50 bc	1.00 a	1.00 a	7.47 bcdefg	0.17 a	0.35 a	0.07 a	0.12 ab
Barc-2		49 c	94 d	235.00 bcdefg	1.00 a	1.00 a	6.21 abc	0.72 abcde	1.15 cdef	0.80 bcd	0.30 fghijk
Barc-4		49 c	95 e	95.00 a	1.00 a	1.00 a	6.76 abcde	0.20 a	0.35 a	0.20 a	0.27 efghijk
Clark		49 c	95 e	241.30 bcdefg	1.00 a	1.00 a	5.78 ab	0.30 a	0.55 abcd	0.19 a	0.19 bcdef
L76-1988		43 b	70 c	221.20 bcdefg	1.00 a	1.00 a	6.63 abcde	1.25 ef	2.34 h	1.01 cde	0.22 bcdefgh
L82-1449-II		35 a	56 b	318.30 hijk	6.00 de	1.00 a	10.14 ij	0.31 a	0.60 abcde	0.55 abc	0.37 jkl
LS 6161 R		49 c	95 e	266.70 defgh	5.00 cd	1.00 a	4.96 a	0.30 a	0.72 abcde	0.18 a	0.16 abcd
Magoye		49 c	95 e	291.80 ghij	6.00 de	1.00 a	7.78 bcdefgh	0.50 ab	1.19 def	0.42 ab	0.25 cdefghi
Williams		43 b	70 c	240.50 bcdefg	2.00 ab	1.00 a	6.52 abcd	1.18 cdef	1.26 ef	0.54 abc	0.22 bcdefgh
Barc-14	200	49 c	94 d	351.70 ijk	6.00 de	2.00 b	12.05 jk	1.00 bcdef	1.68 fgh	1.37 def	0.32 hijk
Barc-17		49 c	94 d	177.70 bc	2.00 ab	2.00 b	9.02 fghi	0.55 ab	0.77 abcde	0.47 abc	0.26 defghij
Barc-2		49 c	95 e	380.00 kl	4.00 bc	2.00 b	12.30 k	1.21 def	1.59 fgh	1.98 g	0.38 kl
Barc-4		56 d	98 g	238.30 bcdefg	1.00 a	1.00 a	6.26 abc	1.21 def	2.12 gh	0.57 abc	0.15 abc
Clark		56 d	97 f	208.80 bcde	2.00 ab	2.00 b	8.53 defghi	0.59 ab	1.52 fg	0.39 ab	0.16 abcd
L76-1988		56 d	98 g	215.80 bcdef	2.00 ab	2.00 b	9.72 hi	0.28 a	0.44 ab	0.42 ab	0.37 jkl
L82-1449-II		35 a	56 b	218.50 bcdefg	2.00 ab	2.00 b	8.70 efghi	1.18 cdef	2.18 gh	1.70 fg	0.32 hijk
LS 6161 R		49 c	95 e	365.00 jkl	4.00 bc	2.00 b	6.79 abcde	0.20 a	0.28 a	0.26 ab	0.35 ijkl
Magoye		49 c	95 e	278.30 efghi	4.00 bc	2.00 b	9.23 ghi	0.17 a	0.48 ab	0.18 a	0.22 bcdefgh
Williams		49 c	95 e	440.00 l	8.00 f	3.00 c	13.25 k	0.50 ab	1.19 def	1.42 efg	0.45 l
Barc-14	250	56 d	98 g	280.20 efghi	2.00 ab	1.00 a	7.58 bcdefg	0.27 a	0.48 ab	0.14 a	0.16 abcd
Barc-17		56 d	98 g	196.20 bcd	1.00 a	1.00 a	7.48 bcdefg	0.29 a	0.51 abc	0.26 ab	0.25 cdefghi
Barc-2		56 d	98 g	245.00 cdefgh	1.00 a	1.00 a	6.52 abcd	0.35 a	0.65 abcde	0.42 ab	0.31 ghijk
Barc-4		56 d	97 f	168.00 ab	1.00 a	1.00 a	7.64 bcdefgh	0.42 a	0.79 abcde	0.33 ab	0.22 bcdefgh
Clark		56 d	98 g	287.70 fghi	1.00 a	1.00 a	6.77 abcde	0.17 a	0.34 a	0.27 ab	0.35 ijkl
L76-1988		56 d	98 g	213.00 bcdef	2.00 ab	1.00 a	8.39 defghi	0.40 a	0.67 abcde	0.44 abc	0.30 fghijk
L82-1449-II		35 a	54 a	232.70 bcdefg	2.00 ab	1.00 a	5.99 abc	0.36 a	0.68 abcde	0.26 ab	0.19 bcdef
LS 6161 R		56 d	98 g	270.00 defgh	3.00 b	2.00 b	5.98 abc	0.62 abc	1.19 def	0.43 ab	0.20 bcdefg
Magoye		56 d	98 g	240.80 bdcefg	2.00 ab	1.00 a	7.32 bcdefg	2.64 g	4.02 i	0.55 abc	0.20 bcdefg
Williams		56 d	97 f	273.50 efgh	2.00 ab	1.00 a	6.97 abcdef	0.67 abcd	1.11 bcdef	0.41 ab	0.18 bcde
$LSD_{(0.05)}$		1.57	0.55	75.36	1.02	0.72	2.13	0.56	0.67	0.58	0.11
% CV		1.90	0.40	18.20	26.40	29.70	16.50	4.00	27.90	14.70	27.60

[a]SC = silicon concentration, DF=Days to 50% flowering, DM=Days to 50% maturity, PH = plant height, PPP =number of pods per plant, SPP= number ofseeds per pod, HSM=100 seed mass, DRM =dry root mass, DSM = dry shoot mass, SY = seed yield, HI = harvest index

Means in a column followed by the same alphabets are not significantly different at p=0.05

In experiment II seed yield had significant positive correlation with plant height, number of pods per plant, number seeds per pod, hundred seed weight, dry root mass, dry shoot mass and harvest index (Table 2.9). No or weak associations were found between seed yield with days to maturity, days to 50% flowering and days to 50% maturity. Harvest index showed a significant positive correlation with plant height, number of pods per plant, number of seeds per pod hundred seed weight and see yield (Table 2.9) with the exception of dry root mass and dry shoot mass which show a non-significant weak negative correlation. The non-significant weak correlation was as observed for days to 50% flowering and days to 50% maturity in relation to harvest index. This suggests that high harvest index is associated with reduced dry root and shoot mass. Thus increased seed yield and harvest index could be achieved by selecting increased plant height, number of pods per plant, number of seeds per pod and hundred seed weight. Also high dry root and shoot masses are an indication of increased seed yield.

Table 2.9 Correlation coefficients for pair wise comparison among agronomic traits of soybean during experiment two [a].

Traits	DF	DM	PH	PPP	SPP	HSM	DRM	DSM	SY
DM	0.89**								
PH	-0.10	0.07							
PPP	-0.26	-0.03	0.77**						
SPP	-0.04	0.12	0.50**	0.54**					
HSM	-0.10	-0.03	0.23	0.30	0.38*				
DRM	-0.04	-0.08	0.07	-0.03	0.01	0.12			
DSM	-0.03	-0.08	0.08	0.03	0.07	0.08	0.96**		
SY	-0.31	-0.20	0.48**	0.38*	0.52**	0.48**	0.50**	0.51**	
HI	-0.15	-0.02	0.50**	0.47**	0.51**	0.36	-0.03	-0.05	0.57**

[a]DF= days to 50% flowering, DM=days to 50% maturity, PH=Plant height, PPP= number of pods per plant, SPP=number of seeds per pod, HSM=100 seed mass, DRM= dry root mass, DSM=dry shoot mass, SY=seed yield, HI=harvest index, MS=Mean square, F.Pro= F Probability *, **,=significant difference at 0.05 and 0.01 probability levels respectively.

Data from experiment ll on the 10 agronomic traits of soybean was subjected to principal component analysis in order to identify the most significant traits. Three principal components (PC) were identified contributed to 80% of the variation (Table 2.10). PC1 with 39% of total explained variance correlated well with plant height, number of pods per plant, number of seeds per pod, 100 seed weight, seed yield and harvest index (Table 2.10). Dry root and shoot mass correlated highly and positively with PC2 that 22% of total variance while days to 50% flowering and maturity well with PC3 which explained 19% of total variance (Table 2.10).

Table 2.10 Principal component analysis with total variances contributed by eight agronomic traits among 10 soybean genotypes collected from experiment ll.

Trait	Principal components		
	PC1	PC2	PC3
Days to flowering	-0.11	0.01	0.98
Days to maturity	0.06	-0.07	0.98
Plant height	0.80	0.05	0.03
Number of pods per plant	0.81	-0.06	-0.12
Number of seeds per pod	0.80	0.05	0.10
Hundred seed weight	0.85	0.10	-0.01
Dry root mass	-0.01	0.98	-0.01
Dry shoot mass	0.02	0.98	-0.01
Seed yield	0.66	0.57	-0.22
Harvest index	0.76	-0.03	-0.06
Eigenvalues (explained variance)	3.89	2.19	1.89
Proportion of total variance (%)	39	22	19
Cumulative variance (%)	39	61	80

The combined analysis of variance between experiment I and II indicated a significant differences ($P<0.05$) for the three way interaction i.e. genotype, silicon concentration and experiments on plant height, 100 seed weight, dry root mass, dry shoot mass, seed yield and harvest index. But no significant difference was shown by days to 50% maturity, pods per plant and seeds per pod (Table 2.11).

Table 2.11 Comparison of analysis of variance for observed agronomic traits of 10 selected soybean genotypes at three silicon concentrations during experiments one and two[a]

Source of variation	df	DM		PH		PPP		SPP		HSM		RM		SM		SY		HI	
		MS	F.Pro	MS	F.Pro	MS	F.Pro	MS	F.Pro	MS	F.Pro	MS	F.Pro	MS	F.Pro	MS	F.Pro	MS	F.Pro.
Replication	2	0.07		2055.00		1.62		0.41		1.73		0.15		0.37		0.32		0.02	
Genotypes (g)	9	2861.60	*	32119.00	*	35.85	*	0.28	*	18.02	*	4.64	*	17.76	*	3.34	*	0.04	*
Silicon Concentration (SC)	2	1724.87	*	1189.00	*	16.71	*	3.91	*	117.25	*	3.72	*	16.02	*	6.91	*	0.17	*
Set	1	0	NS	396378.00	*	2.45	NS	8.02	*	632.66	*	3.60	*	6.45	*	8.72	*	0.04	*
g x SC	18	306.60	*	2518.00	**	3.34	*	0.21	**	10.64	*	0.41	*	2.37	*	0.59	*	0.03	*
g x Set	9	0	NS	8954.00	*	1.59	**	0.12	NS	34.17	*	3.76	*	11.02	*	1.87	*	0.03	*
SC x Set	2	0	NS	6683.00	*	14.82	*	3.21	*	36.63	*	4.16	*	11.46	*	3.76	*	0.13	*
g x SC x Set	18	0	NS	3352.00	*	0.88	NS	0.17	NS	10.46	*	0.62	*	2.19	*	0.38	*	0.02	*
Error	118	0.11		1513.00		0.87		0.12		4.43		0.13		0.44		0.11		0.01	

[a]df=Degrees of freedom, DM=days to 50% maturity, PH=Plant height, PPP=number of pods per plant, SPP=number of seeds per pod, HSM=100 seed mass, DRM= dry root mass, DSM=dry shoot mass, SY=seed yield, HI=harvest index, MS=Mean square, F.Pro= F Probability, * and **,=significant difference at 0.05 and 0.10 probability levels respectively.

2.4 DISCUSSION

The response of each of the selected soybean genotypes to the various concentrations of silicon gave different results for most of the agronomic traits observed in each of the two experiments conducted. This is attributed to the genotypic; environmental factors i.e. silicon application, as well as the interaction of the two. These collectively would influence growth and development in the crop (Lersten and Carlson, 2004).

In experiments I and II the days to maturity showed a significant difference amongst the genotypes for each level of silicon used (Table 2.3 and 2.7). This was also seen in days to flowering in experiment two (Table 2.7). Experiments conducted by Miyake and Takahashi (1985) on various soybean genotypes did not show any significant difference for plants that received silicon with those that did not in the early stages of development. These differences may be attributed to the environmental conditions or the naturally flowering and maturing times of each genotype. According to Patterson (1992) conducted experiments on soybean (*Glycine, max* L.) (var. Williams) grown at various temperatures, the reproductive development of soybean is influenced by the day and night temperatures. However, the silicon may have influenced the early stages of development in the experiments. Recent studies conducted by (Li *et al*., 2004) on soybean showed that soil available silicon increased the early stage development i.e. seed germination and seedling growth rate. However this study was not conducted on various soybean genotypes at different levels of silicon.

In experiment II genotypes on average produced high plant height, number of pods produced per plant, number of seeds produced per pod and 100 seed weight at 200 ppm Si which gave high harvest index values (Tables 2.8 and 2.9). These results were also noted in experiment I (Tables 2.3 and 2.5) with the exception of number of seeds produced per pod as all genotypes in experiment one produced one seed. In crops such as sugarcane and maize the application of silicon has proved to be an effective growth promoter (Savant *et al*., 1999; Cengiz *et al*., 2006). It was also found that for experiment I the dry root mass and dry shoot mass resulted in a negative relationship to the harvest index. In both experiments the PCA identified plant height, number of pods per plant, number of seeds per pod, 100 seed weight, seed yield and harvest index

(Table 2.6 and 2.10) as the most important traits that will be useful for future selection of soybean genotypes.

According to Venkateswarlu and Visperas (1987) the leaf of a plant is the potential source from which the fruit will develop. Studies on tomatoes (*Solanum lycopersicum* L.) by (Kang *et al*., 2011) showed that the rate of leaf growth is decreased upon on set of fruit development. This would account for genotypes with high harvest indices due to high seed yield but having a low shoot mass.

Cianzio (2007) reported that the most important trait for soybean is seed yield as it is the most important to farmers. The genotype that produced the highest seed yield values in experiment one was Williams at 200 ppm Si (Table 2.3). In experiment II Williams at 200 ppm also produced a high seed yield of 1.42 g/plant (Table 2.8). However, in experiment II Barc-2 at 200 ppm produced the highest seed yield (1.98 g/plant) (Table 2.8). Genotypes with the highest harvest index value in experiment I was Williams at 200 ppm Si (0.58) (Table 2.3) which was higher than the highest harvest index for experiment two which was for Williams at 200 ppm (0.45) (Table 2.8). The harvest index is a value that is calculated by dividing the seed yield over the sum of the dry biomass and seed yield (Sinclair, 1998). Thus the harvest index values are influenced by the dry root and shoot mass. This would account for genotypes producing a high dry root and shoot masses but have a low harvest index.

Genotypes that produced the highest overall seed yield and harvest index values for the two experiments were Williams and Barc- 2 at 200 ppm Si. Further research is required to determine the response of these 10 genotypes to 200 ppm Si under actual field conditions.

2.5 CONCLUSION

The performances of test soybean genotypes seem to be influenced by the level of silicon as well as the genotype and the interaction between the two. From the results most of the soybean genotypes used showed a positive response in most of the agronomic traits, at a silicon concentration of 200 ppm. Principal component analysis revealed that selection of soybean genotypes should be based on plant height, number of pods per plant, number of seeds per pod, 100 seed weight, seed yield and harvest index as per the findings in this study for the genotypes used. Genotypes, Williams and Barc-2 at 200 ppm Si performed the best compared to other tested soybean genotypes in many of the agronomic traits. These genotypes also produced high seed yield and harvest index. Field experiments are required to determine the genetic diversity of the selected genotypes.

2.6 LITERATURE CITED

AMAZA, P.S., OLAYEMI, J.K., BILA, Y. and IHEANACHO, A. 2007. *Baseline socioeconomic survey report: agriculture in Borno State*, Nigeria, IITA, Nigeria, Pp 3-74.

ARNON, D.I., and STOUT, P.R. 1939. Molybdenum as an essential element for higher plants. Plant Physiology 14: 599-602.

CENGIZ, K., LEVENT. T., and HIGGS, D., 2006. Effect of silicon on plant growth and mineral nutrition of maize grown under water-stress conditions. Journal of Plant Nutrition 29: 1469-1480.

CIANZIO, S.R. 2007. *Breeding major food staples*. (Eds) Kang, M.S., and Priyadarshan, P.M., Blackwell Publishing, UK. Pp 245-250.

DUXBURG, M.R., BIRCH, E.B., and PARSONS, M.J. 1990. *Soybeans in Natal. Agricultural production guidelines for natal.* Department of Agriculture and Development. Natal, RSA.

ESPTEIN, E. 1994. The anomaly of silicon on plant biology. Proceedings of the National Academy of Science 91:11-17.

EPSTEIN, E. 1999. Silicon. Annual Review of Plant Physiology and Plant Molecular Biology 50: 641-664.

EPSTEIN, E and BLOOM, A.J. 2003. Mineral Nutrition of Plants: Principals and Perspectives, (2ed). John Wiley and Sons, New York.

FAO, 2004. The role of soybean in fighting world hunger. (http:// www.fao.org): Accessed: 16 August 2011.

FARGIONE, F., HILL, F., TILMAN, D., POLASKY, S and HAWTHRONE, P. 2008. *Land clearing and the biofuel carbon department.* American Association for the Advancement of Science, New York, Pp 1235-1237.

GENSTAT, 2009. The statistical analyses software (GENSTAT®) 12th Ed, VSN International Ltd., Hemel Hempstead, UK.

KANG, M. Z., YANG, L. L., ZHANG, Z. G., and de REFFYE, P. 2011. Correlation between dynamic tomato fruit-set and sink-source ratio: a common relationship for different plant densities and seasons?. Annals of Botany 5: 805-815.

KUPFER, C and KAHNT, G. 1992. Effects of application of amorphous silica on transpiration and photosynthesis of soybean plants under varied soil and relative air humidity conditions. Journal of Agronomy and Crop Science 168: 318–325.

LERSTEN, N. R., and CARLSON, J.B., 2004. Vegetative morphology. I*n Soybeans: Improvements, Production, and Uses*, (Eds) Boerma, H.R. and Specht, J.E. USA. pp 15-58.

LI, Q., MA, C.C., LI, H.P., XIAO, Y.L., and LUI, X.Y. 2004. Effects of soil available silicon on growth, development and physiological functions of soybean. China Journal of Applied Ecology 15:73-76.

MA, J.F., 2004. Role of silicon in enhancing the resistance of plants to biotic and abiotic stresses. Journal of Soil Science and Plant Nutrition 50: 11-18.

MA, J.F., MIYAKE, Y., and TAKAHASHI, E. 2001. Silicon as a beneficial element for crop plants. In Silicon in Agriculture, (Eds). Datnoff, L.E., Snyder, G.H., and Korndorfer, G.H., Elsevier Science, Amsterdam, Pp 17-39.

MA, J.F., and TAKAHASHI, E. 2002. Soil, fertilizer, and plant silicon research in Japan. Elsevier Publications, Amsterdam, Netherlands, Pp 107–180.

MIYAKE, Y., and TAKAHASHI, E. 1985. Effect of silicon on the growth of soybean plants in a solution culture. Soil Science and Plant Nutrition31: 625–636.

MPEPEREKI, S. 2001. Soybean N_2 fixation: A conceptual model for maximising benefits from research and extension for smallholder farmers. African Crop Science Conference proceedings 5: 861-865.

NAMC, 2011. The South African Soybean Value Chain. The Markets and Economic Research Centre of the National Agricultural Marketing Council. (http://www.namc.co.za/) : Accessed 03 August 2011.

PANDLEY, A.K., and YADAV, R.S. 1999. Effect of antitranspirants on physiological traits and yield of wheat under water deficit conditions. Indian Journal of Agricultural Research 33: 159–164.

PATTERSON, D.T. 1992. Temperature and Canopy development of Velvetleaf (*Abutilontheophrasti*) and Soybean (*Glycine Max*). Weed Science Society of America. Weed Techonology 6:68-70.

SAVANT, N.K., KORNDORFER, G.H., DATNOFF, L.E., and SNYDER, G.H. 1999. Silicon nutrition and sugarcane production: A Review. Journal of Plant Nutrition 22:1853-1903.

SINCLAIR, T. R., 1998. Historical changes in harvest index and crop nitrogen accumulation. Crop Science 38: 638-643.

SOMMER, M., KACZOREK, D., KUZYAKOV Y., and BREUER, J. 2006. Silicon pools and fluxes in soils and landscapes – a review. Journal of Plant Nutrition and Soil Science 169: 310–329.

SPSS BASE., (2001) 11.0 for windows user's guide. Chicago: SPSS Inc

VENKATESWARLU, B. and VISPERAS, R.M. 1897. *Source-sink relationships in crop plants*.IRRI Research Paper Series 125. Pp 19

WILLIAMS, D.E., and VLAMIS, J. 1957. The effect of silicon on yield and manganese-54 uptake and distribution in the leaves of barley plants grown in culture solutions. Plant Physiology 32: 404–409.

CHAPTER THREE

3. RESPONSE OF SELECTED SOYBEAN GENOTYPES TO SILICON AND *Trichoderma harzianum* (ECO-T®) APPLICATIONS

Abstract

Silicon (Si) and *Trichoderma harzianum* have been reported to promote growth and productivity in various important crops such as wheat, rice, and soybeans. This study was conducted to determine the agronomic responses of 10 selected soybean (*Glycine max* L.) genotypes with and without silicon and *Trichoderma harzianum* (Eco-T®) applications. A field experiment was conducted at Ukulinga research farm of the University of KwaZulu-Natal, Pietermaritzburg during 2010/2011 using the randomized complete block design. Data collected included number of days to 50% flowering, 50% maturity, plant height, number of pods per plant, number of seeds per pod, 100 seed weight, root mass, shoot mass, seed yield and harvest index. Results showed significant interactions among genotypes, silicon and Eco-T® applications. Silicon applied at 200 ppm with Eco-T® on average was more effective which enhanced growth, seed yield and high harvest indices on the soybean genotypes. Principal component analysis revealed plant height, number of pods produced per plant, number of seeds per pod, hundred seed weight, seed yield and harvest index as the most influential traits to make selections in soybean genotypes under the applications of silicon and Eco-T® . Significant correlations were found amongst all considered traits with seed yield and harvest indices. Genotypes Williams and LS6161R were the best seed yielders at 63.70 and 56.85 g/plant, respectively when grown with the applications of 200 ppm Si with Eco-T®. A combined use of silicon at 200 ppm and Eco-T® would be effective in increasing the harvest index and seed yield as well as other agronomic traits in soybean.

Keywords: Eco-T®, *Glycine max*, harvest index, seed yield, silicon, soybean, *Trichoderma harzianum*,

3.1 INTRODUCTION

Soybean (*Glycine max* L.) is one of the important food legumes and a valuable component of the traditional cropping systems. China was the largest producer of soybean before the 1950s when USA started extensive production of soybean making the world's top soybean producer preceding Brazil and Argentina (Qiu and Chang, 2010). The world total production of soybean in 2007 was estimated at 220.5 million tons with South Africa producing approximately 205 thousand tons (FAO, 2009).

Soybeans is one of the most important cultivated grain legumes with a high protein content of about 40% and oil content of 20%. Soybean is a valuable protein rich food source. It is also classified as an oilseed. It is used as human food and as a fodder for livestock and in the poultry industry. The soy extracted from soybeans is used in the production of various food items such as milk and cheese. Soybean is increasingly become important in the production of plant based diesels (de Kleijn *et al.*, 2002, Fargione *et al*., 2008).

Soybeans are planted in crop rotation systems with other crops notably cereals to replenish the soil nitrogen levels. This occurrence is due to the symbiotic relationship between legume plants and nitrogen fixing bacteria (*Rhizobia* spp.) in the soil (Duxburg *et al*., 1990). The nodules on the soybean plants are formed when nod factor produced by the nitrogen fixing bacteria induce plant root hairs to curl. The bacteria then attaches to the root hair and is taken up into the curled root forming a nodule. A mutualistic relationship forms between the plant and bacteria as the plant receives nitrogen from the bacteria and the bacteria in turn obtains nutrition from the plant (Tikhonovich, 1995).

In South Africa, there are various factors that limit soybean production. These constraints include poor soil fertility and pests and diseases amongst others (Amaza *et al*., 2007). The development of chemical fertilizers has allowed for the improvement of various food crops. However the continuous use of certain chemicals to enhance crop yields have led to soil pollution, destruction of soil natural profile and the residues that

remain on foods may make these foods unsafe for human consumptions. Thus certain hazardous chemicals would eventually be banned from use. This prompted the development of bio-fertilizers which have little or no effect on the environment since they are developed from organisms or elements already present in the environment (Wagner, 1997).

Trichoderma harzianum is a fungus that is found in most soils and is often used as a biological control agent for other fungi and bacteria. *T. harzianum* has also shown capabilities in the promotion of plant growth. *T. harzianum* can induce resistance, increase plant growth and increase the uptake of nutrients. Studies conducted by Harman and Taylor (1990) on tomato seedlings (*Solanum lycopersicum* L.) showed that this fungus enhanced plant growth and development, increased mineral uptake and increased tolerance to soil borne diseases. Studies conducted on bean (*Phaseolus vulgaris* L.) (Harman *et al*., 2004) cucumber (*Cucumis sativus* L.) (Chang *et al*., 1986) and pepper (*Capsicum annum* L.) (Kleifeld and Chet, 1992) have shown an increase in crop productivity and nutrient uptake in the presence of *Trichoderma* (Windham *et al*., 1986).

T. harzianum is found to increase seed germination rates and emergence in two muskmelon cultivars (Hamed *et al*., 2011). In sweet corn (cv Supersweet Jubilee) it has reportedly increased the number of deep roots providing extra anchorage and allowing uptake of water in deeper soil profiles thus making the plant, to some extent, drought resistant (Harman, 2000). In soils with an ion imbalance this fungus can secret organic acids that resulted in the breakdown of carbon sources providing solubilization of certain cations. In sum, the application of *T. harzianum* has increased soil fertility and crop productivity in crop plants (Harman *et al*., 2004).

Silicon (Si) is an essential element found in most soils worldwide. The plant available silicon in soils in various areas may be very low due to mono-cropping and the regular application of inorganic fertilizers (Ma and Yamaji, 2006). Reports by Epstein (1994) showed that silicon can be used to reduce biotic, abiotic and chemical stresses on

plants. Further it improves the resistance of plants to diseases and pests by physical and physiological means (Epstein, 1999). Previously silicon was not considered as an essential nutrient for growth. However research on silicon applications in crops such as sugar cane showed a marked difference in growth among different genotypes (Savant *et al*., 1999). Studies on silicon using various food crops such as rice and oat (Hossain, 2002), wheat (Pandley, Yadav, 1999), soybeans (Kupfer and Kahnt, 1992) and cowpeas (Mali and Aery, 2008) indicated a significant increase in plant growth and productivity. However, there is limited information on the simultaneous application of silicon and *T. harzianum* to enhance growth and productivity in soybean or other food security crops. Thus, the objective of this study was to determine the responses of 10 selected soybean genotypes with combined applications of silicon and *T. harzianum* (Eco-T®) under field conditions. Suitable genotypes would be identified with enhanced growth and productivity under silicon, Eco-T® or combined applications.

3.2 MATERIALS AND METHOD

3.2.1 Field study, plant materials and treatments

An irrigated field trial was carried out during 2010/2011 at Ukulinga research farm of the University of KwaZulu-Natal, Pietermaritzburg. The pedigree and seed source of 10 selected soybean genotypes used in this trial are indicated in Chapter 2 section 2.2.1. Genotypes were treated with four different treatments comprising two silicon (Si) levels (0 and 200 ppm) and two levels of Eco-T® (with and without) The treatments involving Si was prepared using soluble silica fortified with potassium (AgriSil™). This silicon was obtained from PQ Silicas South Africa. The solution of potassium silicate (KSi) was made up of silicon at 9.8% of the solution. This percentage was used to calculate and prepare the Si treatment using water to achieve the preferred concentration.

3.2.2 Preparation of silicon concentration

The potassium silicate (KSi) solution contained 9.8% silicon. Therefore in 100 ml of potassium silicate the concentration of silicon is 9.8 ml. The concentration of the silicon treatment was measured in parts per million (ppm). Water was used to dilute the KSi to the preferred silicon concentration i.e. 200 ppm. The amount of potassium silicate used for each concentration was calculated using the formula: $C_1V_1=C_2V_2$. The initial concentration of silicon (C_1) in the potassium silicate solution was 9.8 $x10^4$ ppm and the initial volume was (V_1). The concentration of silicon that is required is (C_2) 100 ppm and the volume that is required is (V_2) 10000 ml. This means that 10.2 ml of KSi when added to 10L of liquid (water) would give a solution that contained silicon in the concentration of 100ppm.

Therefore 20.4 ml of KSi was used to make up the 200ppm treatment. The silicon treatment was prepared as required using measuring equipment.

3.2.3 Seed treatment with *Trichoderma harzianum* (Eco-T®)

A guar gum sticker solution was prepared dissolving 1.5 g of guar gum in 1 liter of sterilized water. This solution was allowed to stir for 1 hr on a magnetic stirrer at 150 rpm. Approximately 1 ml of the sticker solution was placed in beaker with 128 soybean seeds (± 27.56 g). The beaker was swirled to ensure all the seeds were coated with the sticker. After 30 minutes 0.03 g of the commercial strain of *T. harzianum* (Eco-T®), obtained from Plant Health Products, South Africa was sprinkled over the seeds and swirled again. The Eco-T® coated soybean seeds were placed in sterilized Petri dishes and allowed to air dry overnight in the laminar flow for approximately 12-18 hrs. This procedure was carried out for each of the 10 soybean genotypes as indicated in Figure 3.1.

Figure 3.1. Conical flasks with seeds of the 10 soybean genotypes treated with Eco-T®

3.2.4 Experimental design, planting and application of fertilizer

A 10x2x2 factorial experiment was conducted using the randomized complete block design with two replications. The three factors are the ten genotypes, the two levels of Si (0 and 200 ppm), and the two levels of Eco-T® (with and without), respectively. Seeds from the 40 treatment combination were sown at Ukulinga research farm on 15 x 22 m plot. The plants were grown in two blocks with an inter row spacing of 50 cm and an intra row spacing of 10 cm with a population density of 20 000 plants/ha. The seeds were planted at a depth of 2.5 cm and each treatment combination was planted over two rows. A total of 256 seeds were planted per genotype.

Trials were fertilized by broadcasting pellets containing Nitrogen, Phosphorus and Potassium (NPK), in the ratio (2:3:4). The trial received approximately 20kg/ha of this fertilizer following soil analysis provided by The Fertilizer Advisory Service, KwaZulu-Natal Department of Agriculture and Environmental Affairs, Soil Fertility and Analytical Services, Pietermaritzburg from testing three randomly taken field soil samples (Table 3.1). The established trial is depicted in Figure 3.2.

Table 3.1 Nutrient and Lime recommendations from The Fertilizer Advisory Service

Sample	P (mg/L)	K (mg/L)	Ca (mg/L)	Mg (mg/L)	Exchange acidity (cmol/L)	Total cations (cmol/L)	Acid saturation %	pH (KCl)	Zn (mg/L)	Mn (mg/L)	Cu (mg/L)	MIR clay %	MIR organic C %	MIR N %
A	36	408	1882	667	0.07	15.99	0	4.41	2.8	24	7.9	39	1.9	0.31
B	21	264	2976	848	0.03	18.04	0	4.56	1.9	11	5.5	46	1.9	0.32
C	35	424	1824	619	0.10	15.38	1	4.34	2.6	26	7.6	39	1.7	0.30

EA=exchange acidity; TC=total cations; AS=acid saturation; MIR=mid-infrared

Figure 3.2. Ten week old soybean plants grown at Ukulinga research farm, Pietermaritzburg.

3.2.5 Application of silicon

Silicon at 200 ppm was applied before flowering at five intervals, i.e. 2, 7, 10, 39 and 46 days after plating to enhance growth early flowering. The silicon aqueous solution was prepared in 20 liter plastic buckets and two liters of the solution was drenched evenly over each row which was 70 cm in length with 8 plants per row thus approximately 500 ml was applied per plant.

An irrigation system was implemented for the entire trial period. Plants were watered every alternate three day for two hours for the first month subsequent to planting. Thereafter water was reduced to approximately one hour once a week for the remainder of the trial, conditional to rainfall.

3.2.6 Data collection and analysis

The following agronomic traits of soybean were collected and observed for each of the 10 genotypes. Days to 50% flowering was recorded when 50% of the plants flowered in each genotype-treatment combination. Days to 50% maturity was taken when 50% of the plants reach maturity in each genotype-treatment combination. At maturity the following agronomic traits were collected from eight randomly selected plants in the middle rows. The plant height was measured in centimeters (cm) taken from soil surface to plant apex. The number of pods produced per plant and the number of seeds per pod were recorded at maturity. The seed yield (g/plant) was established by collecting and weighing the seeds produced. The weight of hundred seeds (in g/100 seed) was obtained from randomly sampled hundred seeds. Eight sample plants were removed at random for each genotype with each treatment at week 6 to observe the number of nodules produced. Active nodules were determined by cutting a cross section through the nodules, active nodules appeared pink to reddish brown and inactive nodules were whitish brown. The dry root mass and dry shoot masses were obtained by cutting the roots at the rhizoshere and aerial parts (shoots) of the plant respectively. The roots and shoots were dried for approximately 72 hours in a LABOTEC TERM-O-MAT at 70^{o}C (Labotec Oven, Model number 385, South Africa) oven, before weighing to find out the dry root mass (g/plant) and shoot mass (g/plant)

which make up the dry biomass of a plant. The harvest index was then calculated by dividing the seed yield by the sum of the dry biomass and the seed yield.

The data collected for was analyzed using GenStat (Genstat, 2009) to detect significant interactions. Correlation analysis was conducted using the Pearson model on SPSS (2001) to test the relationship between the traits. Principal component analysis was conducted using SPSS (2001). Principal component analysis was used to identify which of the traits contributed to most of the variation among the genotypes. This method of analysis is useful when there are a number of correlated traits involved in the study (Johns et al., 1997).

3.3 RESULTS

The analysis of variance indicated significant interactions among genotypes, Silicon and Eco-T (E) applications on all traits considered in the study (Table 3.2).

A summary of the mean data collected for the selected traits are shown in Table 3.3 for all treatment combinations. Results revealed that days to 50% flowering ranged from 17 to 35 days (Table 3.3). Genotype L82-1449-ll flowered significantly earlier for all treatments between 17 and 19 days (Table 3.3) although treatments with the combination of 200 ppm Si and Eco-T® and Eco-T® alone showed no significant difference at 19 days (Table 3.3). Genotypes Barc-17, L76-1988 and Williams flowered between 20 and 24 days for all treatments (Table 3.3). Several genotypes flowered significantly late between 30 and 35 days (Table 3.3), for all treatment combinations.

The number of days to 50% maturity varied from 50-72 days (Table 3.3), genotype L82-1449-ll matured earlier between 50 and 51 days (Table 3.3) at all treatments which was significantly different to several other genotypes with late maturity. Genotypes Barc-4, Clark and L76-1988 matured relatively late between 70 and 72 days when treated with Si and Eco-T® applications (Table 3.3).

In the experiment mean plant height ranged from 423.50-1157.40 cm. Genotype Barc-17 without Si and Eco-T® had the shortest plant height of 423.50 cm (Table 3.3). This genotype also produced relatively short plant heights for the other treatments. Several

other genotype-treatment combinations (e.g. L82-1449-ll without Si and Eco-T®, Barc-4 and Clark with Si and without Eco-T®) displayed reduced plant heights. Magoye, a landrace soybean genotype, had the highest plant height at 1157.40 cm when grown with combined application of Si and Eco-T® (Table 3.3).

In this study the number of pods produced per plant ranged from 46 to 176 (Table 3.3). Genotypes L82-1449-11 with Eco-T® application produced the lowest number of pods per plant (46) (Table 3.3). Also, genotypes Barc-17 and Barc-14 without Si and Eco-T® produced a low number of pods per plant at 48 and 52, respectively (Table 3.3). These genotypes displayed no significant difference to genotype L82-1449-11 when grown with Eco-T®. The highest number of pods was achieved by genotypes Magoye and LS 6161 R at 176 and 162 pods/plant, respectively, without significant differences when subjected to combine treatment of Si and Eco-T®.

An average of 2 seeds was produced per pod (Table 3.3) for the following treatments for all genotypes; without Si and Eco-T®, with Si and without Eco-T® and without Si and with Eco-T®. Genotypes grown in the presence of the combination treatment of Si and Eco-T® produced on average 3 seeds per pod (Table 3.3).

The present findings indicated that the dry shoot mass values varied from 6.29-41.31 g per plant (Table 3.3). The lowest dry shoot mass was obtained from genotype Barc- 2 grown with Eco-T® (6.29 g/plant) (Table 3.3). Several other genotypes had low dry matter content and showed no significance difference to this genotype. The highest dry matter was obtained by genotype L82-1449-ll grown with Eco-T® (41.31g/plant) which showed no significant difference to genotype Barc-4 (36.36g/plant) grown without Si and Eco-T® applications (Table 3.3).

Table 3.2 Analysis of variance on 12 agronomic traits among 10 soybean genotypes when tested with two levels of Si and two levels of Eco-T®using two replications[a,b]

Source of variation	DF	DFL	DM	PH	PPP	SPP	HSW	TND	AND	DRM	DSM	SY	HI
		MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS
Block	1	0.05	12.80	40545.00	23.70	0.03	0.34	1.51	1.51	62.61	38.84	13.09	0.03
Genotype (G)	9	989.03*	582.69*	530279.00*	21271.90*	0.24**	0.24*	1813.7*	2041.74*	542.66*	2582.68*	2316.60*	0.71*
Silicon (Si)	1	18.05*	156.80*	824586.00*	27695.40*	8.78*	8.78*	2453.1*	877.81*	1.56**	156.28*	2588.25*	0.08*
Eco-T (E)	1	6.05*	72.20*	1121248.00*	37562.80*	6.33*	6.33*	31.25*	74.11*	16.64**	125.71**	9358.19*	0.51*
G x Si	9	156.05*	192.91*	89262.00*	2438.70*	0.26**	0.26*	65.02*	94.01*	43.45*	230.39*	549.82*	0.03*
G x E	9	132.72*	208.09*	80449.00*	2727.70*	0.21**	0.21*	6.55*	35.79*	49.62*	284.08*	607.12*	0.07*
Si x E	1	1.25**	24.20*	212695.00*	8978.20*	2.63*	2.63**	57.80*	13.61*	23.17**	303.40**	85.108**	0.003**
G x Six E	9	111.25*	206.76*	31877.00*	1757.00*	0.20**	0.20*	5.61*	22.96*	68.89*	357.28*	575.31*	0.01**
Error	279	0.40	0.98	5702.00	176.60	0.18	0.18	0.51	0.53	10.50	38.45	63.90	0.01

[a]DF=Degrees of freedom, DFL=days to 50% flowering, DM=days to 50% maturity, PH=Plant height, PPP=number of pods per plant, SPP=number of seeds per plant, HSW=100 seed weight , TND.= total number of nodules per plant, AND.=Number of active nodules per plant, DRM= dry root mass, DSM= dry shoot mass, SY=seed yield, HI=harvest index, MS=Mean square,

[b], * and ** denote significant differences at 0.05 and 0.01 probability levels, respectively

Dry shoot mass varied from 6.29-41.31 g/plant (Table 3.3). The lowest dry shoot mass was obtained from genotype Barc- 2 with Eco-T® (6.29 g/plant) (Table 3.3) while several other genotypes displayed low values without significance difference to this genotype. The highest dry shoot mass was obtained by genotype L82-1449-ll (41.31 g/plant) with Eco-T® which showed no significance difference to genotype Barc-4 that yielded 36.36 g/plant without Si and Eco-T® (Table 3.3).

The seed yield exhibited by the genotypes ranged from 12.29-63.70 g/plant (Table 3.3). Several genotypes produced low seed yield while the minimum was obtained in genotype Barc-17 when grown without Si and Eco-T® (12.29 g/plant). The highest mean seed yield was recorded from genotype Williams (63.70 g/plant) and LS 6161 R (56.84 g/plant) when subjected to a combined treatment of Si and Eco-T® (Table 3.3).

In this study the harvest index values ranged from 0.25-0.86 (Table 3.3). Genotypes Barc-17 with the following treatments: without Si and Eco-T® and with Eco-T® and without Si and Barc-4 without Si and Eco-T® gave the lowest harvest index values at 0.25, 0.33 and 0.33, respectively (Table 3.3). While the highest was achieved by genotypes Williams, LS 6161 R and Barc-2 with combined treatment of Si and Eco-T® at 0.86, 0.83 and 0.80, respectively (Table 3.3).

The coefficient of variations (CV) in the experiment ranged from 1.60-36.70% (Table 3.3). In the study characters such as dry root mass, dry shoot mass and seed yield had relatively high CVs at 36.70, 34.60, and 21.90%, respectively. Other traits had low CVs in this experiment (Table 3.3).

Table 3.3 Mean values on 12 agronomic traits among 10 soybean genotypes when grown with the application of Silicon and *Trichoderma harzianum*[a].

Genotype	Si	*T*	Trait											
			DF	DM	PH	PPP	SPP	HSW	T ND	AND	DRM	DSM	SY	HI
Barc- 17	0	-	22 f	57 e	423.50 a	48 ab	2 a	14.08 a	25 n	22 o	14.84 mno	28.86 mnop	12.29 a	0.25 a
Barc-14	0	-	34 m	67 n	627.90 fghijk	52 abc	2 a	18.53 bcde	27 o	25 q	6.50 abcdef	8.36 abcde	36.58 klmno	0.72 opqrs
Barc-2	0	-	32 k	62 i	656.90 hijkl	92 ijk	2 a	19.73 efghijk	23 m	18 l	8.16 cdefgh	16.56 fghij	41.79 nopqrst	0.63 klmn
Barc-4	0	-	34 m	67 n	606.00 defghij	63 def	2 a	13.43 a	19 i	7 d	16.29 op	36.36 qr	24.08 cdefg	0.33 ab
Clark	0	-	34 m	66 m	625.00 efghijk	81 ghi	2 a	13.09 a	0 a	0 a	13.31 klmno	28.09 lmno	33.03 ijklm	0.44 defg
L76-1988	0	-	23 g	58 f	552.50 cde	72 efg	2 a	17.01 b	18 h	8 e	5.00 abc	14.05 efghi	18.97 abcd	0.50 fgh
L82-1449-II	0	-	17 a	50 a	488.80 abc	69 efg	2 a	18.31 bcde	19 i	8 e	9.68 fghij	19.65 ijk	26.02 defghi	0.48 efgh
LS 6161 R	0	-	35 n	65 l	651.10 ghijk	89 ijk	2 a	19.25 defghi	28 p	26 r	6.00 abcd	12.63 bcdefg	38.01 lmnopq	0.66 lmnop
Magoye	0	-	34 m	64 k	655.20 hijk	93 ijkl	2 a	21.11 jklm	27 o	25 q	4.41 ab	8.94 abcde	24.65 cdefgh	0.67 lmnopq
Williams	0	-	23 g	58 f	664.60 ijkl	93 ijkl	2 a	20.5 fghijkl	15 e	10 g	4.45 ab	9.18 abcde	31.82 ghijkl	0.70 nopqr
Barc- 17	0	+	20 d	58 f	468.10 ab	73 efgh	2 a	14.08 a	25 n	23 p	16.42 op	34.66 pq	27.49 efghij	0.35 bc
Barc-14	0	+	35 n	68 o	675.00 jkl	53 abcd	2 a	19.73 efghijk	28 p	26 r	6.78 abcdefg	13.26 defgh	23.03 bcdef	0.56 hijk
Barc-2	0	+	33 l	63 j	648.10 ghijk	102 klmn	2 a	20.69 ghijkl	23 m	8 e	3.61 a	6.29 a	32.16 hijkl	0.76 rst
Barc-4	0	+	33 l	67 n	595.40 defghi	91 ijk	2 a	20.60 fghijkl	19 i	7 d	14.58 lmno	34.09 opq	37.47 lmnop	0.43 cdef
Clark	0	+	34 m	68 o	611.00 defghij	73 efgh	2 a	20.78 hijkl	0 a	0 a	4.89 ab	8.46 abcde	37.89 lmnopq	0.74 pqrs
L76-1988	0	+	24 h	58 f	577.50 defg	72 efg	2 a	18.39 bcde	18 h	7 d	9.32 efghi	20.01 ijk	44.64 pqrstu	0.60 jklm
L82-1449-II	0	+	19 c	51 b	580.90 defg	46 a	2 a	19.57 efghij	19 i	6 c	18.51 p	41.31 r	48.62 stuv	0.45 defg
LS 6161 R	0	+	30 i	59 g	692.50 klm	113 no	2 a	22.56 mno	27 o	25 q	5.29 abc	10.52 abcdef	41.14 nopqrs	0.72 opqrs
Magoye	0	+	35 n	67 n	1016.10 p	136 p	2 a	18.31 bcde	27 o	25 q	5.62 abc	11.37 abcdef	47.29 rstuv	0.74 pqrs
Williams	0	+	22 f	58 f	755.10 mn	105 lmn	2 a	22.56 mno	15 e	9 f	4.42 ab	9.66 abcde	45.35 qrstu	0.76 rst
Barc- 17	200	-	35 n	68 o	488.10 abc	61 cdef	2 a	17.36 bc	23 m	20 n	12.70 jklm	26.2 lmn	17.82 abc	0.33 ab
Barc-14	200	-	33 l	66 m	586.10 defgh	60 bcde	2 a	18.94 cdef	22 l	20 n	5.01 abc	9.27 abcde	27.75 efghij	0.65 lmno
Barc-2	200	-	34 n	67 n	756 .00 mn	119 o	2 a	21.33 klmn	19 i	11 h	6.44 abcde	12.75 cdefg	40.23 mnopqr	0.68 mnopqr
Barc-4	200	-	33l	57 e	543.60 cd	74 fgh	2 a	17.53 bcd	15 e	8 e	12.77 jklmn	26.16 lmn	20.09 abcde	0.38 bcd
Clark	200	-	21 e	56 d	556.40 cdef	66 def	2 a	19.18 defghi	0 a	0 a	11.53 ijkl	22.64 jkl	22.98 bcdef	0.40 bcde
L76-1988	200	-	23 g	55 c	642.10 ghijk	72 efg	2 a	19.08 cdefgh	12 d	6 c	14.15 klmno	31.48 nopq	49.53 tuvw	0.52 ghij
L82-1449-II	200	-	18 b	51 b	585.20 defgh	69 efg	2 a	18.99 cdefg	10 c	7 d	15.95 nop	33.27 opq	50.02 uvw	0.51 fghi
LS 6161 R	200	-	34 m	64 k	649.40 ghijk	107 no	2 a	22.2 lmno	21 k	19 m	4.10 ab	7.37 abcd	29.45 fghijk	0.72 opqrs
Magoye	200	-	35 n	68 o	812.90 no	109 mno	2 a	18.99 cdefg	17 g	14 i	5.36 abc	10.79 abcdef	38.81 lmnopq	0.71 nopqr
Williams	200	-	35 n	69 p	831.30 o	96 jklm	2 a	22.9 no	12 d	9 f	4.61 ab	8.27 abcde	37.14 klmnop	0.74 pqrs
Barc- 17	200	+	23 g	60 h	538.80 bcd	85 hij	3 b	20.83 ijkl	20 j	17 k	9.81 ghij	18.82 hijk	15.17 ab	0.37 bcd
Barc-14	200	+	34 m	57 e	840.60 o	95 jklm	3 b	23.10 o	22 l	19 m	8.86 defghi	17.74 ghijk	32.80 ijklm	0.59 ijkl
Barc-2	200	+	34 m	68 o	1003.10 p	157 q	3 b	25.53 p	17 g	9 f	3.65 a	6.58 ab	41.98 nopqrst	0.80 stu
Barc-4	200	+	35 n	70 q	586.20 defgh	64 cdef	3 b	20.69 ghijkl	10 c	8 e	12.86 jklmn	23.58 klm	34.31 jklmn	0.49 efgh
Clark	200	+	31 j	70 q	650.60 ghijk	86 hij	3 b	20.78 hijkl	0 a	0 a	11.25 hijk	22.31 jkl	44.07 opqrstu	0.56 hijk
L76-1988	200	+	22 f	73 r	989.20 p	72 efg	3 b	18.39 bcde	10 c	7 d	7.16 bcdefg	14.34 efghi	54.60 vw	0.71 nopqr
L82-1449-II	200	+	19 c	51 b	605.90 defghij	92 ijk	3 b	19.57 efghij	9 b	5 b	15.55 mnop	29.96 nop	54.02 vw	0.54 hij
LS 6161 R	200	+	32 k	62 i	730.50 lm	169 qr	3 b	25.53 p	21 k	19 m	4.37 ab	7.19 abcd	56.84 wx	0.83 tu
Magoye	200	+	35 n	62 i	1157.40 q	162 q	3 b	22.2 lmno	18 h	16 j	5.33 abc	8.79 abcde	54.78 vw	0.75 qrst
Williams	200	+	22 f	63 j	1048.20 p	176 r	3 b	25.94 p	10 c	6 c	3.84 a	6.86 abc	63.70 x	0.86 u
$LSD_{(0.05)}$			0.62	0.97	74.33	13.08	0.42	1.73	0.70	0.72	3.19	6.10	7.87	0.09
% CV			2.20	1.60	11.10	14.70	16.40	8.90	4.1	5.8	36.70	34.60	21.90	14.70

[a]Si= silicon concentration (ppm); Eco-T® (- =without , +=with), DF=days to 50% flowering, DM=days to 50% maturity, PH = plant height (cm), PPP = number of pods per plant, SPP= number of seeds per pod, HSW=100 seed weight (gram), DRM = dry root mass (g./plant), TND= total number of nodules per plant, AND.=Number of active nodules per plant, DSM = dry shoot mass (g/plant), SY = seed yield (g/plant), HI = harvest index.

[b]Means in a column followed by the same alphabets are not significantly different at p=0.05.

Table 3.4 shows pair-wise correlations among agronomic traits during the trial. Seed yield showed a significant positive correlation with other traits including plant height, number of pods per plant, number of seeds per pod, hundred seed weight and harvest index (Table 3.4). Weak associations were found between seed yield and days to 50% flowering. Further negative associations were observed between seed yield days to 50% maturity, total number of nodules per plant, number of active nodules per plant, dry root mass and dry shoot mass (Table 3.4).

Strong and significant correlations were noted between harvest index and plant height, number of pods produced per plant, number of seeds produced per pod, hundred seed weight and seed yield. Weak associations were noted for the total number of nodules per plant and number of active nodules produced (Table 3.4). Days to 50 % flowering and days to 50% maturity had positive associations with harvest index. Also a strong negative correlation existed among dry root and shoot masses with the harvest index (Table 3.4).

Therefore the above associations indicate high seed yield and harvest index values are associated with increased plant height, number of pods per plant, number of seeds per plant and hundred seed weight.

Table 3.4 Correlation coefficients showing pair-wise relationship among 12 selected traits on soybean when grown with the application of Silicon and *Trichoderma harzianum* [a]

Traits	DF	DM	PH	PPP	SPP	HSW	TND	AND	DRM	DSM	SY
DF											
DM	0.76**										
PH	0.31	0.36*									
PPP	0.27	0.15	0.76**								
SPP	0.18	0.20	0.47**	0.52**							
HSW	0.21	0.10	0.56**	0.69**	0.62**						
TND	0.25	-0.01	-0.03	0.04	-0.21	-0.01					
AND	0.35*	0.13	0.04	0.08	-0.13	0.02	0.88**				
DRM	-0.42**	-0.37*	-0.54**	-0.56**	-0.44**	-0.60**	-0.20	-0.28			
DSM	-0.43**	-0.39*	-0.54**	-0.55**	-0.45**	-0.60**	-0.19	-0.30	0.99**		
SY	-0.06	0.01	0.64**	0.56**	0.49**	0.49**	-0.24	-0.22	-0.15	-0.15	
HI	0.35*	0.31	0.72**	0.69**	0.58**	0.72**	0.06	0.11	-0.84**	-0.84**	0.62**

[a]DF= days to 50% flowering, DM=days to 50% maturity, PH=Plant height, PPP= number of pods per plant, SPP=number of seeds per pod, HSW=100 seed mass, TND= total number of nodules per plant, AND= Number of active nodules per plant, DRM= dry root mass, DSM=dry shoot mass, SY=seed yield, HI=harvest index

[b], * and ** denote significant differences at 0.05 and 0.01 probability levels, respectively.

Data on 12 agronomic traits of soybean were subjected to the principal component analysis (PCA) to show the most significant traits in this study. Three principal components i.e. PC1, PC2 and PC3 (Table 3.5) contributed 79% of the variation in the study. PC1 alone contributed 46% of the variation which was correlated well with plant height, number of pods per plant, number of seeds per pod, 100 seed weight, seed yield and harvest index. PC2 explained 21% of the variation and correlated with total nodules per plant and number of active nodules (Table 3.5). Days to 50% flowering and days to 50% maturity were represented in PC3 that contributed to 12% of the variation (Table 3.5). Thus, plant height, number of pods produced per plant, number of seeds produced per pod, 100 seed weight, seed yield and harvest are important in selection of soybean genotypes when tested with the application of Silicon and *T. harzianum.*

Table 3.5 Principal component (PC) scores, eigenvalues, total and cumulative variances for 12 traits soybean genotypes when grown with the application of Silicon and *Trichoderma harzianum*.

	Principal Component		
Traits	PC1	PC2	PC3
Days to flowering	0.16	0.22	0.86
Days to maturity	0.11	-0.06	0.94
Plant height	0.79	-0.05	0.22
Number of pods per plant	0.85	0.06	0.06
Number of seeds per pod	0.71	-0.24	0.13
Hundred seed weight	0.85	0.04	0.02
Number of nodules	-0.04	0.94	0.01
Number of active nodules	0.01	0.93	0.14
Dry shoot mass	-0.71	-0.34	-0.42
Dry root mass	-0.71	-0.34	-0.41
Seed yield	0.72	-0.33	-0.21
Harvest index	0.91	0.11	0.24
Eigenvalues (explained variance)	5.47	2.47	1.38
Proportion of total variance (%)	46	21	12
Cumulative variance (%)	46	67	79

3.4 DISCUSSION

The present study identified significant interactions among genotypes, Silicon and *Trichoderma harzianum* applications on 12 important agronomic traits in soybean. (Table 3.2). This provided differential responses of the tested genotypes to the application of Silicon and *Trichoderma harzianum.* According to Lersten and Carlson (2004) this may have been due to the influence of the growing environment and the genetic compositions of tested varieties.

Genotype L82-1449-ll flowered the earliest at 17 days and matured the earliest between 50-51 days (Table 3.3). This early flowering and maturing may be due to the natural flowering and maturing dates rather than response due to the treatments as this general trend is noted for this genotype for all treatment combinations. Genotypes; Barc-14 and Clark without Si and with Eco-T®, Barc-17 and Magoye with Si and without Eco-T®, and Barc-2 with Si and Eco-T® matured late at 68 days (Table 3.3).

During this trial it was noted that genotypes that received silicon or Eco-T® singly or in combinations produced on average increased plant height, number of pods per plant; hundred seed weight; seed yield and harvest indices when compared to the control (Table 3.3). Research done by Kupfer and Kahnt (1992) and Pandley and Yadave (1999) showed that the application of silicon to wheat, soybean, rice and maize have displayed an increase in crop productivity in these crops. In addition Eco-T® has the ability to increase soil fertility, create competition with pathogens on roots and control pathogens by various means (Harman *et al*., 2004). Studies conducted on crops such as corn and tomato have shown a marked increase in productivity when *Trichoderma harzianum* was applied (Björkman *et al*., 1998, Gravel *et al*., 2007). The above mentioned may have been the contributing factors to the increase of the above mentioned traits when Eco-T® was applied. The combined application of Si and Eco-T® provided the highest performance of the studied traits (Table 3.3). This result may be due to the growth promoting nature of silicon and Eco-T®. Also all genotypes that received combined treatment of Si and Eco-T® produced a mean of three seeds per pod (Table 3.3) whereas only 2 seeds were produced by the other treatments (Table 3.3).

The number of total and active nodules produced by genotypes varied significantly. Genotypes that received treatments without Si produced on average a higher number of total nodules formed as well as a higher number of and active nodules. This reduction in nodulation formation was also noted by Mali and Aery (2008) on cowpea (*Vigna unguiculata*) when silicon was applied at concentrations higher than 100 ppm.

In the study high seed yield and harvest index values were positively associated with high plant height, number of pods per plant, number of seeds per pod and hundred seed weight (Table 3.4). High harvest index values were also observed with strong negative correlations to dry root and shoot masses (Table 3.4).

The result from the principle component analysis (PCA) revealed that the components that contributed to most of the variation were plant height, number of pods produced per plant, number of seeds produced per pod, hundred seed weight, seed yield and harvest index (Table 3.5). Therefore for the 10 soybean genotypes used the above mentioned traits would be most important for the selection of genotypes in the presence of Si at 200 ppm with Eco-T®.

Among the tested 10 soybean genotypes Williams and LS 6161 R yielded best at 63.70, and 56.85 g/plant, respectively when grown with combined application of Si and Eco-T®. Also the genotypes had high harvest indices at 0.86 and 0.83, respectively under this treatment (Table 3.3).

Silicon and *Trichoderma harzianum* (Eco-T®) has previously only been used individually to control crop pests such as fungi and to increase crop yields in crops such as rice (Hossain, 2002) and pepper (Kleifeld and Chet, 1992). However, there is no prior research on the combined use of silicon and Eco-T® on food security crops such as soybean. Therefore the results obtained constitutes a primary study on the use of both silicon and Eco-T® used in combination on 10 selected soybean genotypes to determine the response on growth and nodulation formation.

3.5 CONCLUSION

The genotypes on average had a positive response to most of the agronomic traits observed when subjected to Si, Eco-T® singly or in combinations. However the combined treatment of Si and Eco-T® gave increased positive responses to most of the traits. The presence of silicon resulted in low nodule formation and activity. The results from the principle component analysis showed that selection of soybean genotypes should be based on plant height, number of pods produced per plant, number of seeds produced per plant, hundred seed weight, seed yield and harvest index. In this study Williams and LS 6161 R at combination treatment of Si at 200 ppm and Eco-T® at 1g/kg of seed produced the highest seed yield and harvest index values.

3.6 LITERATURE CITED

AMAZA, P.S., OLAYEMI, J.K., BILA, Y. and IHEANACHO, A. 2007. *Baseline socioeconomic survey report: agriculture in Borno State*, Nigeria, IITA, Nigeria, Pp 3-74

BJÖRKMAN, T., BLANCHARD, L.M., HARMAN., G., 1998. Growth enhancement of shrunken-2 (sh2) sweet corn by *Trichoderma harzianum* 1295-22: effect of environmental stress. Journal of the American Society for Horticultural Science 123: 35-40.

CHANG, Y.C., CHANG, Y.C., BAKER, R., KLEIFELD, O. and CHET, I. (1986). Increased growth of plants in the presence of the biological control agent *Trichoderma harzianum.* Plant Disease 70: 145-148.

de KLEIJN, M.J., VAN DER SCHOUW, Y.T., WILSON, P.W., GROBBEE, D.E., and JACQUES, P.E. 2002. Dietary intake of phytoestrogens is associated with a favourable metabolic cardiovascular risk profile in postmenopausal U.S.women: the Framingham study. Journal of Nutrition 132: 276-82.

DUXBURG, M.R., BIRCH, E.B., and PARSONS, M.J. 1990. *Soybeans in Natal. Agricultural production guidelines for natal*. Department of Agriculture and Development. Natal, RSA.

ESPTEIN, E. 1994. The anomaly of silicon on plant biology. Proceedings of the National Academy of Science 91:11-17.

EPSTEIN, E. 1999. Silicon. Annual Review of Plant Physiology and Plant Molecular Biology 50: 641-664.

FAO, 2009. FAOSTAT. Food and Agriculture Organization of the United Nations, Rome, Italy.

(http://www.faostat.fao.org): Accessed 05 November 2011.

FARGIONE, F., HILL, F., TILMAN, D., POLASKY, S and HAWTHRONE, P. 2008. *Land clearing and the biofuel carbon department*. American Association for the Advancement of Science, New York, Pp 1235-1237.

GENSTAT, 2009. The statistical analyses software (GENSTAT®) 12th Ed, VSN International Ltd., Hemel Hempstead, UK.

GRAVEL, V., ANTOUN, H., TWEDDELL, R.J., 2007. Growth stimulation and fruit yield improvement of greenhouse tomato plants by inoculation with *Pseudomonas putida* or *Trichoderma atroviride*: possible role of indole acetic acid (IAA). Soil Biology and Biochemistry 39: 1968–1977.

HAMED, K., JAROODEH, S.V., Aruee, H and MAZHABI, M. 2011.Would *Trichoderma* Affect Seed Germination and Seedling Quality of Two Muskmelon Cultivars, Khatooni and Qasri and Increase Their Transplanting Success? Journal of Environmental Science 5: 169-175.

HARMAN, G. E. 2000. The myths and dogmas of biocontrol: changes in perceptions derived from research on *Trichoderma harzianum* strain T-22. Plant Disease 84:377-393.

HARMAN, G.E and TAYLOR, A.G 1990. *Development of an effective biological seed treatment system. In "Biological control of soil borne pathogens"* (Hornby D and Cook, R.J, Eds). CAB International, Wallinford, UK. Pp.415-426.

HARMAN, G.E., HOWELL, C.R., VITERBO, A., CHET, I. and LORITO, M., 2004. *Trichoderma* species - opportunistic avirulent plant symbionts. Nature Reviews Microbiology2: 43–56.

HOSSAIN, M.T., MORI, R., SOGA, K., WAKABAYASHI, K., KAMISAKA, S., FUJII, S.,YAMAMOTO, R. and HOSON, T. 2002. Growth promotion and an increase in cell wall extensibility by silicon in rice and some other Poaceae seedlings. Journal of Plant Research 115: 23–27.

JOHNS, M.A SKROCH, P.W., NIENHUIS, J., HINRICHSEN, G.B. and MUÑOZ-SCHICK, C. 1997. Gene pool classification of common bean landraces from Chile based on RAPD and morphological data. Crop Science. 37:605–613.

KLEIFIELD, O. and CHET,I. 1992 *Trichoderma* – plant interaction and its effect on increased growth response. Plant Soil 144: 267–272.

KUPFER, C. and KAHNT, G. 1992. Effects of application of amorphous silica on transpiration and photosynthesis of soybean plants under varied soil and relative air humidity conditions. Journal of Agronomy and Crop Science 168: 318-325.

LERSTEN, N. R. and CARLSON, J.B., 2004. *Vegetative morphology. In Soybeans: Improvements, Production, and Uses*, (Eds) Boerma, H.R. and Specht, J.E. USA. pp 15-58.

MA, J.F., and N. YAMAJI. 2006. Silicon uptake and accumulation in higher plants. Trends in Plant Science 11:392-397.

MALI, M and AERY, N.C. 2008. Silicon effect on nodule growth, dry-matter production, and mineral nutrition of cowpea (*Vigna unguiclate*). Journal of Plant Nutrition Soil Science 171: 835-840.

PANDLEY, A.K., and YADAV, R.S. 1999. *Effect of antitranspirants on physiological traits and yield of wheat under water deficit conditions*. Indian Journal of Agricultural Research 33: 159–164.

QIU, L., and CHANG, R. 2010. *The Soybean: Botany, Production and Uses. The Origin and History of Soybean*. Ed Singh G. CAB International, London, UK, Pp 1-15.

SAVANT, N.K., KORNDORFER, G.H., DATNOFF, L.E., and SNYDER, G.H. 1999. Silicon nutrition and sugarcane production: A Review. Journal of Plant Nutrition 22:1853-1903.

SPSS BASE., (2001) 11.0 for windows user's guide. Chicago: SPSS Inc

TIKHONOVICH, I.A., PROVOROV, N.A., ROMANOV, V.I., NEWTON, W.E., 1995. *Nitrogen Fixation: Fundamental and Applications*, 3-8. Kluwer Academic Publishers Group, Dordrecht, The Netherlands, Pp 260-795.

WAGNER, G.M., 1997.Azolla: A Review of its Biology and Utilization. Botanical Review 63: 1-26.

WINDHAM, M.T., ELAD Y., BAKER R. 1986. A mechanism for increased plant growth induced by *Trichoderma* spp. Phytopathology76: 518–521.

CHAPTER FOUR

4. GENETIC DIVERSITY ANALYSIS OF SELECTED SOYBEAN GENOTYPES USING SSR MARKERS

Abstract

Molecular markers are fast, efficient and reliable techniques in detecting differences between genotypes at DNA level. Among these markers, simple sequence repeats (SSRs) or microsatellites have become a useful tool for plant breeders such as in genetic diversity analysis, chromosome locations of desired genes and marker-assisted breeding. This study was conducted to investigate genetic variations between 10 selected soybean genotypes using eight SSR markers. The nuclear DNA of genotypes was isolated using a seed extraction method. The DNA was then quantified on 0.7% agarose gel with ethidium bromide and amplified via the polymerase chain reaction (PCR). A dendrogram was constructed to summarize the genetic relationship between the 10 soybean genotypes. The results indicated that genotypes Williams, LS 6161 R, and Magoye are genetically related agreeing with the findings presented in Chapters 2 and 3 based on phenotypic analysis using important agronomic traits. Thus, the use of SSR markers revealed the genetic relationship between the soybean genotypes complementing the phenotypic analysis.

Keywords: *Glycine max*, microsatellites, SSR markers, soybean

4.1 INTRODUCTION

Soybeans (*Glycine max* L.)are important food crop grown for the high nutritional value with relatively low production costs compared to cereals such as maize and wheat. Novel foods produced from soybeans are promoted for human consumption worldwide to assist in nutritional requirements for the fast expanding world population (Tripathi and Misra, 2005).

Soybeans are native to China and domestications begun approximately 3000-5000 years ago (Hymowitz and Newell, 1981). The wild soybean (*Glycine soja* Sieb. *et* Zucc.) grows widely in China, Japan Taiwan, Russia and Korea (Hymowitz, 2004). The phenotypic variability of the cultivated soybean is very wide encompassing traits such as seed shape, colour and size as well as plant morphology and resistance to abiotic and biotic stresses (Guriqbal, 2010).

The domestication of soybeans has resulted in the loss of genetic diversity. This is largely attributed to modern plant breeding programs and agricultural practices. In modern agriculture only a few selected soybean genotypes are grown widely which are results of intensive plant breeding, leading to a narrow genetic diversity (Gizlice *et al*., 1994). Gai and Zhao (2001) reported that of the 308 ancestral soybean varieties available in China only 38 were released during 1923–1995. The 38 varieties contributed to 54.18 and 56.84% of the nuclear and cytoplasmic genetic material, respectively, to soybean breeding in the country.

Crops with a limited or decreasing genetic diversity such as soybeans present a challenge to plant breeders given that these genetic resources become increasingly vulnerable to newly emerging pathogens and pests and other abiotic constraints. This requires adequate genetic conservation strategies for sustainable plant breeding programs. Traditionally, plant breeders apply morphological and biochemical markers to determine genetic variation among germplasm of various crop species. However, DNA based polymorphisms are more stable, and can reveal subtle changes useful in genetic diversity studies and varietal comparison (Wang *et al*., 2010).

The soybean genome is approximately 1115 Mpb which is relatively smaller when compared to other crops such as maize and barley. However, soybean genome is larger than the rice genome (Arumuganathan and Earle, 1991). The cultivated soybean is an annual allotetraploid (2n=4x=40).Due to its polyploidy nature a considerable number of duplications are present amongst the chromosomes (Pagel *et al*., 2004). This in turn results in reduced plant genetic diversity especially in cultivated soybean genotypes which makes it difficult for further improvements. Therefore it is important to determine the genetic diversity of soybean germplasm for breeding. The use of DNA markers have become a popular tool to identify genetic diversity using various markers such as; restriction fragment length polymorphisms (RFLPs); random amplified polymorphic DNAs (RAPDs); amplified fragment length polymorphisms (AFLPs); single nucleotide polymorphisms (SNPs) and microsatellites or simple sequence repeats (SSRs)(Brown-Guedira *et al*., 2000).

Among various molecular markers, simple sequence repeats (SSRs) or microsatellites have become a useful tool for plant breeders such as in genetic diversity analysis, chromosome locations of desired genes and marker-assisted breeding. Microsatellite markers are tandem repeats of 1-6 nuleotides of DNA. Microsatellites frequently occur in both prokaryotic and eukaryotic genomes (Zane *et al*., 2002). SSR markers are successfully used in different crops in analyzing genetic variation as well as in associational mapping of phenotypic and genotypic traits (Varshney *et al*., 2005). Thus the objective of this study was to investigate genetic variations between 10 selected soybean genotypes using eight SSR markers.

4.2 MATERIAL AND METHODS

4.2.1 Plant materials

Ten soybean genotypes (see Chapter 2 section 2.2.1) obtained from different sources were used for this experiment. Disease-free, dry and quiescent seeds of the 10 genotypes were available to extract nuclear DNA.

4.2.2 DNA Extraction

DNA was extracted from seed material using a seed extraction method. The procedure is outlined as follows: 2g of seed was ground in an electric grinder to achieve a fine meal consistency for each genotype. 70mg of the meal was placed into 1.5ml eppendorf tubes and 700 µl of extraction buffer (50mM Tris-HCL pH to 8; 10mM EDTA; 100mM NaCl; 2-Mercaptoethanol; 1% SDS) was added to the tubes and incubated at 65^{o}C for 10 minutes. After which 200 µl of 3M Sodium acetate was added and the tubes were vortexed well and placed on ice for 10 minutes. Then the tubes were centrifuged at 12 000 rpm for 10 minutes at 4^{o}C. 400 µl of supernatant was added into new 1.5 eppendorf tubes and the same volume of cold ethanol (kept at -20^{o}C) was added. The tubes were mixed by gentle inversion and placed in the freezer to precipitate for 1 hour. Then the tubes were centrifuged at 8000 rpm at 4^{o}C for 3 minutes, a white pellet of DNA formed on the side and/or collected at the bottom of tubes. The supernatant was discarded and the pellet washed twice in 400 µl of 70 % ethanol by centrifuging the tubes at 12 000 rpm at 4^{o}C for 1:30 minutes. The tubes were then left to dry for a few hours to allow the excess ethanol to evaporate. Then 50 µl of PCR Grade water was added to the tubes and incubated in a water bath at 65^{o}C for 10 minutes to re-suspend the DNA pellet.

4.2.3 DNA Quantification

The extracted and re-suspended DNA was quantified on 0.7% agarose gel (3.5 g agarose in 500 ml of 1 x TAE buffer and 25 µl Ethidium Bromide). The florescence of each of the 10 DNA samples extracted was compared by a size standard (lambda [λ] DNA) under UV light (Figure 4.1). DNA was assessed visually and diluted accordingly to estimate the concentration of DNA to the size standard (λ) that would be used for amplification via PCR (Table 4.1).

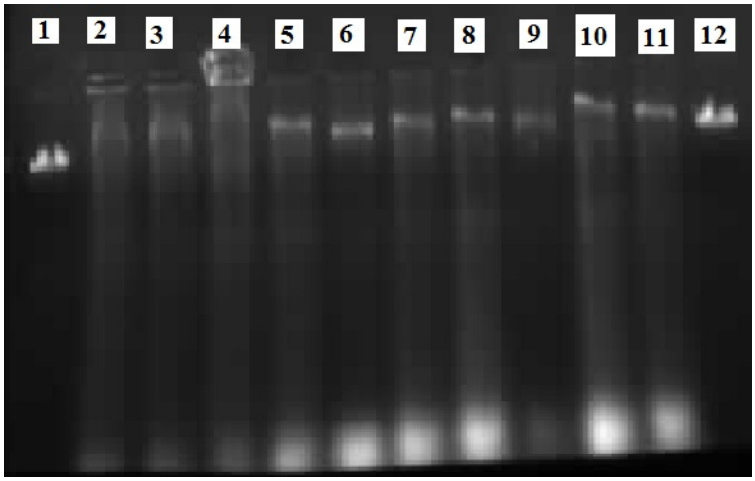

Figure 4.1 Agarose gel (0.7%) with Ethidium bromide showing total DNA isolated from 10 soybean genotypes. Lanes: 11 and 12 are Lambda [λ] DNA and Lanes: 1-10 refer to the 10 genotypes correspondingly numbered 1 to 10 in Table 4.1.

Table 4.1 Dilutions made for each the 10 genotypes before DNA amplification via PCR

Number	Genotype	DNA (µl)	PCR grade water (µl)	Ratio
1	Clark	20	40	1:2
2	Barc-2	20	40	1:2
3	Williams	30	30	1:1
4	LS 616 R	15	45	1:3
5	Magoye	15	45	1:3
6	Barc-14 nodulated	15	45	1:3
7	Barc-17 nodulated	15	45	1:3
8	L76-1988	15	45	1:3
9	L82-1449-ll	15	45	1:3
10	Barc-4	15	45	1:3

4.2.4 SSR primers

Eight primer sets were used for comparison of the 10 selected soybean genotypes viz. Satt373; Satt534; Satt009; Satt242; Satt173; SOYPRP1; Satt005 and Satt001 (Table 4.2). The primers were selected based on availability and high success rate at INCOTEC®, South Africa The primers are developed and published by Cregan *et al*. (1999). Both the forward and reverse primer sequence can be found on Dr. Cregan's website (http://bldg6.arsusda.gov/cregan/soymap.htm) that describes the mapped soybean SSR Loci.

4.2.5 Polymerase chain reaction

Extracted and quantified DNA was amplified in a reaction mix containing 20ul of the following: 1 µl forward primer; 1 µl reverse primer; 10 µl PCR Phire® Buffer mix; Tail;0.4 µl Phire® Hot Start ll DNA polymerase; 2 µl of DNA sample. Amplification of the DNA was done using a BIO-RAD CFX96™ Real-Time SystemThermal cycler (ABI, Foster City, California, USA) with the following parameters: PCR setup protocol used was programmed for initial denaturation for 5 min at 98℃, followed by 35 cycles of 1 min at 98℃, 1 min at 50℃, and 1 min at 72℃, final extension was 10 min at 72℃ (optimization used by INCOTEC®, South Africa). The PCR products, mixed with Gene scan™ -500 Liz® (a size standard) (Applied Biosystems, Foster City, California, USA) and Hi-Di Formamide, were visualized on 3130 Genetic Analyzer (HITACHI) ABI machine (Applied Biosystems) using microsatellites.

4.2.6 Reagents and chemicals used in DNA extraction, quantification and PCR

The following reagents and chemicals were used for DNA extraction, quantification and PCR:

- Extraction buffer : 50mM Tris-HCL pH to 8; 10mM EDTA; 100mM NaCl; 2-Mercaptoethanol; 1% SDS
- 3M Sodium acetate
- Absolute ethanol -20^{o}C
- 70 % ethanol: 70 ml ethanol; 30 ml distilled water

- 1 x TAE buffer: 50 ml 10 X TAE buffer; 500 ml distilled water
- 10 x TAE buffer: 48.40 g Tris; 4.10 g Sodium acetate; 2.86 g EDTA
- Ethidium bromide
- Agrose gel
- Phire®Plant Direct PCR Kit (F-130)
    - PCR Phire® Buffer mix
    - Phire®Hot Start ll DNA polymerase (F-1225)
- Tails (Dye)
    - Vic; Fam; Pet; Ned
- Hi-Di Formamide
- Gene Scan™ - 500 Liz size standard

4.2.7 Data analysis

PCR products were fluorescently labeled and separated by capillary electrophoresis on an ABI 3130 automatic sequencer (Applied Biosystems, Johannesburg, SA), analysis was performed using GeneMapper 4.1. The program GGT 2.0 (van Berloo, 2008) was used to calculate the Euclidean distances between bulked samples, the matrix of the genetic distances were used to create the unweighted pair-group method with arithmetic mean (UPGMA) dendrogram.

4.3 RESULTS

The results in table 4.2 indicate that a total of 36 alleles expressed amongst the 10 soybean genotypes used in the study. The number of alleles expressed by each SSR locus ranged from 1-6 (Table 4.2). The lowest number of expressed alleles was found at locus SATT009 which was 1 and the highest was 6 found at loci SATT001 and SATT173. The polymorphism information content (PIC) values varied from 0.000 at locus SATT009 to 0.7630 at locus SATT001 (Table 4.2). The heterozygosity (HE) values ranged from 0.0000 to 0.7929 (Table 4.2).

Table 4.2 Details of the eight SSR loci used in this study indicating the position on chromosome, size range, number of alleles expressed, PIC values and heterozygosity (He) tested with 10 soybean genotypes.

SSR locus	Chromosomal position	Size range	Expressed alleles	PIC	HE
SATT373	107.24	235-275	5	0.6560	0.6982
SATT009	28.52	171-190	1	0.0000	0.0000
SATT005	75.29	146-204	4	0.5350	0.5800
SATT001	50.56	113-138	6	0.7630	0.7929
SATT534	87.59	170-216	5	0.7067	0.7456
SATT242	14.35	202-225	5	0.7014	0.7400
SATT173	58.4	211-273	6	0.7193	0.7500
SOYPRP1	46.94	180-215	4	0.6102	0.6600
Total			36	4.6916	4.9667
Average			4.5	0.5865	0.6208

The result in table 4.3 shows the fragment size of the expressed alleles for each SSR marker per soybean genotype. SSR marker SATT373 showed 5 different fragment sizes, the common fragment size of 266 was present in Clark, Barc-2, LS6161R, Barc-14, L76-1988 and Barc-4. Marker SATT009 showed no allele expression for genotypes Williams, Magoye, Barc-17 and L82-1449-II and an allele fragment size of 179 was present for the other genotypes (Table 4.3).

SATT005 resulted in 4 different fragments with genotypes Clark, Barc-2, Williams, Barc-14, L76-1988 and Barc-4 displaying a common fragment size 157 (Table 4.3). SATT001 exhibited 6 different allele fragment sizes with most of the genotypes showing a

fragment size of 135 and no allele expression for genotypes Williams and L82-1449-II (Table 4.3).

SATT534 showed 5 different allele fragment sizes with no allele expression for genotype Williams and the most common occurring fragment amongst the genotypes was 205 (Table 4.3). SATT242 displayed 5 different allele fragment sizes with the most frequent occurring size of 217 amongst the genotypes (Table 4.3).

In table 4.3 SATT173 showed 6 different alleles amongst the genotypes with the most common fragment size being 216. SOYPRP1 revealed 4 different allele fragment sizes with the most common size of 210 occurring for most of the genotypes (Table 4.3)

Table 4.3 Fragment size of alleles expressed by eight SSR markers when tested using 10 soybean genotypes.

Genotype	SSR Markers and fragment sizes							
	SATT373	SATT009	SATT005	SATT001	SATT534	SATT242	SATT173	SOYPRP1
Clark	266	179	157	129,135	205	211	254	205
Barc-2	266	179	157	129,135	205	213	216	210
Williams	257	*	157	*	*	217	216	210
LS6161R	242,266	179	178	119,126	199,207	217	216	210
Magoye	268	*	178	117,123	186	217	259	212
Barc-14 nodulated	242,257,266	179	157	126	177,199,205	211	254,268	182
Barc-17 nodulated	263	*	187	119,126	207	205	229	205
L76-1988	266	179	157	135	205	217	216,259	210
L82-1449-II	242	*	184	*	199	222	266	182
Barc-4	266	179	157	135	205	213	216	210

* denotes no allele expression.

The matrix of Euclidean genetic distances shown in Table 4.4 indicates the distances between genotypes. These distances were used to create the preceding dendrogram (Figure 4.2). From the results it is evident that the smallest genetic distance exists between genotypes Barc-4 and Barc-2 (0.5) (Table 4.4) and the largest genetic distance was between genotypes Williams and Clark (5.3) (Table 4.4).

The fragment sizes summarized in table 4.3 and the matrix of Euclidean genetic distances in table 4.4 were used to construct the UPGMA dendrogram (Figure 4.2).The dendrogram revealed 2 distinctive groups i.e. I and II (Figure 4.2). Group I consists of genotypes Clark, Barc-14 nodulated, Barc-2, Barc-4, L76-1988 and Group II consists of genotypes Magoye, Barc-17 nodulated, L82-1449-II, Williams, LS 6161 R.

The dendrogram depicts close linkages between Clark and Barc-14 nodulated; Barc-2 and Barc-4; Barc-17 nodulated and L82-1449-II and between Williams and LS6161R i.e. shown by the genetic distances of 1.2, 0.25, 0.65, and 1.5, respectively (Figure 4.2). Genotypes with the closest link are Barc-2 and Barc-4. While genotypes LS6161R and Clark are the least related and positioned on either side of the dendrogram.

Table 4.4 The matrix of Euclidean genetic distances among 10 soybean genotypes analyzed using eight SSR markers.

Genotype	Clark	Barc-2	Williams	LS6161R	Magoye	Barc-14	Barc-17	L76-1988	L82-1449-11
Barc-2	2.9								
Williams	5.3	4.3							
LS6161R	4.8	3.6	3.0						
Magoye	4.0	3.8	3.5	3.0					
Barc-14	2.3	2.4	3.7	3.4	3.8				
Barc-17	3.7	4.1	3.1	3.2	1.9	3.0			
L76-1988	3.0	1.9	3.9	3.2	3.0	3.0	3.7		
L82-1449-11	3.5	3.7	2.5	3.4	2.4	2.5	1.3	3.4	
Barc-4	2.9	0.5	4.1	3.4	3.7	2.2	3.9	1.8	3.5

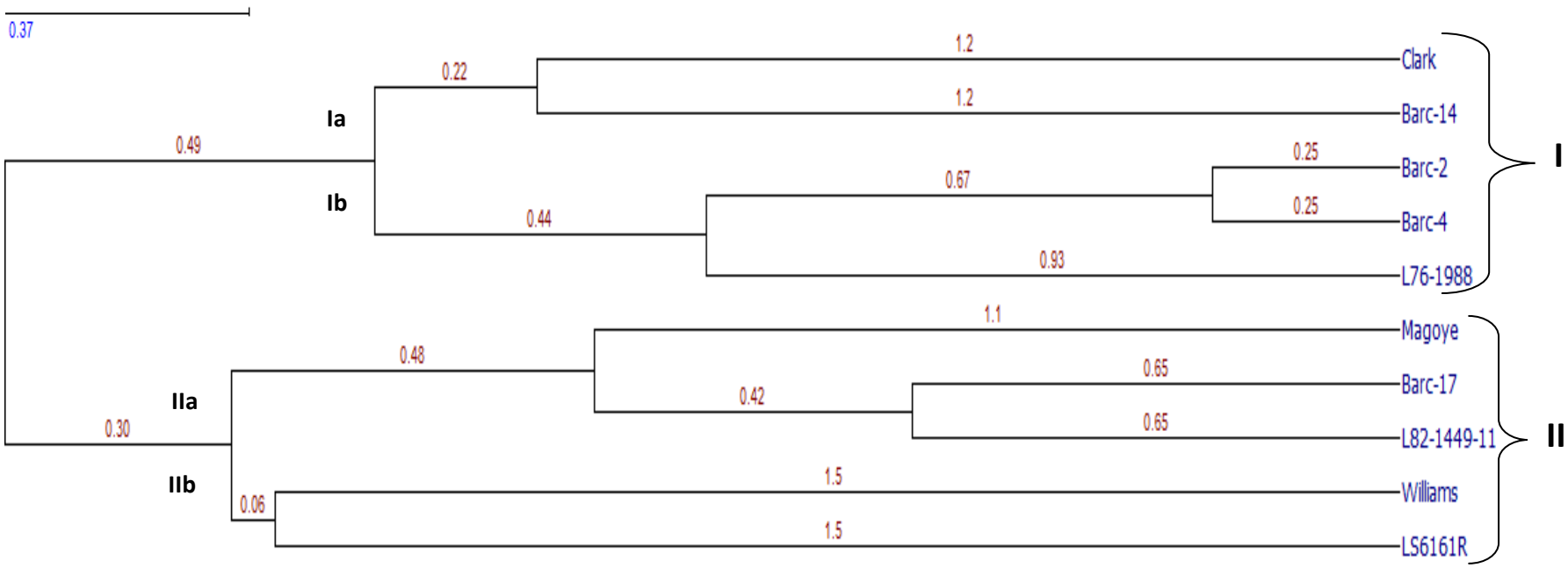

Figure 4.2 UPGMA clustering of ten soybean genotypes analyzed with eight SSR markers using Euclidean's genetic distance. The two groups are denoted by I and II with sub-groups by Ia and Ib and IIa and IIb.

4.4 DISCUSSION

Soybean is an important legume crop that is grown worldwide. However, over the years of soybean production the genetic diversity has decreased. As a result most genotypes lack the diversity to adapt to different environmental stresses. Therefore more strict breeding programs are being developed in the selection of parental sources to avoid crossing closely related genotypes (Thompson and Nelson, 1998).

In order to develop successful breeding programs to aid in the diversification of soybean genotypes, the pedigree of the genotypes need to be determined. SSRs or simple sequence repeats are useful tools in genetic diversity studies and crop variety comparison. Research conducted on soybean by Diwan and Cregan (1997) revealed that approximately 95% of the alleles in the local soybean were explained by 35 soybean genotypes that were distinguished by 20 SSR markers.

The results summarized in Table 4.2 indicate the presence of 36 alleles amongst the 10 soybean genotypes using eight SSR markers. The number of alleles ranged from 1- 6 with the average of 4.5 alleles. The locus SATT009 revealed only one allele while loci SATT001 and SATT173 revealed six alleles (Table 4.2). The PIC, a value used to measure the usefulness of a marker by a measure of the polymorphism (Botstein *et al*., 1980), ranged from 0.0000 to 0.7630 with an average of 0.5865. Markers SATT001, SATT173, SATT242 and SATT534 had PIC values higher than 0.7000 and also high number of expressed alleles, which indicates that these loci were the most informative in distinguishing between the genotypes (Table 4.2). Similar results were obtained with markers SATT173, SATT242 and SATT534 in soybean by other researchers (Priolli *et al*., 2002, Wang *et al*., 2010; Tantasawat *et al*., 2011). The heterozygosity, which occurs when different alleles of the same gene are present at one or more corresponding chromosomal loci (Mhameed *et al*., 1996), ranged from 0.0000 to 0.7929 with an average of 0.6208. High HE values (> 0.70000), were achieved by DNA markers SATT001, SATT173, SATT242 and SATT534 (Table 4.2). This same trend was noted for the PIC values for these markers indicating the high degree of gene diversity. Thus the results suggest that most useful markers in this study were

SATT001, SATT173, SATT242 and SATT534. Markers SATT005, SATT373 and SOYPRP1 were moderately useful while marker SATT009 has the least useful information.

The markers SATT001 and SATT173 had the highest frequency and total number of alleles expressed (6) (Table 4.3). Marker SATT009 showed a high frequency but displayed the least number of alleles (1) (Table 4.3). Previous studies conducted on wild, cultivated and landrace soybeans revealed a higher total number of alleles expressed in the wild soybean, when tested against 40 SSR markers (Wang *et al*., 2010). This is an indication of the greater genetic diversity in wild soybean as compared to that of cultivated soybean. In this study only eight SSR markers were used and it was evident that the USDA sourced Williams and L82-1449-II did not express alleles for markers SATT009, SATT001 as well as SATT534 while the landrace Magoye did not express alleles for marker SATT009.

The results from the matrix of Euclidean genetic distances revealed a close relationship between Barc-2 and Barc-4 (0.5) (Table 4.4). The pedigree of the genotypes in Table 2.1 indicates a common parent (Clark 63(8)) between Barc-2 and Barc-4. Therefore the genetic distance in the above indicates the usefulness of SSR markers for the distinction of closely related genotypes. A similar trend was noted by Chotiyarnwong *et al* (2007) for the distinction between closely related soybean genotypes by 18 SSR markers using 160 soybean genotypes.

The genetic analysis on ten selected soybean genotypes with eight SSR markers allocated the genotypes into two distinctive groups: I and II (Figure 4.2) with two sub groups in each. These groups and sub-groups were created by the results obtained in Tables 4.3 and 4.4. Group I contains two sub-groups i.e. sub-group I [Ia]: (Clark and Barc-14 nodulated), which display a close relationship shown by the same lengths obtained i.e. (1.2) (Figure 4.2) and sub-group I [Ib]: which consists of Barc-2, Barc-4 and L76-1988. Although L76-1988 is included in sub-group I [Ib] and shows a close relationship to Barc-2 and Barc-4, the two latter display closest relationship to each other (0.25) (Figure 4.2) than to L76-1988. This same trend is observed in Group II

which consists of two sub-groups i.e. IIa in which, Magoye shows a close relationship to Barc-17 nodulated and L82-1449-II. However, the two genotypes show a closer relationship to each other than to Magoye. IIb consists of Williams and LS 6161 R which show a close genetic relationship of 1.5 (Figure 4.2). The dendrogram also indicates that the greatest distance occurred between genotypes Clark and LS6161R which was expected since Clark was sourced from the USDA and LS6161R is a local landrace (Table 2.1).

In the preceding chapter (Chapter 2) it was found that genotypes Williams and Barc-2 performed the best when compared to the other genotypes to Si application at 200ppm. The two genotypes are distinct as revealed from the current genetic analysis. Williams belongs to Group II and Barc-2 belongs to Group I (Figure 4.2). However, according to the pedigree shown on Table 2.1, Barc-2 is the product of cross combination of genotype Clark by Hill and Williams is resulted from a cross of Clark by Adams. Therefore, although Barc-2 is not closely related to Williams on this dendrogram, these genotypes may be closely related in some respect due to the common parentage i.e. Clark (Table 2.1).

Furthermore, the results shown in Chapter 3 indicated that genotypes Williams, LS 6161 R, Magoye and Barc-2 had promising phenotypic performances towards combined application of Si and Eco-T®. From the current SSR analysis it is evident that the genotypes are allocated in the same group (Group II), with Williams and LS 6161 R belonging to the same sub-group IIa (Figure 4.2). Therefore similar and positive response of the genotypes to Si and Eco-T® combined treatments may be attributed to the close genetic relationship. Genotype, Barc-2 although not belonging to Group II shares a common parent with Williams suggesting the genetic relatedness in agronomic performance of the best genotypes in Chapter 3.

4.5 CONCLUSION

Enhanced performance of Williams, LS 6161 R, Magoye and Barc-2 was found with regards to important agronomic traits (Chapters 2 and 3) to Si and Eco-T® applications. The present analysis using the SSR markers suggests close genetic relationships among these germplasm. Thus the use of the SSR markers is effective and agreed to phenotypic analysis which can be applied in diversity analysis and establish genetic relationship among soybean genotypes.

4.6 REFERENCES

ARUMUGANATHAN, I., and EARLE, E. D. 1991. Nuclear DNA content of some important plant species. Plant Molecular Biology Reporter 9: 208-219.

BOTSTEIN, D., WHITE, R.L., SKOLNICK, M. and DAVIS, R.W. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. American Journal of Human Genetics 32:314–331.

BROWN-GUEDIRA, G. L., THOMPSON, J. A., NELSON, R. L. and WARBURTON, M. L. 2000. Evaluation of genetic diversity of soybean introductions using RAPD and SSR markers. Crop Science40: 815–823.

CHOTIYARNWONG, O., CHATWACHIRAWONG, P., CHANPRAME, S. and SRINIVES, P. 2007. Evaluation of Genetic Diversity in Thai Indigenous and Recommended Soybean Varieties by SSR Markers. Thai Journal of Agricultural Science 40: 119-126.

CREGAN, P.B., JARVIK, T., BUSH, A.L, SHOEMAKER, R.C., LARK, K.G. and KAHLER, A.L. 1999. An integrated genetic linkage map of the soybean genome. Crop Science 39:1464-1490.

CREGAN, P.B. 2006. Soybean genomics and improvement laboratory. Mapped Soybean SSR Loci July 2006. (http://bldg6.arsusda.gov/cregan/soymap.htm). Accessed 29 May 2012.

DIWAN, N. and CREGAN, P.B. 1997. Automated sizing of fluorescent-labeled simple sequence repeat (SSR) markers to assay genetic variation in soybean. Theoretical and Applied Genetics 95:723-733.

GAI, J.Y. and ZHAO, T.J. 2001. The core ancestors of soybean cultivars in China. Journal of Nanjing Agricultural University 24: 20-23.

GIZLICE, Z., CARTER, J.R. and BURTON, J.W. 1994. Genetic base for North American public soybean cultivars released between 1947-1988. Crop Science 34: 1143-1551.

GURIQBAL, S. 2010. *The Soybean: Botany, Production and Uses*. CAB International. Pp. 494.

HYMOWITZ, T. 2004. Chapter 4: *Speciation and cytogenetics. In: Soybeans: Improvement, production, and uses*. 3rd ed. Agronomy Monograph 16. Madison, Wisconsin. USA. Pp. 97–136.

HYMOWITZ, T. and NEWELL, C. A. 1981. Taxonomy of the genus *Glycine*, domestication and the uses of soybeans. Society for Economic Botany 35:272–288.

MHAMEED, S., SHARON, D., HILLEL, J., LAHAV, E., KAUFMAN, D. and LAVI, U. 1996. Level of Heterozygosity and mode of inheritance of variable number of tandem repeat loci in avocado. Journal of the American Society of Horticultural Science 12:778-782.

PAGEL, J., WALLING, J.G., YOUNG, N.D., SHOEMAKER, R.C. and JACKSON, S.A. 2004. Segmental duplications within the Glycine max genome revealed by fluorescence in situ hybridization of bacterial artificial chromosomes. *Genome* 47: 764-768.

PRIOLLI, R.H.G., MENDES-JUNIOR, C.T., ARANTES, N.E. and CONTEL, E.P.B. 2002.Characterization of Brazilian Soybean Cultivars Using Microsatellite Markers. Genetics and Molecular Biology 2: 185-193.

TANTASAWAT, P., TRONGCHUEN, J., PRAJONGJAI, T., JENWEERAWAT, S. and CHAOWISET, W. 2010. SSR analysis of soybean (*Glycine max* (L.) Merr.) genetic relationship and variety identification in Thailand. Australian Journal of Crop Science 5: 283-290.

THOMPSON, J.A. and NELSON, R.L.1998.Utilization of diverse germplasm for soybean yield improvement. Crop Science 38:1362-1368.

TRIPATHI, A.K. and MISRA, A.K. 2005. Soybean- aconsummate functional food: A Review. Journal of Food Science and Technology 42:111–119.

van BERLOO, R. 2007. GGT graphical genotypes. Laboratory of Plant Breeding Wageningen University. The Netherlands. (http://www.dpw.wau.nl/pv/pub/ggt/): Accessed 1 July 2012.

VARSHNEY, R.K, GRANER, A. and SORRELLS M.E. 2005. Genic microsatellite markers in plants: features and applications. Trends in Biotechnology 23:48–55.

WANG, M., LI, R., YANG, W. and DU, W.2010. Assessing the genetic diversity of cultivars and wild soybeans using SSR markers. African Journal of Biotechnology 9: 4857-4866.

ZANE, L., BARGELLONI, L. and PATARNELLO, T. 2002. Strategies formicro satellite isolation: a review. Molecular Ecology 11:1–16.

CHAPTER FIVE

5.OVERVIEW

AIM

Ten selected soybean genotypes were used in this study to determine the effect of potassium silicate (KSi) and *Trichoderma harzianum* (Eco-T®) when used singly or in combination. Yield, yield components, and nodulation formation of soybean were investigated under controlled and field conditions.

The most significant findings of this study are summarized below:

CHAPTER 2

Soybean genotypes Barc-4, L82-1449-ll, L76-1988, Barc-2, Clark, Barc-14 and Barc-17 were provided by the United States Department of Agriculture (USDA) and Magoye was locally available as landrace. While LS 6161 R was the local genotype purchased from Link Seeds.

Preliminary studies were conducted in two controlled experiments at the University of KwaZulu-Natal in 2010 to determine the optimum silicon and genotype combination. Silicon was applied as an aqueous solution at three different levels i.e. (0, 200 and 250 ppm). Genotype L82-1449-ll flowered early at 17 days across the levels of Si applied while genotype Magoye flowered relatively later at 35 days. In experiment I the number of seeds produced per pod was the same for all genotypes across each Si concentration level and could therefore not be used as a distinctive trait, however in experiment two the number of seeds produced per pod varied among genotypes and with each level of Si.

The results obtained in Chapter 2 indicates that for the agronomic traits observed, overall Si applied at 200ppm showed a positive response to most of the traits such as plant height, number of pods produced per plant, hundred seed weight, seed yield and

harvest index. In both experiments a general trend was noted from the correlations analysis which indicated that an increase in plant height, number of pods produced per pod, hundred seed weight resulted in increased seed yield and harvest index and the principle component analysis carried out indicated that these above mentioned traits contributed to most of the variation with an exception to number of seeds produced per pod in experiment one as per the above reason.

The results in Chapter 2 indicated that Si at 200 ppm produced the best seed yield and harvest index especially in genotypes Williams and Barc-2 and this level of Si was therefore used in the subsequent field trial conducted at Ukulinga research farm of the University of KwaZulu-Natal, Pietermaritzburg during 2010/2011.

CHAPTER 3

Trichoderma harzianum was applied to the field trial in the form of Eco-T® to aid in the growth promotion and to observe its effect on nodulation as well as Silicon in the form of potassium silicate (KSi) at 200ppm.

During the field trial it was noted on average that plants flowered and matured earlier than in the controlled experiments and the plants also grew taller and produced more leaves and roots.

Genotypes that received Si only produced the least number of nodules and the least number of active nodules while those which received Si and Eco-T® in combination produced more nodules as well as more active nodules while genotypes that received0ppm Si without Eco-T® and those which received Eco-T® only produced a high number of nodules and more active nodules.

The genotypes that received Si and Eco-T® in combination showed an overall positive response with the exception of the total number of nodules produced and number of active nodules. A higher plant height, number of seeds produced per pod, number of pods produced per plant, hundred seed weight, dry root and shoot masses, seed yield

and harvest index was noted at the above mentioned treatment. Genotypes Williams, LS 6161 R, Magoye, and Barc-2 were the top producers during the experiment and would be recommended to farmers in this or similar environments.

CHAPTER 4

Varietal comparison was conducted on the 10 soybean genotypes with eight SSR markers. The plant DNA was extracted from seeds and comparison was carried out using capillary electrophoresis.

The results indicated close relationships between the genotypes as expected from the pedigrees. The closest relationships were noted between Barc-2 and Barc-4. And the greatest genetic distance was noted between genotypes Clark and LS6161R. However, the most valuable information obtained was the link shown between top performers Barc-2, Williams, Magoye and LS6161R.

CONCLUSION

Therefore from Chapter 2 it was established that Si at 200ppm is the optimum Si concentration for the ten selected soybean genotype and from Chapter 3 it was identified that Eco-T® is an effective growth enhancer and the combination of these two agents for the ten selected soybean genotypes will result in the best seed yield and harvest index values in soybeans. Also the selection of high seed yielders and high harvest indices will correspond with these plants having a high plant height, number of seeds produced per pod, number of pods produced per plant, hundred seed weight and high root and shoot masses as shown in Chapter 3. The results obtained in Chapter 4 show the linkage between genotypes Williams, LS 6161 R, Magoye and Barc-2 which showed a superior performance overall of the agronomic traits observed in Chapters 2 and 3. This link can be used to explain the success of these genotypes when treated with KSi and Eco-T®. Therefore it can also be concluded that SSR markers is an effective tool that can be used to distinguish between closely related genotypes.

5.2 IMPLICATIONS

The research conducted in this study can be used as a stepping stone for implementing more biological conscious methods in improving commercially viable crops worldwide. The use of potassium silicate (KSi) and *Trichoderma harzianum* (Eco-T®) which, both can be used as bio-fertilizers and bio-control agent's may allow for a reduction in the use of inorganic fertilizers and pesticides.

A close link can be made from the phenotypic and SSR analyses indicating the importance of the use of molecular markers. SSR markers have been shown to be very effective in variety comparison to establish relatedness amongst genotypes. This method saves costs and time by allowing for more effective trial management and by avoiding crossing closely related parental lines which may lack diversity and in turn adaptability.

5.3 RECOMMENDATIONS

In this study selected agronomic traits of only 10 soybean genotypes were observed. In order to have a more comprehensive study more soybean genotypes should be used and a wider range of agronomic traits should be observed.

The research included control trials in the glasshouse and tunnels as well as a field trial. The field trial was conducted in one location over one growing season. Therefore further research is required at several different locations over more than one growing season.

The levels of potassium silicate (KSi) and *Trichoderma* were a recommended amount for soybean. However, the results obtained for the agronomic traits differed for each genotype therefore further research on different levels of KSi and *Trichoderma* should be tested for each genotype other than the levels tested in this study.

Eight SSR makers were used for genetic comparison analysis in this study. Further studies may include more SSR markers in order to give a more detailed variety comparison.