GROWTH, NODULATION AND YIELD RESPONSES OF PROMISCUOUS AND SPECIFIC-NODULATING SOYBEAN CULTIVARS TO *RHIZOBIUM* INOCULATION AND SEAWEED EXTRACT

BY

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DECLARATION

I, Tshepo Prince Raofa declare that i have not previously submitted this mini-dissertation hereby submitted to the University of Limpopo, for the Master of Science in Agronomy at this or any other university; that it is my work in design and execution, and that i duly acknowledged all material contained herein.

Candidate: Mr TP Raofa	Signature	Date

DEDICATION

The dedication of this work goes to my beloved parent Motsatsi Joyce Raofa, my late grandmother Masale Salome Raofa and my daughter Dora Blessing Maake.

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ABSTRACT

Soybean *(Glycine max L.)* is one of the valuable leguminous crops with grain used for human consumption, livestock feeding, bio-fuel (bio-diesel) production, vegetable oil and is a protein resource. The crop also fixes atmospheric nitrogen. The study aimed to evaluate the performance of two soybean varieties to a combination of inoculation and seaweed extract. The research trial was conducted at Syferkuil farm during 2017/2018 summer season in which two soybean varieties (TGx 1937-1F promiscuous) and (PAN 1583R specific-nodulating) were evaluated for their growth, nodulation and yield responses to *rhizobium* inoculation and seaweed extract (0 % rate (0ml/12L), 50 % rate (30ml/12L) and 100 % (60ml/12L). The research trial was laid out as a split-split plot arrangement fitted in RCBD with four replications.

Inoculation significantly influenced grain yield at $P \leq 0.05$, seed nutrient content and total above-ground biomass, except for seed potassium. No inoculation significantly achieved higher primary branches per plant at $P \leq 0.05$, pod number per plant, stem diameter ($P \leq 0.05$), grain yield ($P \leq 0.05$), harvest index and total above-ground biomass at $P \leq 0.001$. The variety TGx 1937-1F had significantly ($P \leq 0.001$) higher nodule number per plant, effective nodules per plant, nodule dry weight, dried shoot biomass, leaf number per plant, pod number per plant, primary branches per plant, stem diameter, plant height, leaf chlorophyll content, total above-ground biomass, grain yield and seed iron (Fe) content. Application of full rate seaweed extract significantly ($P \leq 0.001$) increased primary branches per plant, stem diameter, leaf number per plant, plant height, shelling percentage, total above-ground biomass, grain yield, and seed content of calcium (Ca), potassium, magnesium (Mg), manganese (Mn) and sodium (Na) all at $P \leq 0.05$. Seaweed extract rate at 0 % obtained the highest harvest index ($P \leq 0.001$).

Inoculation and variety TGx 1937-1F interaction exhibited a significant increase on leaf number per plant at $P \leq 0.001$, primary branches per plant at $P \leq 0.001$ and plant height at $P \leq 0.001$. Variety TGx 1937-1F, without inoculation, obtained significantly higher pod number per plant ($P \leq 0.001$), stem diameter at $P \leq 0.001$, grain yield at $P \leq 0.05$ and total above-ground biomass at $P \leq 0.001$. Variety PAN 1583R, without inoculation, obtained significantly higher harvest index and shelling percentage at $P \leq 0.001$. Interaction of inoculation and seaweed extract showed that no inoculation × 100 % rate of seaweed extract significantly ($P \leq 0.001$) increased primary branches per plant, leaf number per plant, stem diameter, pod number per plant and plant height. Interaction of inoculation × 100 % rate of seaweed extract increased grain yield ($P \leq 0.001$) and total above-ground biomass at $P \leq 0.001$. Inoculation × 50 % rate of seaweed extract interaction increased shelling percentage at $P \leq 0.001$. No inoculation × 0 % rate of seaweed extract interaction obtained significantly higher harvest index ($P \leq 0.001$). Interaction of variety and seaweed extract showed that variety TGx 1937-1F × 100 % rate of seaweed extract significantly increased primary branches per plant ($P \leq 0.001$), pod number per plant at $P \leq 0.001$, grain yield at $P \leq 0.001$ and total above-ground biomass at $P \leq 0.001$. The variety TGx 1937-1F × 50 % rate of seaweed extract significantly raised the size of stem diameter ($P \leq 0.01$) and plant height ($P \leq 0.001$). Three-way interactive effects of inoculation × variety TGx 1937-1F × 100 % rate of seaweed extract obtained significantly higher number of shelling percentage at $P \leq 0.001$, leaf number per plant at $P \leq 0.05$ and primary branches per plant at $P \leq 0.001$. Interaction of no inoculation × variety TGx 1937-1F × 100 % rate of seaweed extract obtained significantly high pod number per plant at $P \leq 0.001$, grain yield at $P \leq 0.05$, total above-ground biomass at $P \leq 0.001$ and plant height at $P \leq 0.001$. No inoculation × PAN 1583R × 100 % rate of seaweed extract interaction had a higher harvest index at $P \leq 0.001$. The study showed that inoculation, seaweed extract, or their combination generally enhanced seed nutrient content, especially in variety TGx 1937-1F.

The study further showed that promiscuous soybean (TGx 1937-1F) had higher grain yield, under stressful growing conditions as compared to PAN 1583R variety. This implies that soybean variety TGx 1937-1F, with 50 % or 100 % application rate of seaweed extract could be recommended to smallholder farmers.

Key words: Soybean, inoculation, seaweed extract, phenological development, growth, nodulation, grain yield and seed nutrient content.

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CHAPTER 1

INTRODUCTION

1.1. Background

Soybean *(Glycine max L.)* is a legume species whose grain is widely consumed throughout the world and also contains essential oil. Soybean is produced commercially in South Africa (DAFF, 2011). The crop originated from China and was then introduced to India in 1968 from USA. In many countries soybean is now being considered as one of the important crops. Due to its multiple uses, soybean is also named as the "Golden bean". Soybean grain is extremely useful for human health and the crop is also easy to cultivate. Very small quantities of fresh leaves and fresh green grain of the crop are consumed directly as food by human beings. The crop can also serve as fodder for livestock during winter and cultivated as a commercial legume crop in many African countries. The soybean seeds can be processed for the manufacturing of vegetable oil and then the remaining cake is reserved for livestock feeding. Soybean seeds are also processed for biofuel (biodiesel) production, which is considered to be a clean and renewable source of energy (DAFF, 2011). Soybean grain contains 40 % protein, 5.72-7.00 % soluble sugars (Tefera, 2011), 4 % saponins, 5 % fibre, 18-20 % oil and 30 % carbohydrate (Tefera, 2011) thus making it an important source of food for vegetarians (DAFF, 2011). Soybean grain has a substantial content of vitamin A, B, D, E and K and also contains thiamin and riboflavin (Tefera, 2011). Javaid and Manmood (2010) revealed that soybean can fix a maximum of 200 kg N/ ha annually, thereby limiting the costs and environmental harm of nitrogen fertilizer. Saifaddin (2021) reported that in South Africa, Free State Province is the leading soybean grower with 504 000 metric tons, which is approximately 40.65 % of the total national production. Free State Province is followed by Mpumalanga Province with 34.5%, KwaZulu-Natal with 8.5 %, Northwest with 6.29 %, Gauteng with 5.8 %, Limpopo with 3.6 %, Northern Cape with 0.56 %, Eastern Cape with 0.24 %. Western Cape Province does not produce the crop.

There are two types of soybean cultivars, 'promiscuous' that can nodulate naturally with native soil *Rhizobia*, and 'specific-nodulating' that require inoculation with commercial inoculants for effective nodulation. Promiscuous cultivars form nodules (effective) with the indigenous rhizobia or cowpea-type rhizobia while specific-nodulating soybean requires inoculation with compatible inoculants, such as "*Bradyrhizobium japonicum*", before planting to enable effective nodulation (Gwata *et al.,* 2004). Promiscuous soybean cultivars develop high biomass when nodulation has been effective. The higher biomass can be incorporated into the soil for soil fertility amelioration and this reduces the costs of fertilization, which serves as an advantage to resource-poor smallholder (SH) farmers. Akande *et al.* (2007) reported that plant breeders are continuously developing new soybean cultivars and their development mainly focuses on promiscuity. Such new cultivars need to be evaluated for their growth and yield performance, as they can help to sustain productivity in the low-input cropping systems of the SH farming sector.

Nitrogen (N) is needed for growth and development of the crop as it serves as one of the potential nutrient elements. There are bacteria (rhizobia) which are accountable for atmospheric N fixation in relation to legume crops. Sessitsch *et al.* (2002) reported that these bacteria (rhizobia) are responsible for facilitating a symbiotic relationship with legumes and results in nodulation on their roots and reduce atmospheric N to make it available to the host plants. Nodules develop on legume roots to facilitate biological N fixation. Therefore, effective nodule formation of soybean plants by effective rhizobia results in large N amounts being fixed through the symbiotic association between the plant and bacteria. The study of Gwata *et al.* (2004) revealed that soybean nodulates poorly in various soil types of tropical areas and therefore presence of compatible rhizobial strains is therefore important for N to be fixed. For example, Abaidoo *et al.* (2000) revealed that in some locations of Nigeria promiscuous cultivars were found to have poor nodulation. Sanginga *et al.* (1997) and Mpepereki *et al.* (2000) mentioned that the International Institute of Tropical Agriculture (IITA) soybean scientists bred promiscuous soybean varieties (Tropical Glycine crosses (TGx)) for natural nodulation in the late 1970s and 1980s; and also encouraged the utilization of inoculants in soybean production. All these developments were made to inspire extensive adoption and growing of soybean by SH farmers. Soybean production costs could be lowered due to the ability of the promiscuous soybean cultivar to have effective nodulation with the indigenous rhizobia. This can give SH farmers a greater advantage in soybean production as they will only need access to the seed to be able to produce the crop. This can further bring more benefits in improving the household diet from the soybean high protein, oil content and enhanced yield due to inputs of biologically fixed N. The study of Mpepereki *et al.* (2000) emphasized that the improvement in soil fertility will also contribute to the feasibility or sustainability in the soybean cropping system.

Inoculation can be defined as the technique of applying effective rhizobia onto the legume seed prior to planting to facilitate the nodulation process. The study of Balesevic-Tubic *et al.* (2011) reported that the addition of inoculant with a pertinent strain of bacteria for N fixation is one of the greatest methods to make sure of effective nodulation and to obtain higher yield of soybean. Shiferaw *et al.* (2004) emphasized that BNF saves costs and determines the sustainability of soybean production. The study of Abaidoo *et al.* (2007) revealed that African soils are characterized by poorer BNF activities and lead to low yields of soybean when no addition of N fertilizers or soybean rhizobia has been considered.

Besides enhancement of nodulation by rhizobial strains for BNF, the use of seaweed extracts as biofertilizers can also be considered to obtain high grain yield and total above-ground biomass of soybean. A "biofertilizer" is a substance that is comprised of microbes, which contribute towards promotion of plant growth and development by increasing the translocation and transportation of essential nutrients (N and P) to the plants (Sumkiman *et al.,* 2014). Karthikeyan and Shanmugam (2014) reported that seaweed extracts are rich in mineral elements that contain viz calcium (Ca), sulphur (S), phosphorous (P), iron (Fe), magnesium (Mg), chlorine (Cl), zinc (Zn), potassium (K) and copper (Cu). The seaweed extracts are regarded as the new type of products currently used in crop production as bio-fertilizers to enhance crop growth by activating N and P uptake. Their sources are various species of marine algae, which seem to be beneficial to crop production. They are considered as the most important group of organisms which can be mostly used in crop nutrition for growth stimulation (Ismail and El-Shafay, 2015). Seaweed extracts as bio-fertilizers intensify crop production through phosphate solubilization, plant hormone production and N fixation processes. They come in different forms from a liquid extract that can be useful as a soil drench, granular or powder form and even foliar spray as a manure and soil conditioner (Thirumaran *et al.,* 2009). Michalak and Chojnacks (2015) reported that liquid fertilizers are extracted from natural resources like micro-algae to serve as alternatives to fertilizers for crops as they are rich in micro and macro-elements, and growth regulators and the exploitation of these liquid fertilizers in modern agriculture has been extensively surveyed. Furthermore, liquid fertilizers and chopped powdered algal manures prepared form different marine algae varieties are being used (Michalak and Chojnacks, 2015). Panda *et al.* (2012) and Craigie (2011) reported that seaweed liquid fertilizers and other seaweed extract products are useful in achieving high agricultural production by mostly improving seed germination, seedling development, increased plant resistance to environmental stress and, also growth, development and yield of the plant. Panda *et al.* (2012) revealed that seaweed extract is beneficial as it is comprised of growth hormones like Auxins, Gibberlins, Polythymines, Betane and ethylene that contribute to the addition of trace elements, vitamins, amino acids, micro-elements and anti-biotics. Moreover, seaweeds are also used as soil amendments.

1.2. Problem statement

A key challenge for agricultural researchers is to have a good understanding on how and when the developed technologies are being implemented and the constraints affecting the implementation of the developed technologies. This has led to the search for information which is relative to the mechanisms underlying the developed technologies (Doss, 2006). The interaction of specific-nodulating rhizobia with both promiscuous and specific-nodulating soybean cultivars is not yet well known in soybean production. There is a problem of soybean low yields under low input conditions especially in marginal rainfall areas of Limpopo Province. The effect of application of seaweed extract as bio-fertilizer on promiscuous and specific nodulating soybean cultivars to maximize their growth and yield is also not known. There is a scarcity of literature on soybean growth and yield response to the combination of inoculation and seaweed extract under low input conditions.

1.3. Rationale

Soybean is one of the fairly drought resistant crops, which makes its cultivation under dry-land conditions possible in Limpopo Province which is characterized mostly by marginal rainfall. The consideration of promiscuous soybean cultivars as one of the stable crops that can be grown by Smallholder (SH) farmers has prospects to ameliorate low soil fertility characteristic of low input systems as soil with poor fertility status is a key problem in soybean production (Sanginga and Woomer, 2009). Maingi *et al.* (2006) mentioned that the SH farmers in African countries, including South Africa, lack resources which make the affordability of the expensive N fertilizer inputs needed to obtain high crop yield on poor fertile soils to be difficult. This often results in greatly reduced yields, and failure to achieve food and nutrition security. Soybean benefits crop production as it fixes atmospheric N which assists in reducing the constraint of N deficiency in soils and improves food and nutrition security of the households that are resource poor, often with limited access to adequate protein (animal), by providing a cheap protein alternative (Chianu *et al.,* 2008). The SH farmers need cheap sources of plant nutrients and any management approach that can enhance the tolerance of their crops to abiotic stress is important as they are largely located in hot areas with erratic rainfall conditions (Sanginga and Woomer, 2009). Promiscuous soybean cultivars have large biomass which can facilitate soil fertility amelioration. There is also a need to evaluate if the performance of either promiscuous or specific-nodulating soybean varieties can be improved by seaweed extracts as bio-fertilizers and inoculation. Seaweed extracts have potential to enhance growth and yield of crops through enhancing their nutrient uptake, and tolerance to abiotic stress (Karthikeyan and Shanmugam, 2014). Therefore, the use of seaweed extracts and inoculants for crop growth stimulation through N and P recovery is advantageous to SH farmers as they are relatively cheaper than inorganic fertilizer and are also environmentally friendly. This will encourage the adoption and facilitate soybean production by SH farmers. Therefore, the combination of inoculation and seaweed extracts on soybean growth and yield is worthy of investigation.

1.4. Purpose of the study

1.4.1. Aim

The study aimed to evaluate the performance of two soybean types to a combination of inoculation and application of seaweed extract.

1.4.2. Objectives

The objectives of this study were:

i. To determine the influence of inoculation on soybean growth, nodulation and yield.

ii. To determine the influence of variety on soybean growth, nodulation and yield.

iii. To determine the influence of seaweed extract on soybean growth, nodulation and yield.

iv. To determine the interaction influence of inoculation and variety on soybean growth, nodulation and yield.

v. To determine the interaction influence of inoculation and seaweed extract on soybean growth, nodulation and yield.

vi. To determine the interaction influence of variety and seaweed extract on soybean growth, nodulation and yield.

vii. To determine the interaction influence of variety, inoculation and seaweed extract soybean growth, nodulation and yield.

1.5. Hypotheses

The hypotheses of this study were:

i. Inoculation has no influence on soybean growth, nodulation and yield.

ii. Variety has no influence on soybean growth, nodulation and yield.

iii. Seaweed extract has no influence on soybean growth, nodulation and yield.

iv. Interaction of inoculation and variety has no influence on soybean growth, nodulation and yield.

v. Interaction of inoculation and seaweed extract has no influence on soybean growth, nodulation and yield.

vi. Interaction of variety and seaweed extract has no influence on soybean growth, nodulation and yield.

vii. The interaction of variety, inoculation and seaweed extract has no influence on soybean growth, nodulation and yield.

CHAPTER 2

LITERATURE REVIEW

2.1. Soybean description, origin, uses and early history

Soybean *(Glycine max* L.*)* is a member of Fabaceae family. It is mostly considered as the most widely grown oil seed and protein crop worldwide (Tefera, 2011). It is a grain legume indigenous to the Manchurian region of China (DAFF, 2011). In the late 1800s soybean was introduced into Africa, from North America and was first reported in South Africa in 1903 (Probst and Judd, 1973). The soybean grain is comprised of approximately 37-42 % protein (Medic *et al*., 2014), 28 % lipids (edible oil) (Foster *et al*., 2009), 5.72-7.00 % soluble sugars and 30 % carbohydrates (Tefera, 2011). The soybean seed or grain also contains 8 % seed coat, 2 % hypocotyls and 90 % cotyledons (Rani and Grewal, 2009). Soybean seed is consumed by human beings in a smaller percentage as dried beans or fresh green vegetable and can produce products that include soy protein, soy milk, soy flour, tofu and several retail food products (Riaz, 2006). Soybean as animal feed (hay) is also important as it comprises of 16 to 19 % crude protein and 50 to 55 % (Total N) if harvested when 50 % of the pods have immature beans (Rob, 2012). According to Fageria *et al.* (2005), soybean can be considered as a cover crop and has desirable attributes such as fixing atmospheric N and improving soil organic matter amendment. According to Mpepereki *et al.* (2000), the large biomass of soybean has a positive effect on soil fertility amelioration and weed control.

It has been reported that soybean has been considered as a versatile crop due to its multiple uses by United Soybean Board (USB) of America (USB, 2008). The board is focused on increasing soybean market through modern cropping system in soybean research and technology such as coatings, plastics, lubricants and bio-diesel. Presently, the oil that is extracted from the soybean seeds is made into shortenings, margarine and cooking oil (USB, 2008). Soybean oil is also useful in printing inks, caulking compounds and industrial paints (Akande *et al*., 2007). The soybean cake with high protein after oil extraction can be used to produce soybean flour for human consumption or mixed into livestock feeding. Soybean proteins are also added in baby formulae, sport drinks and weight-loss drinks. Soybean grits and flour are processed in industrial use such as commercial bakery to support in thick conditioning and blenching and conditioning (Akande *et al*., 2007).

2.2. Production of soybean in South Africa

Saifaddin (2021) mentioned that the current South African aggregate commercial production of soybeans amounted to roughly 1.24 million metric tons. Free State Province was the leading grower with 504 000 metric tons, which is approximately 40.65 % of the total production. Mpumalanga Province share totalled 34.5 % (429 000 metric tons) of soybean production, KwaZulu-Natal with 8.5 % (105 000 metric tons), Northwest with 6.29 % (78 000 metric tons), Gauteng with 5.8 % (72 000 metric tons), Limpopo with 3.6 % (45 000 metric tons), Northern Cape with 0.56 % (7 000 metric tons), Eastern Cape with 0.24 % (3 000 metric tons). Western Cape Province ranked the last producer with a 0 % (0 metric tons) does not produce the crop.

2.3. Promiscuous versus specific-nodulating soybean

A promiscuous soybean variety is the one which can nodulate naturally with available cowpea type rhizobial strains whereas specific-nodulating soybean variety is the one which needs to be inoculated with commercial inoculants. The specific-nodulating soybean varieties are usually inoculated with rhizobia type (*Bradyrhizobium japonicum)* inoculum before it can be planted to enable it to form effective nodules. Gwata *et al.* (2004) reported that promiscuous variety of soybean can form nodules effectively with the indigenous or cowpea type rhizobia*.* Akande *et al.* (2007) reported that IITA is continuously developing new cultivars of promiscuous soybean.

2.4. Inoculation description and soybean response to inoculation

Inoculation is the proper technique of applying rhizobia (effective) on the seed just before planting any leguminous seed to efficiently enable effective N fixation. Nitrogen (N) fixation is a result of the symbiotic association of bacteria (Rhizobia) and soybean plants. The bacteria can fix atmospheric N into the ammonium (NH_4) form which is beneficial to the plants. One of the best ways to make sure of good nodulation to lead to increased yield of any leguminous crop is through inoculation with appropriate strain of N fixing bacteria (Balesevic-Tubic *et al.,* 2011). Biological nitrogen fixation (BNF) has great advantages for any leguminous crop as it is cost effective and more sustainable (Shiferaw *et al.,* 2004). According to Abaidoo *et al.* (2007), most African soils require chemical nitrogen fertilizer as an input or the external application of soybean rhizobia as the soils are characterized by poorer BNF activities which obviously cannot achieve high yields of soybean.

Rhizobium inoculants have been manufactured worldwide for almost a century (Bashan, 1998). Some legumes, such as soybean in Brazil, inoculation is compulsory due to the fact that there is no nitrogen fertilizer to be applied as an alternative. Seeds inoculated with *Bradyrhizobium* culture resulted in significantly tall plants with more nodule number, seed grains per pod, pod number per plant and mass of the seed as compared to control treatments (Singh, 2005). *Bradyrhizobium japonicum* inoculation was reported to cause significantly higher plant growth, nodulation and soybean yield (Javaid and Mahmood, 2010). Schulz *et al.* (2005) mentioned that soybean yield increase of 40 % was found in soybean inoculated with *Bradyrhizobium japonicum as* compared to the uninoculated soybean.

2.5. Seaweed extract description and response of various crops and vegetables

Seaweed extracts are regarded as the new type of marine algae products currently utilized in crop production as bio-fertilizers to enhance crop growth by activating N and P nutrient uptake by the plant (Sumkiman *et al.,* 2014). The seaweed extract sources are various types of marine algae species, which seem to add a great value or potential in crop production. These seaweeds are a subject of interest in agricultural industry (Sumkiman *et al.,* 2014). Seaweed extract can come as liquid or in powder form. Application of seaweed extract can be through either foliar spraying or drench methods (Ismail and El-Shafayb, 2015). Foliar spray has been reported to be an active method which has showed positive impacts on growth and yield of grasses, flowers, cereals, and vegetables such as cabbages, spinach, tomatoes and spices (Pramanick *et al.,* 2014). Seaweed extract was also reported as a stimulant to various aspects of plant growth and development such as enlargement of shoot, increased photo-assimilate production rate through photosynthesis, good health, development of the root system, seed germination, phytogenic fungi control, insects or other pests control and absorption of minerals (Davari *et al.,* 2012; Badar *et al.,* 2015; Alves *et al.,* 2016).

Seaweed extracts when applied to plants by small amounts (0.2 to 1.0 %) indicate the presence of active compounds with remarkable growth variables (Crouch, 1990). Aqueous alkaline seaweed extract of *Ascophyllum nodosum* (Algifert 25) on soil, exhibited higher chlorophyll content in the leaves of tomatoes (Tiny Tim cultivars)), dwarf French beans (*Phaseolus vulgaris*), wheat (Brigadier cultivars), barley and maize cultivars than control treatment when treated by the very same amount or volume of water (Blunden *et al.,* 1996). Blunden (1977) evaluated the nutritional content of different kelp extracts and found that the volume of nutrients supplied to crops via foliar spray application failed to enhance plant performance. Funugreek treated with 50 % concentration of seaweed extract improved amino acid, nitrogen content, protein, carbohydrates and polyphenols concentrations as compared to control treatment plants (Pise and Sabale, 2010).

Seaweed extracts are biodegradable, environmentally friendly and non-toxic as compared to chemical fertilizers (Ramarajan *et al.,* 2013). Seaweed extracts are thus economically viable as they result in the highest productivity (Michalak and Chojnacka, 2015). Seaweed extracts are also essential in playing a major function in intramural system of plant growth by interconnecting with major processes of metabolism such as protein synthesis and metabolism of nucleic acid (Alam *et al.,* 2014; Michalak and Chojnacka, 2015). Growth retardants are responsible in enhancing association of source and sink in plants, thereby activating the transportation and translocation of photo assimilates to the seeds (Hurde and Parjosavulecs, 1981). Hurde and Parjosavulecs (1981) further observed that soybean seeds with various rates of seaweed extract such as TIBA, CCC, Atonik and Amen-Chen T6AS63 exhibited decrease in plant height with increase in concentration of these growth retardants. Literature on the effects of seaweed extracts and inoculation on soybean is limited and is found mostly on vegetable crops where seaweed extracts enhanced growth variables (Zodape *et al.,* 2010).

Tanner and Ahmed (1974) observed that reproductive dry matter production rate and seed yield were higher when soybean was treated with TIBA. Hurde and Parjosavulecs

(1981) observed soybean seeds treated with different rates of seaweed extract such as CCC, TIBA, Atonik and Ame-Chen T6AS63 had a reduction in plant height with an increment in concentration of these growth retardants. It is very important to have a good understanding on the growth variables of soybean responsible for yield in order to achieve higher productivity of soybean. Barthakur (1980) studied the effect of spraying soybean cultivar Bragg with 25, 50 and 100 g TIBA/ ha at 25 or 40 days after sowing and found increase in yield by 18, 18 and 23.7 %, respectively. Chung and Kim (1989) tested the spraying of soybean with TIBA, ABA or DGLP at different growth stages and noticed that TIBA and ABA increased podding rate and pod number per plant, but, all treatments increased the number of seeds per pod and seed yield and only TIBA increased 100-seed weight.

CHAPTER 3

MATERIALS AND METHODS

3.1. Description of the study site

The research trial was conducted at the experimental farm of the University of Limpopo during 2017/ 2018 summer season, the farm area is also known as Syferkuil farm (23 °49' S, 29 ° 41' E and 1261.6 m altitude). The Syferkuil farm area is located near Mankweng in Limpopo Province (Capricorn district). The climate of the research site is classified as semi-arid. It is characterized by cool dry winters and hot dry summers. The mean annual rainfall of Syferkuil area is ±500 mm that falls mostly in summer. The range of the summer mean daily temperatures is 28 °C to 30 °C (Mpangane *et al.,* 2004). The soil at Syferkuil farm is classified as "sandy loam, of the Hutton form, *Glenrosa* family" (Moshia, 2005).

3.2. Experimental design, treatments and procedures

The research trial was laid out in a split-split plot arrangement fitted in a randomized complete block design (RCBD) with four (4) replications. Two inoculation levels (inoculation and no inoculation) occupied the main plots; two soybean variety types occupied the sub plots while three rates of seaweed extract occupied the sub-sub plots. One promiscuous soybean variety (TGx-1937-1F) and one specific-nodulating soybean variety (PAN 1583R) were used in this study. Seaweed extract (FLAMMA) was applied at 3 rates: 0 (control), 50 % of recommended rate (30 ml/ 12 L) and recommended (100 %) rate (60 ml/ 12 L). The Inoculant used in this trial was *Bradyrhizobium japonicum* strain WB74. Inter-row spacing was 60 cm and intra-row spacing was 15 cm where each plot had 6 rows of 3 m length, occupying 10.8 m^2.

The soybean seeds were inoculated using *Bradyrhizobium japonicum* strain WB74 just before planting. The seeds were well mixed with a slurry of the inoculant, mollyflo sticker and sugar to make sure that all seeds were treated well. After mixing, the seeds that were inoculated were placed under the shade to dry. The treated seeds were planted into moist soil in the shadow of the researcher (human being shadow during planting) and immediately covered with soil to avoid contact with direct sunlight. Seaweed extract was sprayed according to the treatments on the foliage at onset of flowering. It was applied following label instructions and was applied early in the morning. No inputs such as pesticides and fertilizers were applied and irrigation was applied only when prolonged moisture stress was observed. Weeding was conducted manually by using a hand hoe.

3.3. Soil sampling

Before planting, four soil samples were collected in a random manner from different places in each block using a soil auger at depths of 0- 15 cm and 0- 30 cm to determine physio-chemical properties which included soil pH, organic carbon (%), phosphorus (P), mineral nitrogen (NH_4^+ and NO_3^-) and electrical conductivity (EC). The composite soil samples were achieved by thoroughly mixing all soil samples from each block. At harvest maturity, soil sampling was done to the same depths on a plot basis. The soil samples were taken for analysis at the Soil Science laboratory of the University of Limpopo. The pH meter (GLP 21-Cripson) was used to determine soil pH, EC meter (Meter Toledo) was used to determine EC, Walkley-Black method was used to determine organic carbon (%) (Walkley and Black, 1943), Colorimetric method was used to determine mineral nitrogen (Okalebo *et al.,* 2002) and phosphorus by Bray 1 method (Bray and Kurtz, 1945).

3.4. Data collection

3.4.1. Phenological development and growth variables

The collected phenological data were: days to 50 % flowering and days to 50 % physiological maturity. When pods had turned brown and seeds shook loose the days to 50 % physiological maturity were recorded. Growth variables from four (4) plants per plot were recorded at physiological maturity and these included the following: leaf number per plant, leaf area, stem diameter, primary branches per plant and plant height. Leaf number and primary branches per plant were counted manually. Plant height was measured using a metre ruler, stem diameter (using a Vernier caliper (R5 pro)), and leaf area (using leaf area metre (AM350)). Chlorophyll meter (CCM 200 plus) was used to measure leaf chlorophyll content.

3.4.2. Plant biomass and nodulation

Four representative plants from the net plot (7.2 m^2) were selected in a random manner at 50 % flowering to early podding growth stage to record nodulation performance. A spade was used to carefully uproot the soybean plants, placed them in brown bags and took them to the University of Limpopo Plant Production laboratory for processing. Tap water was used to wash the roots to remove soil and the detached nodules were trapped using a sieve. Tap water was also used to wash the roots after separating plants into shoots and roots. Picking of nodules was conducted manually from the roots and the following were recorded: (a) nodule number per plant, (b) effective nodules per plant, (c) dry weight of nodules and (d) dried shoot biomass. To evaluate the effectiveness of the nodules, fresh ones were cut up to observe the colour inside. Active nodules were represented by pinkish to reddish colour. The oven was set at 65 °C to dry soybean shoots to a constant weight to determine biomass (dried shoot biomass). For nodule dry weight, oven was also set at 65 °C to dry nodules for 24 hours and electronic weighing scale was used to determine the dried nodule weight.

3.4.3. Grain yield and yield components

At harvest maturity the yield and yield components were recorded from the net plot (7.2 m^2). Pod number per plant was recorded from four consecutive plants at 50 % physiological maturity. Unshelled, shelled weight and 100 seeds weight were determined using an electronic weighing scale (CBK 15M). Grain yield and total above-ground biomass were also determined from the same net plot area (7.2 m^2) and also weighed using an electronic weighing scale (CBK 15M). The following formulae were used to calculate harvest index (HI) and shelling percentage (SP):

HI= Grain yield ÷ total above-ground biomass

SP= Shelled grain weight ÷ Unshelled pod weight × 100 %

3.5. Moisture, protein, macro and micro-nutrient content of soybean grain

Harvested soybean seed samples were sent to Limpopo Agro-food Technology Station (LATS) at the University of Limpopo for further analyses. The seeds were analysed for:

moisture %, protein %, nitrogen % and mineral composition such as phosphorus (P), potassium (K), sodium (Na), calcium (Ca), copper (Cu), magnesium (Mg), zinc (Zn), iron (Fe) and manganese (Mn) (Riekert and Bainbridge, 1998).

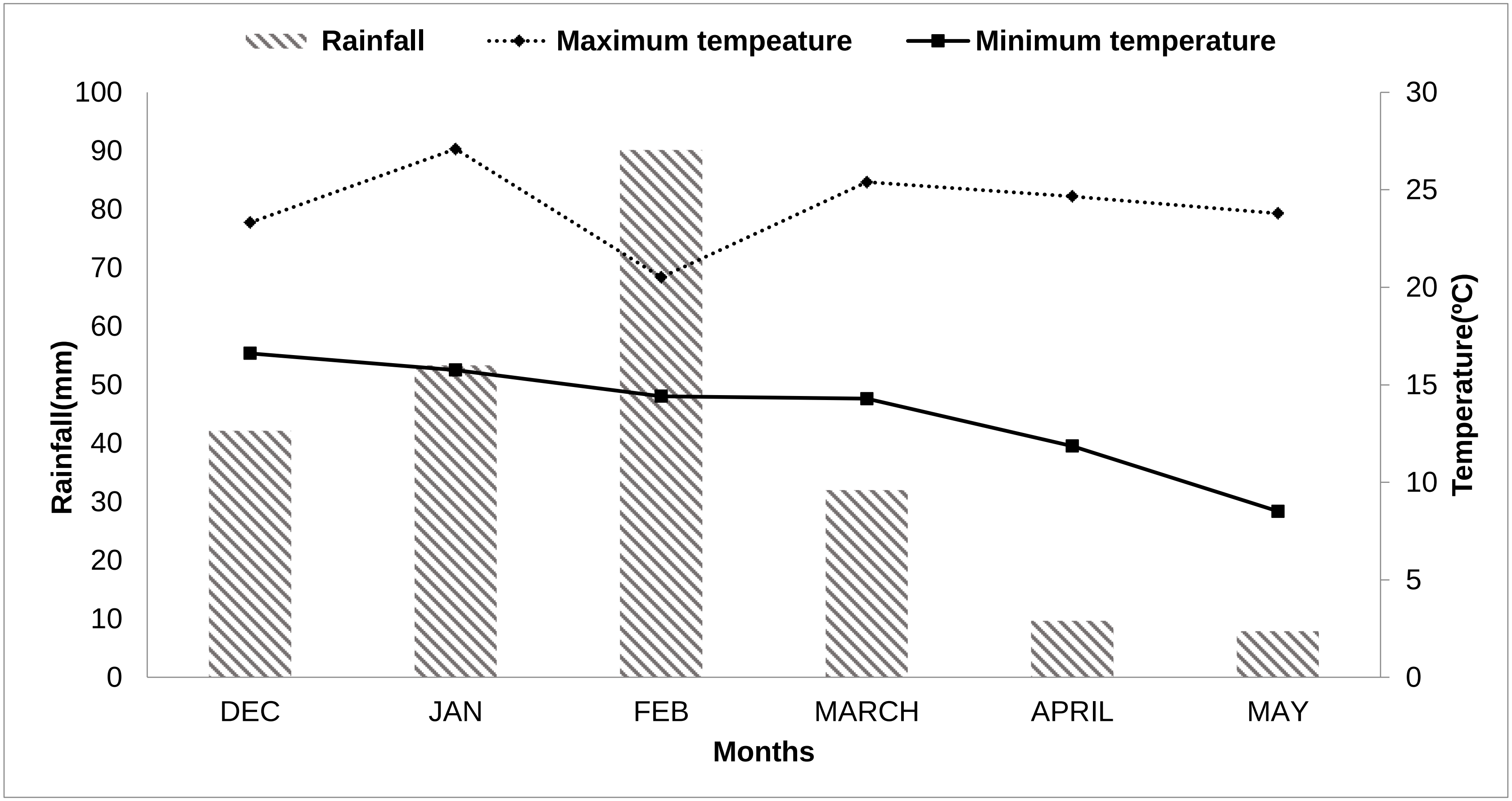
3.6. Data analysis

Collected data were subjected to Analysis of Variance (ANOVA) using Statistix 10.0. Tukey's Honestly Significant Difference was used to run mean separation test at $P \leq 0.05$. Correlation analysis was done between grain yield, growth, nodulation and yield variables.

3.7. Total monthly rainfall and average temperatures during 2017/ 2018 growing summer season

Figure 3.1 shows that adequate rainfall fell from December to March, with a peak in February. The highest total monthly rainfall occurred in February 2018 with approximately 90 mm and the lowest total monthly rainfall occurred in May 2018 with approximately 8 mm. The average maximum temperature increased from December 2017 to January 2018 and then decreased from January to February 2018. Once again there was a decrement from February to May 2018 in average maximum temperature. In this case, the highest average maximum temperature occurred in January 2018 with 27 °C and the lowest occurred in May 2018 with 7 °C. Average minimum temperature had an inverse proportion from December 2017 until May 2018. The highest average minimum temperature was in December 2017 with 16 °C and the lowest average minimum temperature was in May with 9 °C.

Figure 3.1. Total monthly rainfall and average temperatures during 2017/ 2018 growing summer season



3.8. Analysis of soil chemical results before and after planting

Table 3.1 below shows that Electrical conductivity at the subsoil was found to be higher (562.2) than that at the topsoil (181.9). On the other hand, pH (H_2O) was found to be higher at the topsoil (7.85) and lowest at the subsoil (7.69). Soil pH (KCL) was found to be higher at the topsoil (8.8) and lowest at the subsoil (7.69). Organic carbon percentage was higher at the subsoil (1.56 %) compared to 1.12% in the top soil. Phosphorus (mg/kg) on the other hand was found to be 1.42 mg/kg on topsoil and subsoil. Ammonium (NH_4+ mg/kg) was found to be slightly higher at the subsoil (0.09 mg/kg) whereas nitrate (NO_3- mg/kg) was found to be similar (0.22 mg/kg) at both levels.

Table 3.2 shows that treatment $I_1V_2S_3$ (535.08) was found to be the highest in electrical conductivity (µS/cm) and $I_1V_2S_1$ (212.08) was the lowest at the subsoil. At topsoil, $I_2V_1S_2$ (424.58) was found to be the highest and $I_1V_2S_1$ (225.08) was found to be the lowest in EC (µS/ cm). $I_1V_2S_3$ (8.8) was found to be highest in soil pH (H_2O). $I_2V_2S_1$ (8.49) was found to the lowest in pH (H_2O) at topsoil. Then $I_1V_2S_1$ (8.76) was the highest in pH (H_2O) as compared $I_1V_1S_2$ (7.99) which was the lowest at the subsoil. Treatment $I_2V_1S_3$ (8.77) was the highest in pH (KCL) and then treatment $I_2V_2S_3$ (8.34) at the topsoil was the lowest in pH (KCL). $I_1V_1S_3$ (8.74) was found to be the highest in pH (KCL) than $I_2V_1S_1$ (5.51) at the subsoil.

Organic carbon percentage was higher on $I_1V_2S_3$ (1.34 %) while it was the lowest on $I_1V_1S_3$ (0.74 %) at the subsoil. The organic carbon treatment which is $I_1V_2S_3$ (1.34 %) was also higher after harvest as compared to organic carbon percentage of before planting at 0-15cm which was 1.12%. At subsoil, $I_2V_2S_1$ (1.37 %) was the highest in organic carbon (%) than $I_1V_1S_3$ (0.78 %) at the topsoil. $I_2V_2S_1$ (0.34) was found to be the highest in Phosphorus (mg/kg) while $I_1V_1S_1$ (0.10) was the lowest at the topsoil. $I_1V_1S_2$ (0.16) was higher in Phosphorus (mg/kg) than $I_2V_1S_2$ (0.10) at the subsoil. $I_1V_1S_3$ (0.09) was found to be the highest in ammonium (NH_4^+ (mg/kg)) than $I_1V_1S_1$ (0.08) at the topsoil while $I_1V_1S_2$ (0.10) was found to be the highest than $I_2V_1S_3$ (0.08) at subsoil. $I_1V_1S_3$ was the highest by 2.19 than $I_1V_2S_3$ (2.05) which was the lowest in nitrate (NO_3-(mg/kg)) at the topsoil. $I_2V_1S_3$ at 2.20 was found to be the highest in nitrate (NO_3-(mg/kg)) as compared to $I_1V_1S_2$.

Table 3.1. Analysis of soil chemical results before to planting

Depth (cm)	EC (µS/cm)	pH (H_2O)	pH (KCL)	OC (%)	P (mg/kg)	NH_4^+ (mg/kg)	NO_3^- (mg/kg)
0-15	181.9	7.85	8.8	1.12	1.42	0.08	0.22
15-30	562.2	7.69	8.67	1.56	1.42	0.09	0.22

EC= Electrical conductivity, pH= Potential of Hydrogen, H_2O= Water, KCL= Potassium chloride, OC= Organic Carbon, P= Phosphorus, NH_4+ = Ammonium, NO_3+ = Nitrate, %= Percentage, mg/kg= milligrams per kilograms

Table 3.2. Post-harvest soil chemical analysis results

Treatment factor	Depth (cm)	EC (µS/ cm)	pH (H_2O)	pH (KCl)	OC %	P (mg/kg)	NO_4^+ (mg/kg)	NO_3^- (mg/kg)
$I_1V_1S_1$	0-15	281.10	8.54	8.47	1.12	0.10	0.08	2.16
	15-30	323.93	8.31	8.48	0.93	0.11	0.09	2.17
$I_1V_1S_2$	0-15	359.78	8.59	8.43	1.22	0.10	0.08	2.18
	15-30	489.50	7.99	8.49	0.92	0.16	0.10	2.06
$I_1V_1S_3$	0-15	304.63	8.77	8.70	0.75	0.13	0.09	2.19
	15-30	373.28	8.38	8.74	0.78	0.11	0.09	2.14
$I_1V_2S_1$	0-15	225.08	8.67	8.35	1.22	0.15	0.09	2.09
	15-30	212.53	8.76	8.33	1.06	0.14	0.09	2.13
$I_1V_2S_2$	0-15	232.18	8.78	8.55	1.31	0.14	0.09	2.14
	15-30	251.53	8.69	8.44	1.31	0.15	0.09	2.14
$I_1V_2S_3$	0-15	241.38	8.80	8.51	1.34	0.13	0.09	2.05
	15-30	535.08	8.55	8.44	1.09	0.13	0.09	2.07
$I_2V_1S_1$	0-15	322.13	8.52	8.50	1.16	0.12	0.09	2.18
	15-30	366.93	8.46	6.51	1.22	0.12	0.09	2.17
$I_2V_1S_2$	0-15	424.58	8.59	8.50	1.19	0.14	0.09	2.18
	15-30	367.40	8.41	8.50	1.12	0.10	0.09	2.20
$I_2V_1S_3$	0-15	260.35	8.74	8.77	0.87	0.11	0.09	2.08
	15-30	251.35	8.38	8.68	0.94	0.11	0.08	2.12
$I_2V_2S_1$	0-15	432.70	8.49	8.45	1.06	0.34	0.09	2.11
	15-30	335.78	8.47	8.38	1.37	0.13	0.09	2.19
$I_2V_2S_2$	0-15	279.03	8.63	8.61	1.06	0.17	0.09	2.08
	15-30	379.13	8.45	8.53	1.11	0.13	0.09	2.13
$I_2V_2S_3$	0-15	233.23	8.79	8.34	0.90	0.17	0.09	2.18
	15-30	270.05	8.71	8.46	1.23	0.14	0.09	2.10

I_1= without inoculation, I_2= inoculation, V_1= Variety one (TGX 1937-1F), V_2= Variety two (PAN 1583R), S_1= 0 % rate of seaweed extract, S_2= 50 % rate of seaweed extract, S_3= 100 % rate of seaweed extract, %= Percentage, EC= Electrical conductivity, pH= Potential of Hydrogen, H_2O= Water, KCL= Potassium chloride, OC= Organic Carbon, P= Phosphorus, NH_4+ = Ammonium, NO_3+ = Nitrate

CHAPTER 4

RESULTS

4.1. Phenological development

The interactive effects (three-way interaction) of inoculation, variety and seaweed extract on soybean phenological development were found not to have significant differences. The 2-way interactive effects are presented in Tables 4.2 to 4.4.

4.1.1. *Bradyrhizobium japonicum* WB74 inoculation, variety and seaweed extract main effects on soybean phenological development

Inoculation did not significantly influence soybean phenological development (Table 4.1). Soybean variety exhibited significant differences on the phonological development of soybean. The variety TGx 1937-1F took significantly ($P \leq 0.001$) longer to reach 50 % flowering at 82 days compared to 69 days for variety PAN 1583R. The variety PAN 1583R reached 50 % physiological maturity earlier, after 137 days, as compared to variety TGX 1937-1F at 159 days. The application of seaweed extract exhibited no significant influence on phenological development of the two soybean varieties.

Table 4.1. *Bradyrhizobium japonicum* WB74 inoculation, variety and seaweed extract main effects on soybean phenological development

Inoculation	**Days to 50 % flowering**	**Days to 50 % physiological maturity**
Inoculation	74.44a	148.67a
No inoculation	77.42a	148.46a
Significance	ns	ns
$HSD_{0.05}$	-	-
Variety		
TGx 1937-1F	82.08a	159.54a
PAN 1583R	69.79b	137.58b
Significance	**	***
$HSD_{0.05}$	7.44	0.38
Seaweed extract		
0 % SWE	73.19a	148.50a
50 % SWE	76.88a	148.38a
100 % SWE	77.75a	148.81a
Significance	ns	ns
$HSD_{0.05}$	-	-

N B. Means in a column represented by the same letter are not significantly different at P≤0.05. HSD= Honestly significant difference, ns= not significantly different at P≤0.05, **= Highly significantly different at P≤0.01, ***= Highly significantly different at P≤0.001, %= Percentage, SWE= Seaweed extract

4.1.2. *Bradyrhizobium japonicum* WB74 inoculation and variety interactive effects on soybean phenological development

Inoculation and variety interaction had no significant difference on soybean phenological development (Table 4.2).

Table 4.2. *Bradyrhizobium japonicum* WB74 inoculation and variety interactive effects on soybean phenological development

Inoculation × Variety	Days to 50 % flowering	Days to 50 % physiological maturity
Inoculation × TGx 1937-1F	82.17a	159.67a
Inoculation × PAN 1583R	66.75a	137.67a
No inoculation × TGx 1937-1F	82.00a	159.42a
No inoculation × PAN 1583R	72.83a	137.50a
Significance	ns	ns
$HSD_{0.05}$	-	-

N: B. Means in a column represented by the same letter are not significantly different at P≤0.05. HSD= Honestly significant difference, ns= not significantly different at P≤0.05, %= percentage

4.1.3. *Bradyrhizobium japonicum* WB74 inoculation and seaweed extract interactive effects on soybean phenological development

Interactive effect of inoculation and seaweed extract exhibited no significant influences on soybean phenological development (Table 4.3).

Table 4.3. *Bradyrhizobium japonicum* WB74 inoculation and seaweed extract interactive effects on soybean phenological development

Inoculation × Seaweed extract	Days to 50 % flowering	Days to 50 % physiological maturity
Inoculation × 0 % SWE	68.62a	148.75a
Inoculation × 50 % SWE	76.87a	148.13a
Inoculation × 100 % SWE	77.88a	149.13a
No inoculation × 0 % SWE	77.75a	148.25a
No inoculation × 50 % SWE	76.88a	148.63a
No inoculation × 100 % SWE	77.63a	148.50a
Significance	ns	ns
$HSD_{0.05}$	-	-

N: B. Means in a column represented by the same letter are not significantly different at P≤0.05. HSD= Honestly significant difference, ns= not significantly different at P≤0.05, %= percentage, SWE= Seaweed extract

4.1.4. Variety and seaweed extract interactive effects on soybean phenological development

Table 4.4 shows that there was no significant influence on interactive effect of variety and seaweed extract on phenological development of soybean varieties.

Table 4.4. Variety and seaweed extract interactive effects on soybean phenological development

Variety × Seaweed extract	Days to 50 % flowering	Days to 50 % physiological maturity
TGx 1937-1F × 0 % SWE	82.50a	159.50a
TGx 1937-1F × 50 % SWE	81.63a	159.38a
TGx 1937-1F × 100 % SWE	82.13a	159.75a
PAN 1583R × 0 % SWE	63.88a	137.50a
PAN 1583R × 50 % SWE	72.13a	137.38a
PAN 1583R × 100 % SWE	73.37a	137.88a
Significance	ns	ns
$HSD_{0.05}$	-	-

N: B. Means in a column represented by the same letter are not significantly different at P≤0.05. HSD= Honestly significant difference, ns= not significantly different at P≤ 0.05, %= percentage, SWE= Seaweed extract

4.2. Nodulation

The 3-way interaction of inoculation, variety and seaweed extract did not show any significant influence on all the nodulation variables recorded (nodules number per plant, effective nodules number per plant, nodule dry weight) and shoot dry mass. The 2-way interactive effects are presented in Tables 4.6 to 4.8.

4.2.1. *Bradyrhizobium japonicum* WB74 inoculation, variety and seaweed extract main effects on soybean nodulation

Inoculation showed no significant difference on all nodulation variables (Table 4.5). Soybean variety exhibited significance differences on nodule number per plant, effective nodules per plant, nodule dry weight and dried shoot biomass per plant. The variety TGx 1937-1F had significantly higher nodule number per plant (2.46) and effective nodules (2.46) per plant at $P \leq 0.05$, nodule dry weight (0.03 g) and dried shoot biomass (4.95 g) per plant as compared to variety PAN 1583R which obtained the lower nodule number per plant (0.79), effective nodules (0.79) per plant, nodule dry weight (0.01 g) and dried shoot biomass (2.32 g) per plant. Seaweed extract showed no significant effect on nodule variables and dried shoot biomass per plant.

Table 4.5. *Bradyrhizobium japonicum* WB74 inoculation, variety and seaweed extract main effects on soybean nodulation variables and shoot biomass

Inoculation	**Nodule number per plant**	**Effective nodules per plant**	**Nodule dry weight (g)**	**Dried shoot biomass per plant (g)**
Inoculation	1.96a	1.96a	0.03a	3.37a
No inoculation	1.29a	1.29a	0.02a	3.89a
Significance	ns	ns	ns	ns
$HSD_{0.05}$	-	-	-	-
Variety				
TGx 1937-1F	2.46a	2.46a	0.03a	4.95a
PAN 1583R	0.79b	0.79b	0.01b	2.32b
Significance	*	*	**	**
$HSD_{0.05}$	1.69	1.70	0.02	0.11
Seaweed extract				
0 % SWE	1.99a	1.99a	0.03a	3.73a
50 % SWE	1.25a	1.25a	0.02a	3.60a
100 % SWE	1.50a	1.50a	0.02a	3.58a
Significance	ns	ns	ns	ns
$HSD_{0.05}$	-	-	-	-

N: B. Means in a column represented by the same letter are not significantly different at P≤0.05. HSD= Honestly significant difference, ns= not significantly different at P≤0.05, *= Significantly different at P≤0.05, **= Highly significantly different at P≤0.01, %= percentage, SWE= Seaweed extract, g= gram

4.2.2. *Bradyrhizobium japonicum* WB74 inoculation and variety interactive effects on soybean nodulation

Table 4.6 shows that there were no significant differences between all inoculation and soybean variety interactions on plant nodule number, effective nodules per plant, nodule dry weight and dried shoot biomass per plant.

Table 4.6. *Bradyrhizobium japonicum* WB74 inoculation and variety interactive effects on soybean nodulation variables and shoot biomass

Inoculation × Variety	**Nodule number per plant**	**Effective nodules per plant**	**Nodule dry weight (g)**	**Dried shoot biomass per plant (g)**
Inoculation × TGx 1937-1F	2.33a	2.33a	0.04a	4.69a
Inoculation × PAN 1583R	1.98a	1.98a	0.03a	2.49a
No inoculation × TGx 1937-1F	2.58a	2.58a	0.05a	5.21a
No inoculation × PAN 1583R	2.00a	2.00a	0.03a	2.58a
Significance	ns	ns	ns	ns
$HSD_{0.05}$	-	-	-	-

N: B. Means in a column represented by the same letter are not significantly different at P≤0.05. HSD= Honestly significant difference, ns= not significantly different, g= gram

4.2.3. *Bradyrhizobium japonicum* WB74 inoculation and seaweed extract interactive effects on soybean nodulation and shoot biomass

Table 4.7 shows that there were no significant differences between all the interactions of inoculation and seaweed extract on plant nodule number, effective nodules per plant, nodule dry weight and dried shoot biomass per plant.

Table 4.7. *Bradyrhizobium japonicum* WB74 inoculation and seaweed extract interactive effects on soybean nodulation and shoot biomass

Inoculation × Seaweed extract	**Nodule number per plant**	**Effective nodules per plant**	**Nodule dry weight (g)**	**Dried shoot biomass per plant (g)**
Inoculation × 0 % SWE	2.13a	2.13a	0.03a	3.36a
Inoculation × 50 % SWE	1.38a	1.38a	0.02a	3.42a
Inoculation × 100 % SWE	1.88a	1.88a	0.02a	3.34a
No inoculation × 0 % SWE	1.88a	1.88a	0.04a	3.80a
No inoculation × 50 % SWE	1.08a	1.08a	0.01a	3.78a
No inoculation × 100 % SWE	1.13a	1.13a	0.02a	4.11a
Significance	ns	ns	ns	ns
$HSD_{0.05}$	-	-	-	-

N: B. Means in a column represented by the same letter are not significantly different at P≤0.05. HSD= Honestly significant difference, ns= not significantly different, %= percentage, SWE= Seaweed extract, g= gram

4.2.4. Variety and seaweed extract interactive effects on soybean nodulation and shoot biomass

Table 4.8 below shows no significant differences on the interaction of variety and seaweed extract in nodule number per plant, effective nodules per plant, nodule dry weight and dried shoot biomass per plant.

Table 4.8. Variety and seaweed extract interactive effects on soybean nodulation and shoot biomass

Variety × Seaweed extract	**Nodule number per plant**	**Effective nodules per plant**	**Nodule dry weight (g)**	**Dried shoot biomass per plant (g)**
TGx 1937-1F × 0 % SWE	1.98	1.98	0.04a	4.85a
TGx 1937-1F × 50 % SWE	1.52	1.52	0.03a	4.84a
TGx 1937-1F × 100 % SWE	2.07	2.07	0.04a	5.16a
PAN 1583R × 0 % SWE	1.65	1.65	0.02a	3.31a
PAN 1583R × 50 % SWE	2.28	2.28	0.01a	3.35a
PAN 1583R × 100 % SWE	1.38	1.38	0.02a	3.29a
Significance	ns	ns	ns	ns
$HSD_{0.05}$	-	-	-	-

N: B. Means in a column represented by the same letter are not significantly different at P≤0.05. HSD= Honestly significant difference, ns= not significantly different, %= percentage, SWE= Seaweed extract, g= gram

4.3. Soybean growth variables

Three-way interaction of inoculation, variety and seaweed extract showed significant differences on leaf number per plant (P≤0.05), primary branches per plant (P≤0.001) and plant height (P≤0.001) and no significant differences on leaf chlorophyll content and leaf area (Table 4.9). Interactive effect of inoculation × variety TGx 1937-1F × 100 % rate of seaweed extract obtained significantly high leaf number per plant (44) and primary branches per plant (34.25). Interaction of inoculation × variety PAN 1583R × 50 % rate of seaweed extract recorded the lowest leaf number per plant (8.75). Significantly lowest primary branches per plant (5.75) was recorded under the interaction of no inoculation × variety PAN 1583R × 0 % seaweed extract. Interaction of no inoculation × variety TGx 1937-1F × 100 rate of seaweed extract obtained significantly taller plants (57.50 cm). Interaction of no inoculation × variety PAN 1583R × 0 % rate of seaweed extract obtained the shortest plants (17.50 cm).

Table 4.9. *Bradyrhizobium japonicum* WB74 inoculation, variety and seaweed extract interactive effects (3-way) on soybean growth variables

Inoculation × Variety × Seaweed extract	Leaf chlorophyll content (CCI)	Leaf area (cm^2)	Leaf number per plant	Primary branches per plant	Plant height (cm)
Inoculation × TGx 1937-1F × 0 % SWE	21.392a	166.20a	27.00c	25.00c	46.50cd
Inoculation × TGx 1937-1F × 50 % SWE	22.107a	160.68a	35.50b	28.50b	54.00ab
Inoculation × TGx 1937-1F × 100 % SWE	22.840a	160.92a	44.00a	34.25a	56.75a
Inoculation × PAN 1583R × 0 % SWE	18.583a	149.05a	9.50f	6.75h	19.50g
Inoculation × PAN 1583R × 50 % SWE	18.987a	159.88a	8.75f	9.50g	22.75fg
Inoculation × PAN 1583R × 100 % SWE	18.415a	152.10a	9.50f	12.00f	24.50ef
No inoculation × TGx 1937-1F × 0 % SWE	20.608a	140.92a	20.00d	25.00c	45.75d
No inoculation × TGx 1937-1F × 50 % SWE	19.978a	168.25a	37.50ab	28.25b	50.25bc
No inoculation × TGx 1937-1F × 100 % SWE	20.383a	159.78a	42.25a	33.25a	57.50a
No inoculation × PAN 1583R × 0 % SWE	18.825a	144.65a	10.00f	5.75h	17.50g
No inoculation × PAN 1583R × 50 % SWE	19.380a	140.88a	13.75ef	14.25e	27.25ef
No inoculation × PAN 1583R × 100 % SWE	17.61a	157.20a	16.50de	16.75d	28.75e
Significance	ns	ns	*	***	***
$HSD_{0.05}$	-	-	6.57	2.07	5.61

N: B. Means in a column represented by the same letter are not significantly different at P≤0.05. HSD= Honestly significant difference, ns= not significantly different, *= Significantly different at P≤0.05, **= Highly significantly different at P≤0.01, ***= Highly significantly different at P≤0.001, %= Percentage, SWE= Seaweed extract, cm^2= square centimetre, cm= centimetre

4.3.1. *Bradyrhizobium japonicum* WB74 inoculation, variety and seaweed extract main effects on soybean growth variables

Inoculation did not significantly influence leaf chlorophyll content, leaf area, leaf number per plant and plant height (Table 4.10). Inoculation exhibited significant differences on primary branches (20.54) per plant ($P \leq 0.001$) and stem diameter (11.65 mm) ($P \leq 0.01$) which were all highest under no inoculation treatment as compared to inoculation treatment in primary branches per plant (19.33) and stem diameter (9.65 mm).

Variety showed significant differences on leaf chlorophyll content ($P \leq 0.01$), leaf number per plant ($P \leq 0.001$), primary branches per plant ($P \leq 0.001$), stem diameter ($P \leq 0.001$) and plant height ($P \leq 0.001$). No significant difference between soybean varieties was recorded on leaf area. The variety TGx 1937-1F significantly increased leaf chlorophyll content (21.05 CCI) ($P \leq 0.01$), leaf number per plant (34.21) at $P \leq 0.001$, primary branches (29.04) per plant ($P \leq 0.001$), stem diameter (14.38 mm) ($P \leq 0.001$) and plant height (51.79 cm) ($P \leq 0.001$) as compared to variety PAN 1583R which significantly had lower leaf chlorophyll content (17.97 CCI), leaf number per plant (11), primary branches (10.83) per plant, stem diameter (6.92 mm) and plant height (23.38 cm).

Significant differences were achieved by seaweed extract in leaf number per plant, primary branches per plant, stem diameter and plant height. However, no significant difference in leaf chlorophyll content and leaf area was recorded. Seaweed extract rate of 100 % exhibited significantly ($P \leq 0.001$) high leaf number per plant (28.06), primary branches (24.06) per plant, stem diameter (12.35 mm) and plant height (41.88 cm) while seaweed extract rate of 0 % had the lowest leaf number per plant (16.56), primary branches (15.63) per plant, stem diameter (8.45 mm) and plant height (32.31 cm).

Table 4.10. *Bradyrhizobium Japonicum* WB74 inoculation, variety and seaweed extract main effects on soybean growth variables

Inoculation	**Leaf chlorophyll content (CCI)**	**Leaf area (cm^2)**	**Leaf number per plant**	**Primary branches per plant**	**Stem diameter (mm)**	**Plant height (cm)**
Inoculation	20.05a	159.80a	22.17a	19.33b	9.65b	37.33a
No inoculation	18.96a	150.28a	23.33a	20.54a	11.65a	37.83a
Significance	ns	ns	ns	***	**	ns
$HSD_{0.05}$	-	-	-	0.55	1.18	-
Variety						
TGx 1937-1F	21.05a	161.12a	34.21a	29.04a	14.38a	51.79a
PAN 1583R	17.97b	148.96a	11.29b	10.83b	6.92b	23.38b
Significance	**	ns	***	***	***	***
$HSD_{0.05}$	2.14	-	1.69	0.55	0.68	0.75
Seaweed extract						
0 % SWE	19.35a	150.21a	16.56c	15.63c	8.45c	32.31c
50 % SWE	19.36a	157.42a	23.63b	20.13b	11.15b	38.56b
100 % SWE	19.81a	157.50a	28.06a	24.06a	12.35a	41.88a
Significance	ns	ns	***	***	***	***
$HSD_{0.05}$	-	-	1.72	0.59	0.82	1.24

N: B. Means in a column represented by the same letter are not significantly different at P≤0.05. HSD= Honestly significant difference, ns= not significantly different, **= Highly significantly different at P≤0.01, ***= Highly significant at P≤0.001, %= Percentage, SWE= Seaweed extract, cm^2= square centimetre, cm= centimetre, mm= millimetre

4.3.2. *Bradyrhizobium japonicum* WB74 inoculation and variety interactive effects on soybean growth variables

Interaction of inoculation and soybean variety obtained no significant differences on leaf chlorophyll content and leaf area (Table 4.11). Interaction of inoculation and variety had significant differences on leaf number per plant ($P \leq 0.001$), primary branches per plant ($P \leq 0.001$), stem diameter ($P \leq 0.01$) and plant height ($P \leq 0.001$). Inoculation × variety TGx 1937-1F interaction had significantly high leaf number per plant (35.17), primary branches per plant (29.25) and plant height (52.42 cm) while no inoculation × variety TGx 1937-1F significantly increased stem diameter (15.88 mm). Inoculation × variety PAN 1583R interaction recorded the lowest leaf number per plant (09.17), primary branches per plant (09.42), smallest stem diameter (06.44 mm) and shortest plant height (22.25 cm).

Table 4.11. *Bradyrhizobium japonicum* WB74 inoculation and variety interactive effects on soybean growth variables

Inoculation × Variety	**Leaf chlorophyll content (CCI)**	**Leaf area (cm^2)**	**Leaf number per plant**	**Primary branches per plant**	**Stem diameter (mm)**	**Plant height (cm)**
Inoculation × TGx 1937-1F	22.11a	165.93a	35.17a	29.25a	12.87b	52.42a
Inoculation × PAN 1583R	18.99a	153.68a	09.17c	09.42c	06.44c	22.25b
No inoculation × TGx 1937-1F	19.99a	156.32a	33.25a	28.83a	15.88a	51.17a
No inoculation × PAN 1583R	18.94a	154.24a	13.42b	12.25b	07.41c	24.50b
Significance	ns	ns	***	***	**	***
$HSD_{0.05}$	-	-	3.80	1.12	2.01	3.51

N: B. Means in a column represented by the same letter are not significantly different at P≤0.05. HSD= Honestly significant difference, ns= not significantly different, **= Highly significantly different at P≤0.01, ***= Highly significantly different at P≤0.001, cm^2= square centimetre, cm= centimeter, mm= millimetre

4.3.3. *Bradyrhizobium japonicum* WB74 inoculation and seaweed extract interactive effects on soybean growth variables

Table 4.12 shows that inoculation and seaweed extract interaction significantly influenced leaf number per plant ($P \leq 0.001$), primary branches per plant ($P \leq 0.001$), stem diameter ($P \leq 0.001$) and plant height ($P \leq 0.05$) but did not affect leaf chlorophyll content and leaf area. Interaction of no inoculation × 100 % rate of seaweed extract also exhibited highest leaf number per plant (29.38), primary branches per plant (25.00), largest stem diameter (13.89 mm) and plant height (43.13 cm). Interaction of no inoculation × 0 % rate of seaweed extract attained the lowest leaf number per plant (15.00), primary branches per plant (15.38), shortest plant height (8.85 cm) and the smallest stem diameter (31.63 mm).

Table 4.12. *Bradyrhizobium japonicum* WB74 inoculation and seaweed extract interactive effects on soybean growth variables

Inoculation × Seaweed extract	**Leaf chlorophyll content (CCI)**	**Leaf area (cm^2)**	**Leaf number per plant**	**Primary branches per plant**	**Stem diameter (mm)**	**Plant height (cm)**
Inoculation × 0 % SWE	18.99a	162.63a	18.13d	15.88e	8.05d	33.00d
Inoculation × 50 % SWE	20.55a	160.28a	21.63c	19.00d	10.11bc	38.75c
Inoculation × 100 % SWE	20.63a	156.51a	26.75ab	23.13b	10.81bc	40.63ab
No inoculation × 0 % SWE	19.72a	147.79a	15.00d	15.38e	8.85cd	31.63d
No inoculation × 50 % SWE	18.18a	154.56a	25.63bc	21.25c	12.20b	38.75bc
No inoculation × 100 % SWE	19.00a	158.49a	29.38a	25.00a	13.89a	43.13a
Significance	ns	ns	***	***	***	***
$HSD_{0.05}$	-	-	4.14	1.24	2.32	4.19

N: B. Means in a column represented by the same letter are not significantly different at P≤0.05. HSD= Honestly significant difference, ns= not significantly different, ***= Highly significantly different at P≤0.00, %= Percentage, SWE= Seaweed extract, cm^2= square centimetre, cm= centimetre, mm= millimetre

4.3.4. Variety and seaweed extract interactive effects on soybean growth variables

Table 4.13 shows the interaction of variety and seaweed extract which obtained significant differences on leaf number per plant, primary branches per plant, stem diameter and plant height. There were no significant influences on leaf chlorophyll content and leaf area. Interaction of variety TGx 1937-1F × 100 % rate of seaweed extract achieved highest leaf number per plant (43.13) and primary branches per plant (33.75) while variety TGx 1937-1F × 50 % rate of seaweed extract exhibited significantly largest stem diameter (15.99 mm) at $P \leq 0.05$ and tallest plants (57.13 cm) at $P \leq 0.001$. Interaction of variety PAN 1583R × 0 % rate of seaweed extract recorded significantly lowest leaf number per plant (9.63), primary branches per plant (6.25), smallest stem diameter (5.22 mm) and shortest plant height (18.50 cm).

Table 4.13. Variety and seaweed extract interactive effects on soybean growth variables

Variety × Seaweed extract	**Leaf chlorophyll content (CCI)**	**Leaf area (cm^2)**	**Leaf number per plant**	**Primary branches per plant**	**Stem diameter (mm)**	**Plant height (cm)**
TGx 1937-1F × 0 % SWE	21.00a	158.56a	25.50c	25.00c	11.68b	46.13c
TGx 1937-1F × 50 % SWE	20.54a	164.46a	36.00b	28.38b	15.99a	57.13a
TGx 1937-1F × 100 % SWE	21.61a	160.35a	43.13a	33.75a	15.46a	53.13b
PAN 1583R × 0 % SWE	17.70a	151.85a	9.63e	6.25f	5.22e	18.50e
PAN 1583R × 50 % SWE	18.18a	150.37a	11.25de	11.87e	6.81d	25.00d
PAN 1583R × 100 % SWE	18.01a	154.65a	13.00d	14.38d	8.73c	26.63d
Significance	ns	ns	*	***	**	***
$HSD_{0.05}$	-	-	3.65	1.22	1.61	2.15

N: B. Means in a column represented by the same letter are not significantly different at P≤0.05. HSD= Honestly significant difference, ns= not significantly different, *= Significantly different at P≤0.05, **= Highly significantly different at P≤0.01, ***= Highly significantly different at P≤0.001, %= Percentage, SWE= Seaweed extract, cm^2= square centimetre, cm= centimetre, mm= millimetre

4.4. Soybean grain yield and yield components

The 3-way interaction of inoculation, variety and seaweed extract had significant differences on pod number per plant (P≤0.001), shelling percentage (P≤0.001), harvest index (P≤0.001), grain yield (P≤0.05) and total above-ground biomass (P≤0.001) except for 100 seed weight (Table 4.14). Interactive effect of inoculation × variety TGx 1937-1F × 100 % rate of seaweed extract obtained significantly higher pod number per plant (122.50). Interaction of inoculation × variety PAN 1583R × 0 % rate of seaweed extract obtained significantly lower pod number per plant (11.75). Inoculation × variety TGx 1937-1F × 100 % rate of seaweed extract interaction significantly obtained highest shelling percentage (84.45 %). Interaction of no inoculation × variety TGx 1937-1F × 0 % rate of seaweed extract obtained significantly low shelling percentage (53.88 %). Interaction of no inoculation × variety PAN 1583R × 0 % rate of seaweed extract obtained significantly highest harvest index (0.73). No inoculation × variety TGx 1937-1F × 100 % rate of seaweed extract interaction recorded the lowest harvest index (0.29).

No inoculation × variety TGx 1937-1F × 100 % rate of seaweed extract interaction attained significantly highest grain yield (631.23 kg/ha). Inoculation × variety PAN 1583R × 0 % rate of seaweed extract interaction obtained the lowest grain yield (263.08 kg/ha). Interaction of no inoculation × variety TGx 1937-1F × 100 % rate of seaweed extract interaction had significantly high total aboveground biomass (2179.9 kg/ha) while the lowest total aboveground biomass (463.9 kg/ha) was obtained by no inoculation × variety PAN 1583R × 0 % rate of seaweed extract interaction.

Table 4.14. *Bradyrhizobium japonicum* WB74 inoculation, variety and seaweed extract interactive effects on soybean grain yield and yield components

Inoculation × Variety × Seaweed extract	**Pod number per plant**	**100 seed weight (g)**	**Shelling percentage (%)**	**Harvest Index (HI)**	**Grain yield (kg/ha)**	**Total aboveground biomass (kg/ ha)**
Inoculation × TGx 1937-1F × 0 % SWE	89.75d	9.80a	54.77d	0.33c	553.95ab	1703.8d
Inoculation × TGx 1937-1F × 50 % SWE	93.50cd	10.38a	69.29abcd	0.30c	555.34ab	1859.3c
Inoculation × TGx 1937-1F × 100 % SWE	96.75cd	9.70a	84.45a	0.30c	591.49a	1964.1bc
Inoculation × PAN 1583R × 0 % SWE	11.75e	13.41a	67.45bcd	0.37c	263.08c	710.4f
Inoculation × PAN 1583R × 50 % SWE	14.75e	13.73a	66.76cd	0.37c	269.08c	726.7f
Inoculation × PAN 1583R × 100 % SWE	13.75e	14.24a	78.72abc	0.36c	285.56c	788.5f
No inoculation × TGx 1937-1F × 0 % SWE	97.25c	10.25a	53.88cd	0.36c	497.42b	1401.8e
No inoculation × TGx 1937-1F × 50 % SWE	104.75b	10.38a	63.19cd	0.31c	624.20a	2049.2ab
No inoculation × TGx 1937-1F × 100 % SWE	122.25a	10.12a	63.39cd	0.29c	631.23a	2179.9a
No inoculation × PAN 1583R × 0 % SWE	13.75e	13.34a	84.15ab	0.73a	330.02c	463.9g
No inoculation × PAN 1583R × 50 % SWE	16.25e	13.58a	73.37abc	0.52b	340.92c	657.9f
No inoculation × PAN 1583R × 100 % SWE	18.25e	14.93a	66.62cd	0.70a	326.25c	481.1g
Significance	***	ns	***	***	*	***
$HSD_{0.05}$	6.57	-	17.12	0.14	91.49	139.46

N: B. Means in a column represented by the same letter are not significantly different at P ≤ 0.05. HSD= Honestly significant difference, ns= not significantly different, *= Significantly different at P≤0.05, **= Highly significantly different at P≤0.01, ***= Highly highly significantly different at P≤0.00, %= Percentage, SWE= Seaweed extract

4.4.1. *Bradyrhizobium japonicum* WB74 inoculation, variety and seaweed extract main effects on soybean grain yield and yield components

Inoculation showed no significant differences in 100 seed weight and shelling percentage. Significant differences were shown in pod number per plant, harvest index, grain yield and total above-ground biomass ($P \leq 0.001$) as influenced by inoculation (Table 4.15). No inoculation exhibited significant increase in pod number per plant (62.08), harvest index (0.48) and grain yield (458.3 kg/ha). Decreased pod number per plant (53.38), harvest index (0.34) and grain yield (419.8 kg/ha) were shown under inoculation. However, total above-ground biomass was reduced by no inoculation with 1205.6 kg/ha.

Variety had significant differences ($P \leq 0.001$) in pod number per plant, 100 seed weight, shelling percentage, harvest index, grain yield and total above-ground biomass. The variety TGx 1937-1F significantly increased pod number per plant (100.71), shelling percentage (72.84 %), grain yield (575.5 kg/ha) and total above-ground biomass (1859.7 kg/ha). Soybean variety PAN 1583R significantly recorded the lower pod number per plant (14.75), shelling percentage (66.49 %), grain yield (302.5 kg/ha) and total above-ground biomass (938.1 kg/ha). On the other hand, variety PAN 1583R had significantly higher 100 seed weight (13.9 g) and harvest index (0.51). The variety TGx 1937-1F recorded significantly lower harvest index (0.31) and 100 seed weight (8.10).

Seaweed extract did not have significant influence on 100 seed weight and shelling percentage. Application of Seaweed extract at 100 % rate exhibited significantly higher ($P \leq 0.001$) pod number per plant (62.75), grain yield (458.6 kg/ha) and total above ground biomass (1353.4 kg/ha). Seaweed extract rate of 0 % recorded the lowest pod number per plant (53.13), grain yield (411.1 kg/ha) and total above ground biomass (1070.1 kg/ha) while the lowest harvest index (0.37) was recorded under 50 % rate of seaweed extract.

Table 4.15. *Bradyrhizobium japonicum* WB74 inoculation, variety and seaweed extract main effects on soybean grain yield and yield components

Inoculation	Pod number per plant	100 seed weight (g)	Shelling percentage (%)	Harvest Index (HI)	Grain yield (kg/ha)	Total aboveground biomass (kg/ha)
Inoculation	53.38b	10.86a	69.55a	0.34b	419.8b	1292.1a
No inoculation	62.08a	11.11a	72.24a	0.48a	458.3a	1205.6b
Significance	***	ns	ns	***	*	***
$HSD_{0.05}$	0.25	-	-	0.05	27.39	25.33
Variety						
TGx 1937-1F	100.71a	8.10b	72.84b	0.31b	575.6a	1859.7a
PAN 1583R	14.75b	13.87a	66.49a	0.51a	302.5b	938.1b
Significance	***	***	***	***	***	***
$HSD_{0.05}$	1.68	0.5209	2.04	0.04	21.50	25.13
Seaweed extract						
0 % SWE	53.13c	10.70a	69.89a	0.44a	411.1b	1070.13b
50 % SWE	57.31b	10.99a	71.45a	0.37b	447.4a	1323.2a
100 % SWE	62.75a	11.27a	71.92a	0.41a	458.6a	1353.4a
Significance	***	ns	ns	***	***	***
$HSD_{0.05}$	2.43	-	-	0.04	25.57	52.03

N: B. Means in a column represented by the same letter are not significantly different at P ≤ 0.05. HSD= Honestly significant difference, ns= not significantly different, *= Significantly different at P≤0.05, ***= Highly significantly different at P≤0.001, %= Percentage, SWE= Seaweed extract, g= gram, kg= kilograms, ha= hectare

4.4.2. *Bradyrhizobium japonicum* WB74 inoculation and variety interactive effects on soybean grain yield and yield components

There were no significant differences in 100 seed weight (g) as affected by inoculation × variety interaction (Table 4.16). Significant differences were shown on pod number per plant ($P \leq 0.001$), shelling percentage ($P \leq 0.001$), harvest index ($P \leq 0.001$), grain yield ($P \leq 0.05$) and total above-ground biomass ($P \leq 0.001$). Interaction of no inoculation × variety TGx 1937-1F obtained highest pod number per plant (108.08), grain yield (584.3 kg/ha) and total above-ground biomass (1877.0 kg/ha). Interaction of inoculation × PAN 1583R recorded the lowest pod number per plant (13.42), grain yield (272.6 kg/ha) and total above-ground biomass (534.3 kg/ha). No inoculation × variety TGx 1937-1F interaction obtained the highest shelling percentage (75.69 %) while inoculation × variety PAN 1583R interaction lowered the shelling percentage (49.01 %). No inoculation × variety PAN 1583R interaction significantly increased harvest index (0.47). Interaction of inoculation × variety TGx 1937-1F obtained lowest harvest index (0.31).

Table 4.16. *Bradyrhizobium japonicum* WB74 inoculation and variety interactive effects on soybean grain yield and yield components

Inoculation × Variety	**Pod number per plant**	**100 seed weight (g)**	**Shelling percentage (%)**	**Harvest Index (HI)**	**Grain yield (kg/ha)**	**Total above-ground biomass (kg/ha)**
Inoculation × TGx 1937-1F	93.33b	9.93a	73.25ab	0.31b	566.9a	1842.4a
Inoculation × PAN 1583R	13.42d	13.79a	49.01bc	0.37b	272.6c	534.3c
No inoculation × TGx 1937-1F	108.08a	10.28a	75.69a	0.32b	584.3a	1877.0a
No inoculation × PAN 1583R	16.08c	13.95a	52.35c	0.47a	332.4b	741.9b
Significance	***	ns	***	***	*	***
$HSD_{0.05}$	2.40	-	7.76	0.09	50.81	49.72

N: B. Means in a column represented by the same letter are not significantly different at P≤0.05. HSD= Honestly significant difference, ns= not significantly different, *= Significantly different at P≤0.05, ***= Highly significantly different at P≤0.001, %= Percentage, g= gram, kg= kilograms, ha= hectare

4.4.3. *Bradyrhizobium japonicum* WB74 inoculation and seaweed extract interactive effects on soybean grain yield and yield components

Interaction of inoculation × seaweed extract attained significant differences on pod number per plant ($P \leq 0.001$), shelling percentage ($P \leq 0.001$), harvest index ($P \leq 0.001$), grain yield ($P \leq 0.05$) and total above-ground biomass ($P \leq 0.001$) but did not have any significant differences in 100 seed weight (Table 4.17). Interaction of no inoculation × 100 % rate of seaweed extract exhibited significantly highest pod number per plant (70.25) while interaction of inoculation × 0 % rate of seaweed extract obtained the lowest pod number per plant (50.75). Highest shelling percentage (75.60 %) was obtained under interaction of no inoculation × 50 % rate of seaweed extract as compared to inoculation × 0 % rate of seaweed extract interaction which recorded the lowest shelling percentage (64.90 %). Interactive effect of no inoculation × 0 % rate of seaweed extract increased harvest index (0.54) while inoculation × 100 % rate of seaweed extract decreased harvest index (0.33). Interactive effects of no inoculation × 50 % rate of seaweed extract and inoculation × 100 % rate of seaweed extract significantly increased grain yield (482.56 kg/ha) and total aboveground biomass (1376.30 kg/ha), respectively. Interaction of inoculation × 0 % rate of seaweed extract recorded lowest grain yield (413.7 kg/ha) and total above-ground biomass (1207.1 kg/ha).

Table 4.17. *Bradyrhizobium japonicum* WB74 inoculation and seaweed extract interactive effects on soybean grain yield and yield components

Inoculation × Seaweed extract	**Pod number per plant**	**100 seed weight (g)**	**Shelling percentage (%)**	**Harvest Index (HI)**	**Grain yield (kg/ha)**	**Total aboveground biomass (kg/ha)**
Inoculation × 0 % SWE	50.75d	10.61a	64.90c	0.35b	408.5b	932.8c
Inoculation × 50 % SWE	54.13cd	11.00a	68.37b	0.34b	420.3b	1293.0ab
Inoculation × 100 % SWE	55.25c	10.97a	68.78b	0.33b	438.5ab	1376.3a
No inoculation × 0 % SWE	55.50c	10.79a	66.74c	0.54a	413.7b	1207.1b
No inoculation × 50 % SWE	60.50b	10.98a	75.60a	0.41b	482.6a	1353.5a
No inoculation × 100 % SWE	70.25a	11.57a	74.02a	0.49a	478.7a	1330.5a
Significance	***	ns	***	***	*	***
$HSD_{0.05}$	3.49	-	0.09	0.09	58.32	83.43

N: B. Means in a column represented by the same letter are not significantly different at P≤0.05. HSD= Honestly significant difference, ns= not significantly different, *= Significantly different at P≤0.05, ***= Highly significantly different at P≤0.001, %= Percentage, SWE= Seaweed extract, g= gram, kg= kilograms, ha= hectare

4.4.4. Variety and seaweed extract interactive effects on soybean grain yield and yield components

Interaction of variety and seaweed extract did not record any significant differences in 100 seed weight. However, there was significant differences on pod number per plant, shelling percentage, harvest index, grain yield and total above-ground biomass between variety and seaweed extract interactions (Table 4.18). Interaction of variety TGx 1937-1F × 100 % rate of seaweed extract exhibited significantly higher pod number per plant (109.50). The variety PAN 1583R × 0 % rate of seaweed extract interaction reduced pod number per plant (12.75). Significantly higher shelling percentage (75.80 %) was exhibited under interaction of variety TGx 1937-1F × 100 % rate of seaweed extract. Significantly lower shelling percentage of 59.80 was obtained by variety PAN 1583R × 0 % rate of seaweed extract interaction. Interaction of variety PAN 1583R × 0 % rate of seaweed extract obtained significantly higher harvest index (0.55). Both interactions of variety TGx 19377-1F × 50 % rate of seaweed extract and variety TGx 19377-1F × 100 % rate of seaweed extract had significantly lower harvest indices (0.30). Interaction of variety TGx 1937-1F × 100 % rate of seaweed extract achieved highest grain yield (611.36 kg/ha) and total above-ground biomass (2072 kg/ha). Interaction of variety PAN 1583R × 0 % rate of seaweed extract recorded the lowest grain yield (296.55 kg/ha) and total above-ground biomass (587.10 kg/ha).

Table 4.18. Variety and seaweed extract interactive effects on soybean grain yield and yield components

Variety × Seaweed extract	**Pod number per plant**	**100 seed weight (g)**	**Shelling percentage (%)**	**Harvest Index (HI)**	**Grain yield (kg/ha)**	**Total aboveground biomass (kg/ha)**
TGx 1937-1F × 0 % SWE	93.50c	10.03a	70.58bc	0.34c	525.7b	1552.8c
TGx 1937-1F × 50 % SWE	99.13b	10.33a	73.82ab	0.30c	589.8a	1954.2b
TGx 1937-1F × 100 % SWE	109.50a	9.96a	75.80a	0.30c	611.4a	2072.0a
PAN 1583R × 0 % SWE	12.75d	13.38a	59.80c	0.55a	296.6c	587.1e
PAN 1583R × 50 % SWE	15.50d	13.65a	66.69abc	0.45b	305.1c	692.3d
PAN 1583R × 100 % SWE	16.00d	14.58a	66.71abc	0.53a	305.9c	634.8de
Significance	***	ns	***	*	***	***
$HSD_{0.05}$	4.38	-	8.93	0.08	50.19	84.45

N: B. Means in a column represented by the same letter are not significantly different at P≤0.05. HSD= Honestly significant difference, ns= not significantly different, ***= Highly highly significantly different at P≤0.001, %= Percentage, SWE= Seaweed extract, g= gram, kg= kilograms, ha= hectare

4.5. Pearson's correlation for growth variables, nodulation, grain yield and yield components of soybean

Nodule number per plant exhibited positive and significant correlation with effective nodules per plant (r= 1.0), leaf number per plant r= 0.450) and grain yield (r= 0.378) (Table 4.19). There was also a relationship observed between effective nodules per plant with leaf number per plant (r= 0.450), pods number per plant (r= 0.393), stem diameter (r= 0.374) and grain yield (r= 0.378). Leaf number per plant obtained a positive and significant relationship with pod number per plant by r= 0.934, stem diameter by r=0.787, plant height by r= 0.914, leaf chlorophyll content by r= 0.524 and grain yield by r= 0.772. Pod number per plant showed positive and significant relationship with stem diameter (r= 0.860), plant height (r = 0.954), leaf chlorophyll content (r= 0.485) and grain yield (r= 0.765). A strong positive and significant relationship of stem diameter was attained with plant height (r=0.807) and grain yield (r= 0.642. Plant height also exhibited a positive and a significant effect with leaf chlorophyll content by r= 0.534 and grain yield by r= 0.791. Leaf chlorophyll content showed clear positive and significant correlation with grain yield by r= 0.452. Leaf area showed no significant correlation with grain yield.

Table 4.19. Pearson's correlations for growth variables, nodulation, grain yield and yield components of soybean

	NN	ENN	L.NUMBER	P.NUMBER	STEM.D	PH (cm)	L.CHL	LA (cm^2)	GY (kg/ha)
NN	1.000***								
ENN	1.000***	1.000***							
N. LEAVES	0.4495***	0.4495***	1.000***						
N. PODS	0.3927	0.3927*	0.9340***	1.000***					
STEM.D	0.3739	0.3739*	0.7872***	0.8603***	1.000***				
PH (cm)	0.3442	0.3442	0.9139***	0.9544***	0.8069***	1.000***			
L.CHL	0.2477	0.2477	0.5242***	0.4849***	0.3119	0.5341***	1.000***		
LA (cm^2)	0.1228	0.1288	0.1924	0.2073	0.1523	0.2191	0.0714	1.000***	
GY (kg/ha)	0.3776**	0.3776**	0.7721***	0.7649***	0.6417***	0.7911***	0.4527***	0.0274	1.000***

NN= nodule number, ENN= Effective nodule number, L.NUMBER= Leaf number, P.NUMBER= pod number, STEM D= Stem diameter, PH= Plant height, L.CHL= Leaf chlorophyll content, LA= Leaf area, GY= Grain yield, *= Significant at P≤0.05, **= Highly significant at P≤0.01, ***= Highly significant at P≤0.001

4.6. Moisture, protein and macro-nutrient content of soybean grain

Inoculation × variety × seaweed extract interaction was not significant on seed moisture percentage, seed protein percentage, seed nitrogen (N) percentage, seed phosphorus (P) and seed potassium (K) content. The 2-way interactive effects are presented in Tables 4.21 to 4.23.

4.6.1. *Bradyrhizobium japonicum* WB74 inoculation, variety and seaweed extract main effects on moisture, protein and macro-nutrient content of soybean grain

Inoculation, variety and seaweed extract exhibited no significant differences on seed moisture percentage, seed protein percentage, seed nitrogen (N) percentage and seed phosphorus (K) contents (Table 4.20). On the other hand, inoculation showed significant differences ($P \leq 0.05$) on seed K content. Inoculation significantly increased seed K (100.94 mg/L) as compared to no inoculation which recorded the lower seed K content (96.07 mg/L). Seaweed extract at 100 % application rate obtained significantly high seed K content (103.61 mg/L) relative to 0 % rate of seaweed extract which obtained lower seed K content (93.40 mg/L).

Table 4.20. *Bradyrhizobium japonicum* WB74 inoculation, variety and seaweed extract main effects on moisture, protein and macro-nutrient content of soybean grain

Inoculation	**Seed Moisture (%)**	**Seed Protein (%)**	**Seed Nitrogen (%)**	**Seed Phosphorus (mg/L)**	**Seed Potassium (mg/L)**
Inoculation	8.06a	27.8a	4.45a	38.35a	100.94a
No inoculation	7.51a	26.73a	4.29a	37.67a	96.07b
Significance	ns	ns	ns	ns	*
$HSD_{0.05}$	-	-	-	-	1.90
Variety					
TGx 1937-1F	8.13a	27.55a	4.34a	41.14a	100.47a
PAN 1583R	7.44a	26.99a	4.40a	34.88a	96.53a
Significance	ns	ns	ns	ns	ns
$HSD_{0.05}$	-	-	-	-	-
Seaweed extract					
0 % SWE	7.41a	26.20a	4.22a	38.09a	93.40ab
50 % SWE	7.92a	26.97a	4.31a	35.48a	93.50b
100 % SWE	8.03a	28.63a	4.57a	40.46a	103.61a
Significance	ns	ns	ns	ns	*
$HSD_{0.05}$	-	-	-	-	8.67

N: B. Means in a column represented by the same letter are not significantly different at P≤0.05. HSD= Honestly Significant Difference. *= Significantly different at P≤0.05, mg/L= milligrams per litre, %= Percentage, SWE= Seaweed extract

4.6.2. *Bradyrhizobium japonicum* WB74 inoculation and variety interactive effects on moisture, protein and macro-nutrient content of soybean grain

Interaction of inoculation and variety showed significant differences in seed protein (Table 4.21). Insignificant differences were found on seed moisture (%), seed nitrogen, seed phosphorus and seed potassium. Interactions between no inoculation × PAN 1583R had significantly higher seed protein (28.53 %). No inoculation × variety TGx 1937-1F interaction had significantly lower seed protein (25.45 %).

Table 4.21. *Bradyrhizobium japonicum* WB74 inoculation and variety interactive effects on moisture, protein and macro-nutrient content of soybean grain

Inoculation × Variety	**Seed Moisture (%)**	**Seed Protein (%)**	**Seed Nitrogen (%)**	**Seed Phosphorus (mg/L)**	**Seed Potassium (mg/L)**
Inoculation × TGx 1937-1F	8.26a	28.50a	4.58a	40.63a	102.02a
Inoculation × PAN 1583R	7.87a	27.07b	4.32a	36.06a	99.86a
No inoculation × TGx 1937-1F	8.00a	25.45b	4.09a	41.64a	98.92a
No inoculation × PAN 1583R	7.01a	28.53a	4.48a	33.70a	93.21a
Significance	ns	*	ns	ns	ns
$HSD_{0.05}$	-	17.73	-	-	-

N: B. Means in a column represented by the same letter are not significantly different at P≤0.05. HSD= Honestly Significant Difference, ns= non-significant (P ≤ 0.05), *= Significantly different at P≤0.05, mg/L= milligrams per litre, %= Percentage

4.6.3. *Bradyrhizobium japonicum* WB74 inoculation and seaweed extract interactive effects on moisture, protein and macro-nutrient content of soybean grain

There were no significant differences between interactions of inoculation x seaweed extract on seed moisture content (%), seed protein (%), seed nitrogen, seed phosphorus (mg/L) and seed potassium (mg/L) (Table 4.22).

Table 4.22. *Bradyrhizobium japonicum* WB74 inoculation and seaweed extract interactive effects on moisture, protein and macro-nutrient content of soybean grain

Inoculation × Seaweed extract	**Seed moisture (%)**	**Seed Protein (%)**	**Seed Nitrogen (%)**	**Seed Phosphorus (mg/L)**	**Seed Potassium (mg/L)**
Inoculation × 0 % SWE	8.44a	25.51a	4.10a	39.20a	101.75a
Inoculation × 50 % SWE	7.35a	28.83a	4.61a	36.07a	96.92a
Inoculation × 100 % SWE	8.38a	29.07a	4.64a	39.77a	104.15a
No inoculation × 0 % SWE	6.38a	26.90a	4.34a	36.98a	95.05a
No inoculation × 50 % SWE	8.49a	25.09a	4.02a	34.88a	90.08a
No inoculation × 100 % SWE	7.66a	28.20a	4.51a	41.15a	103.07a
Significance	ns	ns	ns	ns	ns
$HSD_{0.05}$	-	-	-	-	-

N: B. Means in a column represented by the same letter are not significantly different at P≤0.05. HSD= Honestly Significant Difference, ns= non-significant (P≤0.05), *= Significantly different at P≤0.05, mg/L= milligrams per litre, %= Percentage, SWE= seaweed extract

4.6.4. Variety and seaweed extract interactive effects on moisture, protein and macro-nutrient content of soybean grain

Tables 4.23 shows no significant differences between the interactions of variety × seaweed extract on seed moisture (%), seed protein (%), seed nitrogen (%), seed phosphorus (mg/L) and seed potassium (mg/L).

Table 4.23. Variety and seaweed extract interactive effects on moisture, protein and macro-nutrient content of soybean grain

Variety × Seaweed extract	**Seed Moisture (%)**	**Seed Protein (%)**	**Seed Nitrogen (%)**	**Seed Phosphorus (mg/L)**	**Seed Potassium (mg/L)**
TGx 1937-1F × 0 % SWE	7.57a	25.65a	4.16a	41.52a	100.87a
TGx 1937-1F × 50 % SWE	8.90a	27.34a	4.38a	39.40a	96.67a
TGx 1937-1F × 100 % SWE	7.92a	27.97a	4.47a	42.49a	103.88a
PAN 1583R × 0 % SWE	7.25a	26.76a	4.27a	34.67a	95.93a
PAN 1583R × 50 % SWE	6.94a	26.59a	4.25a	31.55a	90.33a
PAN 1583R × 100 % SWE	8.13a	29.29a	4.67a	38.43a	103.33a
Significance	ns	ns	ns	ns	ns
$HSD_{0.05}$	-	-	-	-	-

N: B. Means in a column represented by the same letter are not significantly different at P≤0.05. HSD= Honestly Significant Difference, ns= non-significant (P≤0.05), mg/L= milligrams per litre, %= Percentage, SWE= Seaweed extract

4.7. Micro-nutrient content of soybean grain

There were no significant effects observed from the interaction of inoculation × variety × seaweed extract on seed iron, seed magnesium, seed manganese, seed sodium, seed calcium and seed copper content. The 2-way interactive effects are presented in Tables 4.25 to 4.27.

4.7.1. *Bradyrhizobium japonicum* WB74 inoculation, variety and seaweed extract main effects on micro-nutrient content of soybean grain

Inoculation had no significant influence in seed iron, seed magnesium, seed manganese, seed sodium, seed calcium and seed copper (Table 4.24). Variety had a significant difference on seed iron (mg/L) at $P \leq 0.05$ but did not show any significant differences in seed Mg, seed Mn, seed Na, seed Ca and seed Cu content. The variety TGx 1937-1F had significantly higher Fe (3.22 mg/L) content than PAN 1583R (2.22 mg/L). Application of seaweed extract had a significant influence on seed Mg, seed Mn, seed Na and seed Ca but did not significantly influence seed Fe and seed Cu. Seaweed extract rate of 100 % had significantly higher amounts of seed Mg (26.12 mg/L), seed Mn (0.54 mg/L), seed Na (3.67 mg/ L) and seed Ca (41.83 mg/L) while 50% rate of seaweed extract had significantly the lowest amounts of seed Mg (21.98 mg/L), seed Mn (0.48 mg/L), seed Na (1.13 mg/L) and seed Ca (27.60 mg/L).

Table 4.24. *Bradyrhizobium japonicum* WB74 inoculation, variety and seaweed extract main effects on micro-nutrient content of soybean grain

Inoculation	**Seed Iron (mg/L)**	**Seed Magnesium (mg/L)**	**Seed Manganese (mg/L)**	**Seed Sodium (mg/L)**	**Seed Calcium (mg/L)**	**Seed Copper (mg/L)**
Inoculation	2.39a	24.38a	0.51a	2.21a	39.39a	0.62a
No inoculation	3.05a	23.88a	0.53a	2.85a	38.82a	0.68a
Significance	ns	ns	ns	ns	ns	ns
$HSD_{0.05}$	-	-	-	-	-	-
Variety						
TGx 1937-1F	3.22a	24.50a	0.53a	3.00a	41.19a	0.68a
PAN 1583R	2.22b	23.76a	0.51a	2.06a	38.02a	0.62a
Significance	*	ns	ns	ns	ns	ns
$HSD_{0.05}$	0.78	-	-	-	-	-
Seaweed extract						
0 % SWE	3.02a	24.28ab	0.54a	2.80ab	37.38ab	0.67a
50 % SWE	2.25a	21.98b	0.48b	1.13b	27.60b	0.62a
100 % SWE	2.89a	26.12a	0.54a	3.67a	41.83a	0.65a
Significance	ns	*	*	*	*	ns
$HSD_{0.05}$	-	2.96	0.05	1.95	13.89	-

N: B. Means in a column represented by the same letter are not significantly different at P≤0.05. HSD= Honestly Significant Difference, ns= not significant, *= Significantly different at P≤0.05, mg/L= milligrams per litre, %= Percentage, SWE= Seaweed extract

4.7.2. *Bradyrhizobium japonicum* WB74 inoculation and variety interactive effects on micro-nutrient content of soybean grain

Table 4.25 below shows no significant differences of inoculation and variety interactions on seed magnesium, seed manganese, seed sodium, seed calcium and seed copper.

Table 4.25. *Bradyrhizobium japonicum* WB74 inoculation and variety interactive effects on micro-nutrient content of soybean grain

Inoculation × Variety	**Seed Magnesium (mg/L)**	**Seed Manganese (mg/L)**	**Seed Sodium (mg/L)**	**Seed Calcium (mg/L)**	**Seed Copper (mg/L)**
Inoculation × TGx 1937-1F	24.62a	0.51a	2.44a	35.86a	0.64a
Inoculation × PAN 1583R	24.14a	0.50a	1.99a	33.78a	0.59a
No inoculation × TGx 1937-1F	24.38a	0.55a	3.00a	42.52a	0.71a
No inoculation × PAN 1583R	23.38a	0.51a	2.14a	35.27a	0.64a
Significance	ns	ns	ns	ns	ns
$HSD_{0.05}$	-	-	-	-	-

N: B. Means in a column represented by the same letter are not significantly different at P ≤ 0.05. HSD= Honestly Significant Difference, ns= non-significantly different (P≤0.05), mg/L= milligrams per litre

4.7.3. *Bradyrhizobium japonicum* WB74 inoculation and seaweed extract interactive effects on micro-nutrient content of soybean grain

Interaction of inoculation and seaweed extract had significant influences on seed manganese ($P \leq 0.05$) and seed copper ($P \leq 0.05$) while no significant differences were found on seed iron, seed magnesium, seed sodium and seed calcium (Table 4.26). Interactive effect of no inoculation × 100 % rate of seaweed extract exhibited significantly higher contents of seed Mg (0.58) and seed Cu (0.72). Significantly lowest contents of seed Mg (0.47) and seed Cu (0.57) were found under the interactive effects of no inoculation × 50 % rate of seaweed extract and inoculation × 100 % rate of seaweed extract.

Table 4.26. *Bradyrhizobium japonicum* WB74 inoculation and seaweed extract interactive effects on micro-nutrient content of soybean grain

Inoculation × seaweed extract	**Seed Iron (mg/L)**	**Seed Magnesium (mg/L)**	**Seed Manganese (mg/L)**	**Seed Sodium (mg/L)**	**Seed Calcium (mg/L)**	**Seed Copper (mg/L)**
Inoculation × 0 % SWE	2.35a	25.20a	0.55ab	2.66a	35.55a	0.67a
Inoculation × 50 % SWE	2.29a	22.40a	0.48ab	2.16a	26.70a	0.61b
Inoculation × 100 % SWE	2.52a	25.54a	0.49ab	3.02a	33.20a	0.57b
No inoculation × 0 % SWE	3.68a	23.37a	0.53ab	2.94a	39.22a	0.67b
No inoculation × 50 % SWE	2.21a	21.57a	0.47b	2.30a	28.50a	0.62a
No inoculation × 100 % SWE	3.26a	26.70a	0.58a	3.32a	37.47a	0.72a
Significance	ns	ns	*	ns	ns	*
$HSD_{0.05}$	-	-	0.19	-	-	0.18

N: B. Means in a column represented by the same letter are not significantly different at P≤0.05. HSD= Honestly Significant Difference, ns= non-significantly different (P≤0.05), *= Significantly different at P≤0.05, mg/L= milligrams per litre, SWE= Seaweed extract

4.7.4. Variety and seaweed extract interactive effects on micro-nutrient content of soybean grain

Table 4.27 shows no significant differences in seed iron, seed magnesium, seed manganese, seed sodium, seed calcium and seed copper content.

Table 4.27. Variety and seaweed extract interactive effects on micro-nutrient content of soybean grain

Variety × Seaweed extract	**Seed Iron (mg/L)**	**Seed Magnesium (mg/L)**	**Seed Manganese (mg/L)**	**Seed Sodium (mg/L)**	**Seed Calcium (mg/L)**	**Seed Copper (mg/L)**
TGx 1937-1F × 0 % SWE	3.53a	24.62a	0.56a	3.06a	38.53a	0.68a
TGx 1937-1F × 50 % SWE	2.89a	22.80a	0.51a	2.04a	33.48a	0.65a
TGx 1937-1F × 100 % SWE	3.22a	26.08a	0.53a	3.40a	39.55a	0.69a
PAN 1583R × 0 % SWE	2.51a	23.95a	0.53a	2.53a	34.23a	0.65a
PAN 1583R × 50 % SWE	2.07a	21.17a	0.45a	1.99a	28.72a	0.58a
PAN 1583R × 100 % SWE	2.56a	26.16a	0.54a	3.15a	34.12a	0.61a
Significance	ns	ns	ns	ns	ns	ns
$HSD_{0.05}$	-	-	-	-	-	-

N: B. Means in a column represented by the same letter are not significantly different at P≤0.05. HSD= Honestly Significant Difference, ns= non-significantly differently (P ≤ 0.05), mg/L= milligrams per litre, %= Percentage, SWE= Seaweed extract

CHAPTER 5

DISCUSSION

5.1. Phenological development

In this study, inoculation showed no influence on soybean phenological development. The introduced rhizobial strain did not respond or failed to compete with the high population of the native rhizobial strain in the soil to have any impact on growth of soybean. It is also possible that inoculation does not affect phenological development of soybean. On the evaluated varieties, the variety TGx 1937-1F found to be a late flowering and maturing variety as it has taken 82 days to reach 50 % flowering stage and 159 days to reach its 50 % physiological maturity. On the other hand, the variety PAN 1583R was an early flowering and maturing variety taking 69 days to reach 50 % flowering and 137 days to reach 50 % physiological maturity. Variety TGx 1937-1F is unlikely to fully develop under rainfed conditions in the environment of Limpopo province as the area mostly experiences short rainy seasons. Findings from this study agree with Maphosa (2015) who confirmed that TGx 1937-1F takes long to mature under the Syferkuil environment. The results suggest that the variety TGx-1937-1F is disparate to have a successful maturity under the local dry-land conditions which are characterized by short and erratic rainfall conditions. Therefore, this variety TGx 1937-1F requires supplementary irrigation towards the end of growing season. It will also need to be planted at beginning of the rainy season to avoid declining temperatures from April onwards. Thus, the variety TGx 1937-1F may be better suited to irrigation schemes than for dry-land production.

Application of sea-weed extract and its interactions with variety, and with inoculation failed to have any effect on soybean phenological development in the present study. Literature on effect of seaweed extract on phenological development of soybean is scarce, hence this aspect may still justify further enquiry. This may be because seaweed extract did not activate the growth hormone production, such as auxins, cytokinins, gibberellins and solubilization of nutrients (N, P and K), to enhance phenological development of the soybean varieties tested.

5.2. Nodulation

Nodulation results obtained in the present study revealed that inoculation showed no effect on nodule number per plant, effective nodules per plant and nodule dry-weight per plant. The poor performance of inoculation on nodulation may be due to the indigenous rhizobial strains competing with the newly introduced strains or certain environmental conditions such as drought stress which reduced the effectiveness of newly introduced rhizobia strain. This was rather unusual as positive results for nodulation were expected from inoculation, especially on the specific nodulating soybean type. The results do not agree with Pule-Meulenberg *et al.* (2011) who mentioned that inoculation significantly showed an influence on soybean nodule number per plant and nodule mass per plant. Meghvansi *et al.* (2005) and Tahir *et al*. (2009) also reported that inoculated soybean plants resulted in higher nodule number than in uninoculated soybean plants.

The variety TGx 1937-1F had higher nodule number per plant, effective nodules number per plant and nodule dry weight than the variety PAN 1583R. This suggests that indigenous rhizobia was available in the soil at the test site and that the variety TGx 1937-1F was compatible with the indigenous rhizobial strains in the field. Generally, nodulation results in this study showed that both varieties had poor nodulation suggesting lower populations of compatible *rhizobia strain* at the test site, possibly because of periods of very low soil moisture during parts of the year**.** Weather data shows that between December 2017 and January 2018 the rainfall was low (41 mm and 52 mm) whilst average temperatures were fairly high between 24 °C and 27 °C, (Figure 1). Nodulation process start occurring during the vegetative growth stage of the crop especially in soybean cultivars. The vegetative growth stage of the crop occurred over the period December 2017 to January 2018 when the rainfall distribution was low. This suggests that the crop underwent moisture stress which led to lower populations of *rhizobium* bacteria at the test site because *rhizobium* bacteria do not thrive well under harsh environmental conditions such as moisture stress. Thus the population of the *rhizobium* bacteriain the soil may have been lowered by extreme hot summer temperature conditions as *rhizobium* bacteria does not thrive under extreme hot conditions (Figure 1). The maximum mean temperatures ranged from 24 °C to 27 °C between December 2017 and January 2018 and this should have resulted in high evapotranspiration that lowered soil moisture levels. Even though, the variety TGx 1937-1F nodulated better than variety PAN 1583R, poor nodulation of soybean cultivars due to moisture deficiency and extreme hot temperatures was also reported by Abaidoo *et al.* (2000), and this could apply to the present study. Soil phosphorus status was also very low which might have been one of the reasons for poor nodulation (Table 3.1 and 3.2). Phosphorus is responsible for root initiation and also nodulation, and the low concentration impacts negatively on root growth and development of nodules on roots. The study of Singleton *et al.* (1992) reported that phosphorus has some direct positive effects on soil *rhizobia* apart from its effect on acceleration of nodulation and plant growth. The study in other words implies that phosphorus at optimum concentration has significant effects on soil *rhizobia* for effective nodulation process. Nodule dry weight was found to be lower under no inoculation, variety PAN 1583R, 50 % and 100 % rate of seaweed extract treatments. Nodule dry weight in this study was lower (0.03 g) as compared to those reported by Maphosa (2015). The study of Osunde *et al.* (2003) also reported nodule dry weight of 1.13 and 0.60 g/plant for soybean varieties especially those of the TGx series (promiscuous varieties) which is high compared to the results in this study.

Seaweed extract showed no significant influence on nodule number per plant, effective nodules per plant and nodule dry-weight per plant. This can be due to failure of seaweed extract to produce growth substances and activation of enhanced nutrient uptake. Seaweed extract as a bio-fertilizer was expected to pro-activate the nodulation process and thus contributing towards the large dried shoot biomass. Seaweed extracts are reported to enhance growth of plant by increment of the transportation of nutrients (primary) to the host plant (Jensen, 1993). Jensen (1993) reported that seaweed extract is rich in mineral elements. These mineral elements are the primary determinants of root initiation, growth and development which may facilitate nodulation. Kavipriya *et al.* (2011) revealed that the maximum shoot length which contribute towards biomass of the green gram was found at 0.3 % concentration of *Turbinaria conoides* (seaweed extract). The findings of the present disagree with the Kavipriya *et al.* (2011) study on shoot biomass.

5.3. Soybean growth variables

Inoculation exhibited a clear effect on stem diameter and primary branches per plant. Increased primary branches per plant and stem diameter could be because the applied inoculant could effectively form active nodules which promoted fixation of atmospheric nitrogen which led to the enhancement of growth development. The fixed atmospheric nitrogen significantly enhanced vigorous growth and development of the crop which possibly resulted in higher primary branches per plant and thicker stem diameter. The insignificant effects of no inoculation treatment agree with Karasu *et al*. (2011) who emphasized that no inoculation treatment significantly failed to influence primary branches per plant on three dwarf chickpea *(Cicer arietinum)* cultivars over three years. Maphosa (2015) also reported that no inoculation treatment showed no significant effect on primary branches per plant of soybean cultivars.

In the present study, inoculation showed no effect on leaf number per plant, plant height, leaf chlorophyll content and leaf area. These could be due to the presence of indigenous rhizobia which sometimes compete with newly introduced strains in the field or the effectiveness of newly introduced strain was adversely affected by insufficient amount of soil moisture content in the field. This non-response could be also due to the very low phosphorus and N levels found at the trial site (Table 3.1 and 3.2). Phosphorus is important for the root initiation, BNF and also a vital component of the energy unit, some of which is required by the nitrogenase enzyme in BNF. The lower phosphorus concentration in the soil makes the inoculated *Rhizobium* bacteria not to thrive well in the field because insufficient amount of phosphorus has adverse impacts on BNF, roots initiation which is the primary crop growth and development determinant. Nitrogen (N) is the primary determinant of growth and development, and its insufficiency in the soil results in stunted crop growth and poor development. Nitrogen is also regarded as a major essential element of the photosynthesis apparatus including chlorophyll content. Plants utilize chlorophyll to manufacture sugars from water and carbon dioxide (Bassi *et al.,* 2017). The inability of plants to use energy to produce sugars from water and carbon dioxide also reduces growth and development of the crop. Our results agree with Maphosa (2015) and Ndlovu *et al.* (2016) who mentioned that inoculation with

Bradyrhizobium japonicum and *Rhizobium phaseoli* on soybean and dry bean cultivars, respectively, showed no significant effect on plant height. Plant height was increased when soybean variety was inoculated with *Bradyrhizobium japonicum* (Lamptey *et al.,* 2014) and these results disagree with the current study. Inoculation results of the present study also disagree with Maphosa (2015) who reported that inoculation significantly increased leaf chlorophyll content.

The variety TGx 1937-1F had the highest leaves number per plant, primary branches number per plant, stem diameter, plant height and leaf chlorophyll content as compared to variety PAN 1583R. The increased in leaves number per plant, primary branches number per plant, stem diameter, plant height and leaf chlorophyll is the vigorous growth habit on variety TGx 1937-1F as compared to variety PAN 1583R, because the two varieties have genotypic differences. Promiscuous soybean varieties are known to have more vigorous vegetative growth than specific-nodulating ones. Leaves number per plant and primary branches number per plant may differ depending on the growth habit of the plant (upright or bushy growth habit). In this study the short variety (PAN 1583R) produced fewer primary branches compared to the tall variety (TGx 1937-1F). Some studies did not show any significant influence of soybean variety on primary branches per plant (Maphosa, 2015; Bereke and Hailemariam, 2012) but these disagree with the findings of the current study. The variety TGx 1937-1F had also an influence on stem diameter as it was found to have bigger stem diameter over variety PAN 1583R. Growth and development of the plant is the determinant of the stem diameter. Plants with vigorous growth and development are likely to have largest stem diameter (Tanaka and Sharaiwa, 2009). Large stem diameter makes translocation and transportation of nutrients and minerals highly possible (Tanaka and Sharaiwa, 2009). The variety TGx 1937-1F probably needed a thick stem to carry the large shoot growth. An increased in leaf chlorophyll content of variety TGx 1937-1F may be due to increased growth vigour through enhancement of photo-assimilate production through photosynthesis, and could possibly result into large leaf area. Previous report by Maphosa (2015) agrees with the findings of the study by indicating that soybean varieties obtained the highest leaf chlorophyll content. Maphosa (2015) also reported that soybean varieties increased plant height.

Seaweed extract at 100 % application rate increased leaf number per plant, primary branches per plant, stem diameter and plant height. These results suggest that seaweed extract as bio-fertilizer at 100 % application rate activated uptake of nutrients such as N and P and production of growth hormones, such as auxins, cytokinins, gibberellins, gibberellins, ethylene, abscisic acid, betaine and polyamines, by the soybean crop to increase leaf number per plant and attain bigger stem diameter. The activated N and P uptake and produced plant hormones could have accelerated growth and development of the crop through cell enlargement or auxin-induced cell division. These produced plant hormones might have also enhanced activated the phloem and xylem of the crop for efficient transportation and translocation of nutrients and mineral particles. The primary branches per plant, as increased by application of seaweed extract, might be because of the increased translocation of assimilates from roots and stem, also increasing plant height of soybean crop. The increased plant height might be possibly by stimulating auxins which function by softening the cell wall thus further raising its plasticity. The results are in line with Shinde *et al.* (2010) who revealed that TIBA (seaweed extract) application at 40ppm (T5) increased primary branches number per plant of soybean variety and Ramesh *et al.* (2013) who mentioned that seaweed extract (NAA) at 40 ppm (T7) increased soybean plant height.

Interaction of inoculation × variety TGx 1937-1F increased leaf number per plant, primary branches per plant and plant height. This suggests that the promiscuous soybean was compatible with the newly introduced *Bradyrhizobium japonicum* strain. This result can also suggest that there was no adverse competition between *Bradyrhizobium japonicum* and the indigenous rhizobia in the soil. The fixed N enhanced vigorous growth and development of the variety TGx 1937-1F resulting in higher leaf number per plant, primary branches per plant and taller plants. These results agree with Maphosa (2015) and Ndlovu *et al.* (2016) who mentioned that the interaction of inoculation and variety increased growth variables of soybean and dry bean cultivars, respectively. The variety TGx 1937-1F stem diameter was higher without inoculation. This implies that the genetic make-up of the variety TGx 1937-1F attributed to its thick stem, which may be necessary to carry the large biomass of its shoot. It is not clear how inoculation could have reduced stem thickness of variety TGx 1937-1F.

Application of 100 % rate of seaweed extract without inoculation increased leaf number per plant, primary branches per plant and stem diameter. Seaweed extract as a bio-fertilizer could have promoted the production of plant hormones and solubilization of phosphorus as well as the activation of nitrogen uptake by soybean variety. When the crop produced sufficient auxins and gibberellins as well as solubilization of phosphorus and activation of nitrogen uptake, translocation of assimilates to the crop is activated hence increased plant growth. An increase in leaf chlorophyll content implies that the combination of inoculation and 100 % rate of seaweed extract promoted growth vigour of the soybean crop through enhancement of photosynthesis (production of photo-assimilates) and this could also translate into a larger leaf area.

Interaction of variety and seaweed extract showed that variety TGx 1937-1F × 100 % rate of seaweed extract interaction increased leaf number per plant and primary branches per plant. The variety TGx 1937-1F × 50 % rate of seaweed extract interaction increased stem diameter and plant height. These results suggest that application of seaweed extract at 50 % and 100 % rates stimulated the growth and development of the variety TGx 1937-1F through internal mechanisms such as cell elongation and division of the plant shoots, thereby stimulating ribonucleic acid and protein synthesis in plant cells besides the fact that the variety is expected to have a vigorous growth habit naturally. Craigie (2011) mentioned that seaweed extracts enhance crop growth and development due to their contribution to the internal mechanisms of the growth of the plant by interacting with major metabolic processes.

Three-way interactive effects of inoculation × variety TGx 1937-1F × 100 % rate of seaweed extract obtained significantly high leaf number per plant and primary branches per plant. Interaction of no inoculation × variety TGx 1937-1F × 100 % rate of seaweed extract obtained significantly taller plants. Generally, this could be due to the fact that the newly introduced strains did not compete with the indigenous Rhizobial strain in the soil which effectively fixed atmospheric N. Seaweed extract enhanced growth and development in TGx 1937-1F probably due to the production of hormones which enhanced transportation of amino acids, cell division, cell enlargement and nutrient uptake (N, P and K) (Davari *et al.,* 2012; Badar *et al.,* 2015; Alves *et al.,* 2016). This promotes the growth of roots, shoots and buds of the crop hence higher leaf number per plant, primary branches per plant and plant height (Davari *et al.,* 2012; Badar *et al.,* 2015; Alves *et al.,* 2016).

5.4. Soybean grain yield and yield components

No inoculation showed a clear effect on pod number per plant, grain yield, total above-ground biomass and harvest index. This is because the genetic make-up of a soybean variety has a vigorous growth habit which produces more pods number per plant, highest grain yield, total above-ground biomass and harvest index to no inoculation influence. On the other hand, inoculation showed no effect on dried shoot biomass, 100 seeds weight and shelling percentage. The newly introduced strain of rhizobia failed to be effective probably because of a large population of native rhizobia within the soil or unfavourable environmental conditions which caused the failure of the effectiveness of rhizobia bacteria. This has adverse impacts on the reproduction capacity of the crop, because of reduced nitrogen that reduces plant growth leading to poor production of photo-assimilates from the photosynthesis process (Dekalp-Asgrow, 2019). Poor photo-assimilate production may lead to poor podding rate from poor flowering rate, pod filling and seed mass. The podding rate and pod filling are primary determinants of 100 seed weight and shelling percentage. Therefore, poor podding rate and pod filling lead to poor 100 seed weight and shelling percentage (Dekalp-Asgrow, 2019). Maphosa (2015) and Ndlovu *et al.* (2016) also the negative effect of inoculation on pod number per plant, grain yield and harvest index of soybean and dry bean, respectively, which agrees with the current study. Our results disagree with Adeyeye *et al.* (2017) who revealed that grain yield was significantly enhanced by inoculation in soybean. The results on 100 seed weight agree with the findings by Ezekiel-Adewoyin *et al*. (2017) who indicated that inoculation showed no significant influence on 100 seed weight of soybean.

The variety of PAN 1583R had higher harvest index and 100 seed weight as compared to the variety TGx 1937-1F. This was expected as promiscuous soybean cultivars are known to develop their shoot biomass at the expense of seed production. Also, the seed of TGx 1937-1F is much smaller than that of PAN 1583R, which are genetic characteristics. The variety TGx 1937-1F had higher pod number per plant, grain yield, shelling percentage and total above-ground biomass as compared to the variety PAN 1583R. This reflects the inherent genetic differences of these soybean varieties also their specific responses to environmental conditions in the field. The higher pod number per plant and higher shelling percentage translates into higher grain yield for variety TGx 1937-1F. Ikeogu and Nwofia (2013) also reported that pod number per plant influences soybean grain yield. The high performance of promiscuous variety on grain yield was not expected due to its bulk biomass and low harvest index which are associated with variety. However, it must be noted that the overall yields obtained in this study were very low due to harsh environmental conditions which were experienced in the field. The trial was established late in the growing season and the performance of the crop was generally poor probably because the soybean crop was adversely affected by low temperatures towards 50 % physiological maturity. At Syferkuil farm declining temperatures kick in from the end of April whereas the current study was harvested in May for variety PAN 1583R and end of June for variety TGx 1937-1F (Figure 1). Aniekwe (2015) reported that promiscuous varieties, such as TGx1844-18E and TGx1904-2F, achieved a yield size of 11.35 t/ha and 11.18 t/ha which were the highest grain yields per hectare among soybean varieties in their study. Maphosa (2015) also reported that variety TGx-1937-1F resulted in 5582 kg/ha of grain yield. Sanginga *et al.* (2002) also presented grain yield which ranged from 1340 and 1494 kg/ha for three promiscuous varieties in Nigeria. So all these reported soybean grain yields, such as by Aniekwe (2015) and Maphosa (2015), are higher than the grain yield (631 kg/ha) achieved in this study. The growing conditions of Maphosa (2015), Aniekwe (2015) and Sanginga *et al.* (2002) were similar to the current study as they were conducted during summer growing season where irrigation was supplemented in case of moisture stress. The studies of Maphosa (2015), Aniekwe (2015) and Sanginga *et al.* (2002) were conducted in 2012/ 2013, 2007/ 2008 and 1994/ 1995 summer growing seasons.

The variety TGx 1937-1F had smaller seed sizes as compared to variety PAN 1583R which had a much larger sizes. This may be due to genetic differences. Smaller seed size of variety TGx 1937-1F may be an advantage under disputable environmental conditions as the lower seed rates are needed for crop establishment and result in good plants stands. A larger seed size of PAN1583R can be preferred by seed companies as they may be easier to handle and are better suited to machine planting of the soybean crop. The variety TGx 1937-1F also obtained significantly larger dried shoot biomass and total above-ground biomass in this study. The variety TGx 1937-1F showed vigorous growth habit which produced more leaves, lateral branches and taller plant height. TGx varieties are bred to have high plant biomass. The large dried shoot biomass and total above-ground biomass will contribute towards the financial viability of the SH farmers as it can be either incorporated into the soil for soil fertility amelioration, used for mulching to protect the soil against harsh conditions such as extremely high temperatures and also used for fodder during dry winters.

The vigorous shoot biomass can also play important roles in weed growth suppression and moisture conservation by reducing the irradiance level that can reach the soil. The higher dried shoot biomass and total above-ground biomass of variety TGx 1937-1F as compared to variety PAN 1583R was expected since promiscuous soybean varieties have tall plants and large biomass, but lower harvest index than specific-nodulating varieties. The results from the present study agree with Mpepereki *et al.* (2000) who mentioned that promiscuous varieties large leaf biomass contributes to the benefits of the SH farmers by giving both fodder and organic manure (as green manure) to feed livestock and soil fertility amelioration, respectively. However, the long growth duration may restrict the successful growth of this type of soybean to irrigation schemes to avoid moisture stress during the reproductive growth stages, given the short rainy season experience in most parts of the Limpopo Province.

Seaweed extract rate at 0 % increased harvest index. Seaweed extract at 100 % rate increased shelling percentage, grain yield and total above-ground biomass. The soybean economic product is grain seeds hence the harvest index (HI) can be referred to as the ratio of the economic product to biological yield (seeds to the above-ground biomass at harvest maturity). Harvest index is likely to be a genetic characteristic of soybean, hence no response to seaweed extract. These results disagree with Mahajan (2014) who reported that the (T_4) seaweed extract (SWE) granules at 12.5 kg/ha and seaweed foliar spray at 120 ml/ha (25, 40 and 60 DAS) obtained significantly higher harvest index (0.325) as compared to control. The increased shelling percentage and grain yield may be because, seaweed extracts at a 100 % rate may have enhanced the source- sink association supply of photo-assimilates to the seed rather than to the pod shells.

Grain yield results of this study agree with Mahajan (2014), who reported significantly high soybean yield in the (T4) application of seaweed extract (SWE) granules at 12.5 kg/ha and foliar spray of seaweed at 120 ml/ ha (25, 40 and 60 DAS). Debashish Swain and Avijit Sen (1996) also reported that biozyme granules (derived from the seaweed *Ascophyllum nodosum*) increased growth, yield and yield traits of soybean. The significantly higher total above-ground biomass obtained under 100 % seaweed extract might be because seaweed extracts are comprised of more than one plant growth-promoting substance group i.e. zeatin, isopentenyl, BAP (benzyl aminopurine), toplin, auxin and auxin like compounds which directly increase plant biomass especially cytokinins (Lane *et al,* 2006). These plant growth substances promote vigorous vegetative growth of the crop which possibly increased the total above-ground biomass of the soybean variety in this study.

Interaction of no inoculation × variety TGx 1937-1F resulted in increased pod number per plant, grain yield and total above-ground biomass. These results imply that the genetic make-up of the variety TGx 1937-1F is of vigorous growth habit hence high performance of the reproduction parts. The vigorous growth habit of the variety TGx 1937-1F also contributed towards high total above-ground biomass. No inoculation × variety PAN 1583R interaction had a higher shelling percentage and harvest index, suggesting that these are its genetic characteristics

Interaction of inoculation and seaweed extract showed that no inoculation × 100 % increased pods number per plant while no inoculation × 50 % rate of seaweed extract increased shelling percentage and grain yield. No inoculation × 0 % rate of seaweed extract interaction increased harvest index. Inoculation × 100 % rate of seaweed extract interaction increased total above-ground biomass. The increase in pod number per plant implies that the applied seaweed extract resulted in efficient transportation of photo-assimilates from the stem and leave to reproductive parts of the soybean variety thereby increasing pod number per plant. The increased shelling percentage and grain yield suggest that seaweed extract activated the production of photo assimilates through photosynthesis and plant hormones, thereby increasing reproduction and productivity levels (Alves *et al.,* 2016). These possibly result in high shelling percentage hence a high grain yield. The increase in total above-ground biomass implies that seaweed extract as bio-fertilizer generally promoted crop growth and development because it contains plant growth hormones, micro (calcium, magnesium, iodine, zinc, copper) and macro-elements (nitrogen, potassium, sulphur and phosphorus) (Sageetha and Thevanathan, 2010).

Interaction of variety and seaweed extract showed that variety TGx 1937-1F × 100 % rate of seaweed extract increased pods number per plant, shelling percentage, grain yield and total above-ground biomass. This suggests that seaweed extract application at 100 % promoted the translocation of assimilates to effectively maximize plant growth (Badar *et al.,* 2015; Michalak and Chojnacka, 2015) and reproduction of the variety TGx 1937-1F. The enhanced plant growth and reproduction of the variety TGx 1937-1F possibly resulted in a higher pod number per plant and high total above-ground biomass and filled with grain seeds. Harvest index was varied significantly by variety PAN 1583R × 0 % rate of seaweed extract interaction. This implies that the variety PAN 1583R has a good genetic make-up which also obtained more pods number per plant which possibly contributed towards high biological yield. The high biological yield may have direct effects on the economic yield.

The 3-way interaction of no inoculation × variety TGx 1937-1F × 100 % rate of seaweed extract increased pods number per plant, grain yield and total above-ground biomass. Therefore, as this is the same as the variety TGx 1937-1F × 100 % rate of seaweed extract influence alone, it implies that the influence of 100 % rate of seaweed extract as bio-fertilizer on the variety TGx 1937-1F also enhanced vigorous growth habit and development as well as its productivity level. The enhanced vigorous growth habit and development, as well as its productivity level, possibly resulted in higher pod number per plant, high grain yield and total above-ground biomass. Inoculation × variety TGx 1937-1F × 100 % rate of seaweed extract interaction increased shelling percentage. This implies that the variety TGx 1937-1F has a good genetic makeup to enhance more pod numbers which contributed to a higher shelling percentage. No inoculation × PAN 1583R × 100 % rate of seaweed extract interaction had a higher harvest index at $P \leq 0.001$. This is because seaweed extract is comprised of production hormones that could have direct effects on the pods yield which favoured significant increase in harvest index.

5.5. Pearson's correlation

Correlation analysis showed that nodule number per plant, number of effective nodules per plant, leaf number per plant, pod number per plant, stem diameter and plant height showed a positive correlation with grain yield (r^2= 0.02). Leaf chlorophyll content also revealed a positive and significant relationship with grain yield (r^2= 0.02). This implies that nodules number per plant, effective nodules number per plant, leaves number per plant, pods number per plant, stem diameter, plant height and leaf chlorophyll content need to be measured in future studies as they influence grain yield. Grain yield is one of the primary indicators of successful soybean production. Our results agree with Ndlovu *et al.* (2016) who reported a positive correlation of nodules number per plant, nodule dry weight, plant height with grain yield in dry beans. Bekere *et al.* (2012) also mentioned a significant association between nodulation and growth variables of the soybean crop.

There was a weak positive correlation of leaf area with grain yield (r^2=0.02). This suggests that leaf area did not contribute much towards the increment of the reproduction parts which led to grain yield. The leaf number per plant, pod number per plant and plant height also have shown a very strong and positive association with leaf chlorophyll content. This implies that leaf chlorophyll content is a good indicator of soybean growth vigour. Maphosa (2015) also reported a strong positive correlation between leaf chlorophyll content, pod number per plant, stem diameter and plant height with grain yield of soybean. Leaves number per plant, pod number per plant and stem diameter have shown a very strong and positive relationship with plant height. This implies that a tall plant has a high leaf number per plant, pod number per plant and thick stem.

Nodule number per plant, effective nodules per plant and pod number per plant had a clear and positive association with stem diameter. The stem diameter might affect the efficiency of nutrient translocation in the soybean plant. A significant correlation of the pod number per plant with stem diameter is possibly because the bigger stem diameter means increased translocation of nutrients to the reproductive parts. Large stem diameter makes translocation and transportation of nutrients and mineral particles possible. Thus, this will promote vigorous soybean growth and development. Effective nodules per plant and leaf number per plant had a positive correlation with pod number per plant. Atmospheric N fixation through effective nodules enhance crop growth and development which leads to an increase in leaf number per plant. The increased leaf number per plant possibly enhanced photo-assimilate production through photosynthesis. The level of photo-assimilates has direct positive effects on reproductive parts of the crop which possibly determined pod number per plant. Effective nodules number per plant had a positive relationship with leaf number per plant. This might be due to the increase in plant growth which was enhanced by fixed atmospheric nitrogen from effective nodules. The increased plant growth normally enhances leaf number per plant. Increased leaf number can also result in higher levels of photosynthesis which can then supply more energy for the BNF activity in the nodules.

The significant positive association between grain yield and growth variables such as plant height, leaf number per plant, leaf chlorophyll content, stem diameter, pod number per plant, nodule number per plant, and effective nodules per plant implies that plants that could nodulate tend to have highest plant growth rate which results in increased leaf chlorophyll content, yield components and grain yield. This indicates that nodulation and growth had a positive impact on the yield of the soybean variety. Lamptey *et al.* (2014) reported that soybean grain yield is largely dependent on yield components and growth traits. The positive correlation between grain yield and pod number per plant agree with the findings of the study of Makondo (2016). Our findings suggest that important yield traits to evaluate in soybean research are pod number per plant, leaf number per plant, stem diameter, plant height and primary branches number per plant.

5.6. Moisture, protein, macro and micro-nutrients content of soybean grain

Inoculation exhibited an increase in soybean seed potassium (K). The increase in nitrogen availability caused by BNF may induce or stimulate greater potassium availability (Thummanatsakun and Yampracha, 2018). Furthermore, the inoculated bacteria may have benefitted from optimum soil pH reported in this study at both 0-15 cm and 15-30 cm depths before and at harvest maturity (Tables 3.1 and 3.2). The optimum soil pH obtained in this study possibly increased *rhizobium* bacteria ability to supply K to plants.

This is because there is a symbiotic relationship between *rhizobium* bacteria and K. The *rhizobium* bacteria derive nutrition from the plant and generate ammonium ($NH4^+$) from atmospheric N by a process catalysed by the enzyme nitrogenase which also favours the availability or the supply of potassium to the plant (Barta, 1982). Mallarino *et al.* (2013) reported that potassium availability is highest at a soil pH of 6.0. Mike (2012) reported that the optimal pH range is between 6.3 and 6.5 duly because this range maximizes the availability of nutrients and BNF while reducing cyst nematode (SCN) of the soybean.

The seed K is essential in the development of seed as it raises carbohydrate and sugar accumulation within the seed (Fontes, 2001). Rosolem *et al.* (1984) mentioned that K sources may have direct effects on seed size, which may affect or improve seed quality physiologically. Khan *et al.* (2004) also mentioned that K fertilization can raise seed mass. Jeffers *et al.* (1982) found that the fertilization of K in soybeans improves normal seedlings in the testing of germination. In other words, soybean germination of the seed was enhanced by fertilization of K. The seed K in the human body functions to make the body-work properly by enhancing the proper function of nerves by moving nutrients into cells and waste products out of cells, and body muscles to contract and maintains regular heartbeat (DGAC, 2020). The seed K results in the present study disagree with Maphosa (2015) who reported that inoculation exhibited no significant effect on seed K in soybean. Inoculation showed no effect on seed moisture percentage, seed protein percentage, seed nitrogen percentage, seed calcium, seed copper, seed iron, seed magnesium, seed manganese, seed sodium and seed phosphorus. These might be because the overall (before planting and at harvest maturity) soil chemical analysis showed low levels at both depths of 0-15 cm and 15-30 cm except for soil pH (Table 3.1 and 3.2). The low status of soil nutrients, especially organic carbon percentage, P, NH_4^+ and NO_3^-, may have negatively impacted the translocation of nutrients to the soybean seeds. Our results agree with the study of Maphosa (2015) who reported a lack of significant effect of inoculation on seed Mg, Na and P content of soybean. This study of Maphosa (2015) disagrees on seed N %, protein % and Ca % where there were significant effects on seed N % which increase by 10.3 % in seed N %, 3.66 % in seed protein and 2.0 % in seed Ca.

The tested variety TGx 1937-1F had higher seed iron (Fe). The increase in seed Fe might be because the variety TGx 1937-1F has a genetic make-up that promoted the efficient movement of oxygen throughout the roots, leaves and other parts of the crop thereby producing the greener colour which is a good indicator of a healthy plant hence high Fe (Ghasemian *et al.,* 2010). A crop that is rich in Fe can move oxygen efficiently throughout the roots, leaves and other parts. Fe plays a vital role in metabolic processes such as respiration, photosynthesis and DNA synthesis. The soybean products specifically from variety TGx 1937-1F when used for human consumption will contribute towards an increment and efficient flow of blood in the human body as this element (Fe) is essential for blood production. It would be of interest to check if this holds for all promiscuous soybean varieties.

Seaweed extract at 100 % rate increased seed Ca, seed K, seed Mg, seed Mn and seed Na. This suggests that seaweed extract rate at 100 % influenced the mobilization of Ca, K, Mg, Mn and Na in the soil and mainly the genetic efficiency of the soybean variety in the uptake, translocation and storage of these elements. This was expected since seaweed extract is reported to be rich in mineral elements such as, Ca, Mg, K, chlorine (Chl), sulphur (S), P, iodine, zinc (Zn) and Cu (Jensen, 1993) and it functions to improve the nutrient uptake of the plant to make all nutrients available. Increased soybean nutrient uptake traits as affected by the 100 % rate of seaweed extract provide evidence that seaweed extract as bio-fertilizer can improve the nutrition security of legume crops, including soybean seed.

Mahan and Escott-Stump (2008) mentioned that Ca is essential and required to increase bone mass and density, and also needed for transmission of human nerve and heart muscle function regulation. Ca ions are essential in playing a key role in the contractility of human muscles. Mahan and Escott-Stump (2008), reported that Mg functions in the connective and skeletal tissue formation, growth and reproduction, metabolism of carbohydrate and lipids. Mahan and Escott-Stump (2008) also reported that the balance between K, Na and Mg. P and Mg are essential in enzymatic reactions and plays a key function in the metabolism of energy (Ervin *et al.,* 2004).

Interaction of inoculation and variety TGx 1937-1F increased seed N percentage. This means that inoculation through nodulation efficiency enabled the variety TGx 1937-1F to fix atmospheric nitrogen which normally activated the uptake of nitrogen to be available in high percentage in the seed. Variety PAN 1583R without inoculation increased protein percentage. The variety PAN 1583R may have a large tap root system which enabled the efficient uptake of N. This indicates that soybean is a rich source of cheap protein. Protein plays a major function in the building the body and health maintenance and it remains an important element in man's diet (Olatunji *et al.,* 2011). Tahir *et al.* (2009) reported a 9 % increase in N and protein of soybean due to inoculation with *Rhizobium* strains of bacteria. Maphosa (2015) also reported that inoculation significantly influenced seed crude protein of TGx-17402F, TGx-1835-10E and Dundee by 30.9%, 13% and 4.9%, respectively, while TGx-1937-1F failed to respond to *Bradyrhizobium japonicum* strain WB74 introduction. Furthermore, uninoculated variety TGx-1937-1F seed had slightly higher crude protein as compared to the inoculated (Maphosa, 2015).

In this study, seed N % was increased significantly by inoculation in variety TGx 1937-1F. Nitrogen contained in soybean seed is also essential as it contains amino acids, which are the building blocks that make up the proteins in human hair, muscles, skin and other important tissues. It is also an important part of DNA. A seed N that is increased due to *Bradyrhizobium japonicum* strain WB74 treatment was found in TGx-1740-2F, TGx-1835-10E and Dundee while TGx-1937-1F failed to respond to inoculation (Maphosa, 2015). Thus the results reported by Maphosa (2015) disagree with the findings of this study.

Application of seaweed extract at 100 %, without inoculation, increased seed copper (Cu) and seed manganese (Mn). This result implies that seaweed extract at a 100 % rate promoted the mobilization and uptake of seed copper (Cu) and seed manganese (Mn) to be available in high amounts in the seed. Jensen (1993) reported that the seaweed extract is also rich in mineral elements containing manganese and copper. Manganese is essential in activating decarboxylase and dehydrogenase and is a complex PSII protein, Superoxide Dismutase (SOD) and phosphatase constituent of the seed (Ghasemian *et al.,* 2010). Ghasemian *et al.* 2010 reported that manganese increased seed yield (3367 kg/ha), pod number and seed mass of soybean at a 40 kg/ha application rate. Manganese helps the human body in forming connective tissues, bones, plays a key function in carbohydrate metabolism and fat and absorption of calcium, normal brain and nerve function (Claus Henn, 2010). Copper (Cu) serves as one of the micro-nutrient that may also promote the germination process of soybean seeds by activating enzymes that are involved in the synthesis of lignin (Ganesh Sankar and Sundaramoorthy, 2018; Gyan and Sunita, 2015). Ganesh Sankar and Sundaramoorthy (2018) reported that the maximum (100%) percentage of seed germination was achieved by 5 mg/L concentration of Cu and the minimum seed germination percentage (50%) was achieved at 200 mg/L of Cu. In other words, seed Cu enhances seed vigour and thus seed imbibition. Cu associate with Fe in the human body to help in the formation of blood cells (red), blood vessel keeping, nerves and Fe absorption (Alda and Garay, 1990; Amaravadi *et al.,* 1997).

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

The variety TGx 1937-1F performed better than the variety PAN 1583R variety which had a poor performance generally. In locations where smallholder (SH) farmers have poor resources and have limited access to agro-chemical inputs, even though variety TGx-1937-1F is of late maturing, it could be the better variety to be introduced to them in the growing conditions prevailing in Limpopo Province. The SH farmers can take an advantage of promiscuous variety (TGx-1937-1F) which requires low inputs. However, it could be risky to cultivate the variety TGx-1937-1F under dry-land production given the fact that most SH farmers reside in marginal rainfall areas with a short rainy season. Our results suggest that SH farmers in dry-land locations can utilize a variety TGx 1937-1F with or without inoculation.

Seaweed extract rate of 100 % treatment generally had a significant influence on soybean plant growth and seed nutritional content in this study than 0 % rate of seaweed extract and 50 % rate of seaweed extract, therefore it can be recommended to SH farmers. If the resource-poor Limpopo Province farmers can have efficient access to *rhizobium* inoculants, seaweed extract, and promiscuous soybean, they can get high yield and get all the attainable benefits from the crop such as the source of protein, fodder for livestock feeding and soil fertility amelioration. Future research should include promiscuous varieties with a shorter growth duration than TGx1937-1F.

The combination of inoculation × variety TGx 1937-1F × 100 % rate of seaweed extract increased leaves number per plant, primary branches number per plant, tallest plant height and no inoculation × variety TGx 1937-1F × 100 % rate of seaweed extract combination increased pods number per plant, highest grain yield and total above-ground biomass. Both 3-way interactions mentioned above can be provisionally recommended for future soybean production by SH farmers, whilst further research is conducted. The highest total above-ground biomass will contribute towards sufficient fodder for cattle feeding during dry winters, green manuring to improve soil fertility status and can also serve as mulch more especially in case of mulch tillage to promote conservation agriculture and thus conserving the environment by avoiding the use of inorganic fertilizers. Future research should test more naturally nodulating varieties with shorter growth durations to suit the shorter growing seasons prevalent in the Limpopo province. The varieties could be tested with a range of seaweed extracts at varying application levels on several sites, paying special attention to nodulation and dry matter, in addition to grain yield production. Such trials could also include on-farm sites and be subjected to farmer evaluation and economic analysis.

On soybean seed nutritional content, inoculation, TGx 1937-1F and seaweed extract at 100 % rate can also be recommended to SH farmers as they have improved seed K, seed Fe, seed Ca, seed Mg, seed Mn and seed Na. These improved seed nutritional content will contribute to the soybean high seed quality. The high soybean seed quality will have a greater value towards long life span storage, food and nutrition security in case of human consumption or livestock feeding, good germination and emergence after sowing or direct planting and sufficient moisture content. Future research on inoculation, TGx 1937-1F and seaweed extract at 100 % rate should also focus on seed nutritional content to sustain the high seed quality of soybean grain.

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