THE INFLUENCE OF CULTIVARS, NITROGEN SUPPLY, LOCATION AND WATER STRESS ON AGRONOMIC PERFORMANCE AND OIL QUALITY OF LINSEED (*LINUMUSITATISSIMUM*L.) IN KENYA

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The influence of cultivars, nitrogen supply, location and water stress on performance and oil quality of linseed (*Linumusitatissimum* L.) in Kenya

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University

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DEDICATION

To my husband Mwangi and daughters Immaculate and Joy and son Kevin; for the support you have given me this far, thank you.

To my late parents David and Lucy; though you didn't live to witness this lifetime achievement, I know you are proud wherever you are.

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ACRONYMS AND ABBREVIATIONS

AA	Arachidonic Acid
ALA	Alpha Linolenic Acid
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
BP	Base Pairs
CAN	Calcium Ammonium Nitrate
CIA	Chloroform Isoamyl Alcohol
CTAB	CetylTrimethyl Ammonium Bromide
DARwin	Dissimilarity Analysis and Representation for Windows
DAS	Days After Sowing
DHA	Docosahexaenoic Acid
DNA	Deoxyribonucleic Acid
EC	Electrical Conductivity

EDTA	Ethylenediaminetetraacetic Acid
EPA	Eicosapentaenoic Acid
FAME	Fatty Acid Methyl Esters
FAOSTAT	Food and Agriculture Organization Statistics
FASW	Fraction of Available Soil Water
FID	Flame Ion Detection
KALRO	Kenya Agricultural and Livestock Research Organization
KARI	Kenya Agricultural Research Institute
LA	Linoleic Acid
LCPUFA	Long Chain Polyunsaturated Fatty Acids
LSD	Least Significant Difference
NQI	Nutritional Quality Index
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction

PIC	Polymorphic Information Content
PROC GLM	General Linear Model Procedure
PUFA	Polyunsaturated Fatty Acids
RWC	Relative Water Content
SAS	Statistical Analysis Systtem
SSR	Simple Sequence Repeat
TBE	Tris Borate EDTA
UPGMA	Unweighted Pair Group Method with Arithmetic Means

ABSTRACT

Flaxseed,also known as linseed, belongs to the Linaceae family. It is emerging as a major ingredient in health promoting foods (functional foods or nutraceuticals), as well as in animal feedsdue to high omega-3 fatty acid and lignans content.Its current production in Kenyais minimal and the extent of genetic variability within the Kenyan germplasm in unknown. This study therefore addressed four related objectives as a contribution towards increased cultivation of linseed in Kenya namelyto assess 1) genetic variation among linseed cultivars in Kenya using SSR markers; 2) effect of nitrogen fertilizer application on growth and seed yield; 3) effect of location and nitrogen fertilizer application on oil quantity and quality; and 4) effect of water deficit on growth of linseed cultivars.

Thirteen linseed cultivars (Jawhar, CI-1652, Norlea, S19/31, S19/12, Summit, CI-1525, Raja, S19/21, S/10/03, Concurrent, S25/61 and 7331) were characterized using 28 SSR markers. DNA extraction followed a modified CTAB protocol.Twenty six of the 28 SSRs showed amplifications with at least one of the genotypes with sizes of the amplification products varying between 50 and 200bp. A total of 46 alleles were detectedat 26 loci, with an average of 1.769 alleles perlocus. The average polymorphic information content was 0.2033.The tested linseed cultivars fell into three main clusters.

Growth and yield performance of five linseed cultivars(Summit, S19/21, Raja, Jawhar andS19/12) were evaluated in two agro-ecologically different locations,Njoro and

Juja,for two seasons, February-June and July-December 2012 under0, 75 and 150kgN/hafertilizer rates. There were no interactions between nitrogen and cultivars in the two sites and seasons. There were significant differences in the number of tillers, dry weight, number of heads and seed yield ($p<0.05$) but not number of leaves and plant heightamong the cultivars in both sites. While vegetative growth was not significantly affected by addition of nitrogen fertilizer ($P>0.05$) at both sites in both seasons, capsules and seed yield increased with application of nitrogen. Raja yielded moreseed than the other cultivars at both locations and seasons and would therefore be recommended as a choice cultivar.

The oil content and fatty acid profiles of resultant seeds from above field experimentwere determined to give an indication of the influence of location on oil content and fatty acid profiles on linseed germplasm.Oil was extracted using soxhlet apparatus and fatty acid profiles determined using gas chromatography. There were inter-cultivar differences in oil content and fatty acid profiles depending on season and location. Therefore with the right cultivar, good quality linseed could be produced in both Juja and Njoro without supplemental nitrogen fertilizer application.

Incidences of drought are becoming increasingly common in Kenya. A studyto evaluatethe effect of water deficit stress on growth of three linseed cultivars(S19/12, Summit and Raja) was conducted in a greenhousein JKUAT inFebruary-May and August-November 2014.Stressed pots had watering completely withheld from the 4th

week after sowing while the well watered control was maintained at 90% field capacity throughout the experiment. The cultivars did not significantly differ in growth in both seasons. Water stress caused20-40% reduction in growth and begun when 30-80% of available soil water had been used up. Relative water content declined after 25-67% of available soil water had been used up.

The study therefore concludes that Raja could grow well and produce good quality oil in regions similar to Juja and Njoro without addition of nitrogen fertilizer provided soil water levels don't fall below 0.2-0.7fraction of available soil water.

CHAPTER ONE

GENERAL INTRODUCTION

1.1: Background Information

Flaxseed (*Linumusitatissimum*L.), also known as linseedbelongs to the family Linaceae (El-Beltagi,Salama, & El-Hariri, 2007), and is the only economically significant species in this family (Savita,Kenchanagoudar, Parameshwarappa, and Rudranaik. 2011). It is thought to be one of the oldest cultivated crops with evidence of cultivation dating back thousands of years (Newkirk, 2008), and it is cultivated for either seed oil or fibre(Diederichsen & Hammer, 1995; Diederichsen& Richards, 2003;Vaisey-Genser and Morris, 2003; Tour´e & Xueming, 2010). Linen textiles were important in ancient civilizations such as Egypt and Greece, and it is mentioned 22 times in the Bible'sOld Testament (Diederichsen & Hammer, 1995). The Chinese predominantly used flax as oil, dating as far back as 2,000 years, possibly 5,000 years, based on archeological evidence (Pan, 1990). The Romans used it for their sails, and as the empire declined, so did flax production (Buchanan, 2012). However, Charlemagne revived its production in the Roman Empire in the 8th Century, and French immigrants took it to North Americain the 17thCentury (Vaisey-Genser & Morris, 2003). During the industrial revolution, linoleum flooring was patented in Britain, but the invention of the cotton gin made cotton fabric cheaper than linen, and led to a collapse of the flax industry in the 1920s (Vaisey-Genser & Morris, 2003). In the 1980s, consumer emphasis on environmentally friendly, natural products renewed producer interest in linseed in North

America and Europe and, in the 1990s, linseed gained interest as a functional food (Vaisey-Genser & Morris, 2003).

Linseed oil is used as an ingredient in the manufacture of linoleum flooring, plasticizing agents, bitumen, paints, asphalt, wood impregnants, lacquers, granulated pesticides, adhesive and sealing compounds, inks, paper and cardboard, cosmetics, medicinals, copolymers for containers including plastic bottles for water (Tolkachev &Zhuchenko, 2000). In all these applications, linseed oil is an environmentally friendly, biodegradable component.A functional food is one that has health-promoting properties beyond benefits due to traditional nutrients such as carbohydrates, protein, fat, vitamins and minerals.The health promoting components of linseed are mainly the omega-3 fatty acid, alpha-linolenic acid, and a number of lignans. Humans can convert dietary alpha-linolenic acid into longer, more unsaturated fatty omega-3 fatty acids such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). While the omega-6 fatty acid, linoleic acid, is the most predominant polyunsaturated fatty acid (PUFA) in most mammalian tissues, DHA is the major PUFA in the brain and in the retina (Swanson,Block and Mousa, 2012). Adequate levels of DHA and EPA in the body are associated with a reduced risk for major physiological dysfucntionsincludingbut not limited to atherosclerosis, cardiovascular diseases and Alzheimer's disease (Swanson *et al.*, 2012).

The lignan content of linseeds is approximately 100 times more than other plant seeds, and some metabolites of linseed lignans are associated with decreased risk of prostate and breast cancer (Tolkachev & Zhuchenko, 2000).

Linseed is also gaining in importance as a component of pet foods and animal feeds (Vaisey-Genser and Morris, 2003). Supplementation of animal feeds including poultry, cattle feed and fish feed with linseed or linseed oil is mainly aimed at increasing the omega-3 fatty acid content in the animal derived products, which improves their value in human nutrition.

It has been reported that fish farming with feeds high in omega-6 fatty acids greatly affects the fatty acid profiles of fish, thus converting fish from a health promoting food to an unhealthy food (Weaver,Takata, Reesman& Van Dyke, 2008). On the other hand, various studies have shown that supplementation of fish feeds with linseed oil and certain biologically active substances such as lipoic acid and sesamin may lead to increased contents of DHA and EPA in farmed fish (Trattner *et al*., 2008).

The world linseed production stood at about 2.3 million tonnes in 2013, when Canada, the leading producer contributed 712,000 tonnes (FAOSTAT, 2013). Ethiopia, which is the leading producer in Africa, contributed 104,948 tonnes, while Kenya produced a paltry 1000 tonnes in 2013 (FAOSTAT, 2013).

According to FAO, Kenya is currently ranked 35th worldwide in linseed production. The figure below shows Kenya's linseed production statistics between 1961 and 2013 (FAOSTAT, 2013). Although production rose from about 600 tonnes in 1961 to 2400 tonnes in 1999, production has declined thereafter by half.

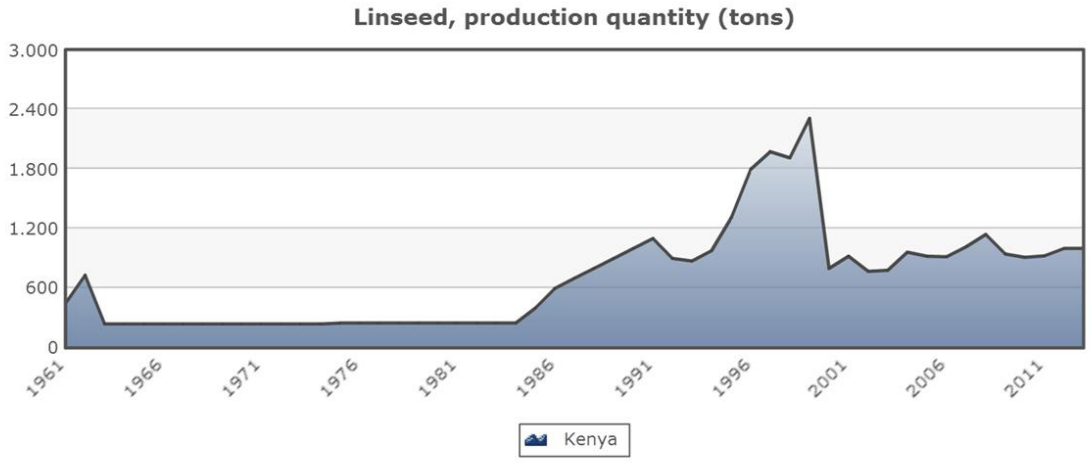


Figure1.1: Linseed production in Kenya,

Source: **http://www.factfish.com/statistic-comparison/ke-et/linseed%2C%20production%20quantity. Downloaded on 25th October 2015**

There is need to increase linseed production in Kenya so that the population can benefit from the good qualities mentioned above. This can be done by improving agronomic practices as well as by increasing the area under linseed cultivation. The latter may include expanding cultivation of linseed to agro-ecological zones where it is currently

not cultivated.However, environmental conditions affect not only the growth and yield of linseed but also the quality of oil. For example, higher temperatures may lead to reduction in the content of alpha-linolenic acid, in favour of linoleic acid, representing a decline in quality (Canvin, 1965). In drier agro-ecological zones, linseed will experience soil moisture deficits, which will affect growth and yield of the crop. Moreover, the response of linseed cultivars to nitrogen and other fertilizers depends on the environmental factors (Berti,Fischer, Wilckens and Hevia,2009). A study on the response of linseed to application of fertilizers and environmental conditions is necessary in Kenya for increased production and quality of the crop.

1.2:Problem Statement

In recent years, there has been a sharp increase in the prevalence of non-communicable diseases such as diabetes, cardiovascular diseases and cancer in Kenya. Although these diseases were previously associated with affluence, they are now equally afflicting people in the lower socio-economic groups.These diseases are mainly caused by chronic inflammation which may be prevented by consumption of health promoting foods such as linseed. Despite linseed having been introduced in Kenya in the 1920s, there are no published reports indicating the genetic variability within such introductions whichwould form a basis for their increased cultivation in Kenya. In spite ofthe benefits of linseed as a functional food, and as a component of animal feeds, its production remains low in Kenya, with an annual production of about 1000 tonnes(FAOSTAT,

2103). There is insufficient information, if any, on the right mineral nutrient rates and water levels as part of proper management practicesfor optimal growth and seed yield of the cultivars in Kenya. The performance of the different cultivars in different agro-ecological zones in Kenya remains largely unknown. Information on the effect of management practices and environmental factors on oil content and quality is also lacking. Currently, linseed is mostly sold in health shops at high prices that most people cannot afford, and its use in animal feeds for improving the quality of animal products remains largely untapped.

1.3:Justification

The Government's strategy to combat chronic non-communicable diseases involves both medical and preventive, nutritional interventions (Republic of Kenya, 2015). For the nutritional interventions to succeed, a variety of foods with health benefits should be readily available and affordable, and linseed is one such food. Linseed contains essential polyunsaturated fatty acids, omega-3 fatty acids, which are known to prevent inflammation and therefore has potential to reduce cases of the chronic non-communicable diseases. The use of fish oils to supplement fish feeds results in farmed fish with omega-3 fatty acid levels, but this is not sustainable because of the dwindling fish populations in the natural water bodies. On the other hand, while linoleic acid-rich vegetable oils are relatively cheap, their use in fish feeds increases the omega-6:omega-3 fatty acid ratios in the fish, thus lowering the value of farmed fish. Various studies

have increasingly shown that the agronomic performance and chemical composition of linseed varies with cultivar, environmental factors andagronomic factors including nitrogen fertilization (Dmitriy,Aleksey & Aleksey, 2012; Muhammad *et al*., 2014; Andruszczak,Gawlik-Dziki, Kraska, Kwiecińska-Poppe, Różyło& Pałys,2015; Raphaëlle *et al*., 2015).Investigatingthe genetic diversityof linseed cultivars in Kenya will form a basis for conducting further studies. Available information on the growth performance and yield of linseed under varying nitrogen fertilizer rates is contradictory and genotype-dependent. Genotype–environment interactions have beenshown to be statistically significant for linseed (Nematallahi and Saeidi (2011), with yields varying considerably depending on location, among other factors, hence the need to investigate growth performance and yield of the linseed in different agro-ecological zones.Published information on effect of water use of linseed is also limited. It is therefore important to understand the effect of water use and nitrogen nutritionon growth and yield variation within linseed germplasm in Kenya.This information will contribute towards increased production of linseed and hence increasing its availability in Kenya. This will in turn lead to reduced cost of linseed and its products which in the end will help improve human health through direct consumption or indirectly as an animal feed.

1.4: Objectives

1.4.1: General objective

To contribute to enhanced linseed production in Kenya through evaluation of selected linseed cultivars for growth performance and oil quality in two agro-ecologically different locations, under different nitrogen fertilizer and water application rates.

1.4.2: Specific objectives

1. To assess the genetic variation among13 linseed cultivarsfound in Kenya.
2. To determine the influence of N-fertilization and location on growth performance and yield of five linseed cultivars.
3. To determine the effect of N-fertilization and location on oil content and fatty acid profiles of five linseed cultivars
4. To determine the influence of water stress on growth performance of 3 selected linseed cultivars.

1.5: Null Hypotheses

1. There is no genetic variation among13 linseed germplasm found in Kenya.
2. N-fertilization and location have no influence on the growth performance and yield on the five linseed cultivars.
3. N-fertilizer and location have no effect on oil content and fatty acid profiles of the five linseed cultivars
4. Water stress does not affect the growth performance of 3 selected linseed cultivars

CHAPTER TWO

LITERATURE REVIEW

2.1:Linseed taxonomy and morphology

Linseed (*Linumusitatissimum* L.) is an annual, self-pollinated species with a genome size of about 370 Mb andbelongs to the family Linaceae and genus *Linum* (Soto-Cerda Diederichsen, Ragupathy and Cloutier,2013; GM Inspectorate, 2010; Jacobsz & van der Merwe, 2010). The genus *Linum* L. has about 200 species including *L. usitatissimum* L. (Friedt,Bickert and Schaub,1996; Friis, 2000). The terms flaxseed and linseed have specific meanings, depending on the region. In Europe for example, flax refers to the seed grown for fibre (linen) production and linseed to oilseed flax grown for industrial and dietetics uses. In North America, the terms are used synonymously although there is a slight preference for using the term flaxseed when referring to the flax used for human consumption. Solin is a generic term for flax cultivars that have less than 3 percent alpha-linolenic acid (ALA) (Oomah & Mazza, 1988). Linseed grown for human consumption and that grown for manufacturing linen belong to the same species but are different varieties (BeMiller, 1973).

Linumusitatissimum L. is an erect annual herb which branches corymbosely above the main stem with a taproot that branches to a depth of up to 60 cm, with fibrous branches that may extend 90-120 cm in light soils (Fernald, 1950, van der Vossen *&* Mkamilo,2007, Canadian Food Inspection Agency, 2012). Two types of

Linumusitatissimum L. are cultivated, namely the linseed type and the flax type, the former being cultivated for the oilseed and the latter for fiber (Canadian Food Inspection Agency, 2012). Although these two types belong to the same species, they markedly differ in morphology, physiology, anatomy and performance (Soto-Cerda *et al.*, 2013). The linseed type is shorter, more branched, larger seeded and grown over a wider area than the flax type, which is mainly grown in cool temperate regions of China, the Russian Federation and Western Europe (Green *et al.*, 2008; Soto-Cerda *et al.*, 2013). *Linumusitatissimum*L. stems are slender and erect with almost opposite leaves in lower part of stem and alternate in upper part;leaves are simple, sessile, linear-lanceolate with entire margins, and are borne on stems and branches; the inflorescence is a loose terminal raceme or cyme; flowers are borne on long erect pedicels, are hermaphrodite, hypogynous and are composed of five sepals, five petals (blue), five stamens, and a compound pistil of five carpels each separated by a false septum;the fruit is a capsule, composed of 5 carpels and may contain up to 10 seeds; the seed is oval, lenticular, 4-6 mm long with a smooth, shiny surface, brown to light-brown in colour (Canadian Food Inspection Agency 2012). The plant has a bushy nature (see Plate 2.1) and since a single inflorescence is produced on each branch, it has several branches in order to produce more seed.

Plate 2.1: Linseed growing in Njoro.

2.2: Origin and distribution

The exact location of origin of linseed is uncertain (Lay & Dybing, 1989, Diederichsen and Hammer, 1995), butthe Mediterranean region and Southwest Asia have both been proposed as possible centres of origin, because of the very diverse forms of flax found in the area between the Mediterranean and India(Millam, Bohus & Anna, 2005; Zeven and Zhukovsky, 1975). Helbaek (1959) suggested that cultivated linseed originated in the Near East, with pale flax, *Linumusitatissimum* L. subsp. *angustifolium* (Huds) Thell. as its progenitor, and that it moved along River Danube into Central Europe, where it became a winter oil plant. On the other hand, Vavilov (1950) suggested five centers of

variation of cultivated linseed, namely Central Asia, Middle East, Mediterranean region and Abyssinian (Ethiopian) region, and such widespread variation was interpreted to mean that domestication of linseed may have occurred in several places simultaneously (Harlan, 1969).The initial use of linseed has also been debated (Diederichsen and Hammer, 1995). Based on archeological evidence, it was proposed that linseed was used first for fibre (Dillman, 1936). However, a more recent comparative study of genetic diversity of the stearoyl-ACP desaturase II (*sad2*) locus from linseed and pale linseed (*L. angustifolium*) showed reduced diversity in the cultivated species, suggesting that linseed may have been initially selected as an oilseed crop (Allaby,Peterson, Merriwether & Fu, 2005).

2.3: Uses of Linseed

Almost every part of the flaxseed plant is utilized commercially, either directly or after processing, and its Latin name, *Linumusitatissimum,* means "very useful" (Singh *et al*., 2012).

2.3.1: Industrial uses of linseed

The various industrial uses of linseed have been listed by Chauhan,Singh&Singh (2009) and Singh,Mridula, Rehal& Barnwal(2011). Among these, the high linolenic acid content makes linseed oil an excellent drying agent in products such as paints, resins, inks, soaps, varnishesand wood. The freshly cold processed oil is also used in the

manufacture of printing and lithographic inks, soft soaps, core oil linings and packing, leather finishing compounds, lubricants, greases, polishes and adhesive. Linseed oil is also used as an emulsifying agent. In addition, the fibre type linseed is used in the textile industry.

2.3.2: Use of linseed as animal feed

As reviewed by Singh *et al.,* (2011), linseed finds application in the important area of animal feeds. The deoiled linseed cake is a favorite cattle feed with an excellent taste and high protein content (about 30%) and the protein has high digestibility (85%). Linseed is also becoming popular as a fat source especially because it improves the omega-3 to omega-6 fatty acid ratio in animal products, and this translates to the products being perceived as healthier than animal products with a higher omega 6 to omega 3 ratio. Whole untreated flaxseed can be used as the fat source in the diet of early lactating cows without any adverse effect on milk production, and such milk has a higher protein content besides the improved omega-3 to omega-6 fatty acids ratio (Petit, 2002). Omega-3 rich egg is a popular product, and incorporation of linseed in poultry feeds is the means by which omega-3 enrichment is achieved (Singh *et al.*, 2011). Linseed also has shown potential as a substitute for more expensive marine oils in fish feeds (Singh *et al.*, 2011). Some studies have also reported that incorporation of linseed in animal feeds leads to improved reproductive performance and health (Turner *et al.*, 2014; Shim,Gui, Arnison, Wang & Reaney,2014)

2.3.3: Linseed as a human food and nutraceutical

In recent years, there has been much interest in foods or food components that promote human health due to components other than traditional nutrients such as proteins, carbohydrates, minerals, and vitamins. Such foods or food components are referred to as nutraceuticals. The term functional food refers to nutraceuticals consumed as food and not as supplements.

Linseed is emerging as an important functional food ingredient, mainly because of its high content of alpha linolenic acid, lignans, and fibre (Bernacchia,Preti& Vinci,2014).

Alpha linolenic acid is an essential omega-3 fatty acid which can be converted in the human body to longer chain omega-3 fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) through the action of elongase and desaturase enzymes, with as high as 12% conversion efficiencies reported. Docosahexaenoic is the most abundant polyunsaturated fatty acid in the human brain and retina, and is required for the optimal development of nervous system and maturation of visual acuity (retina) in preterm and term infants (Neuringer & Connor, 1986; Uauy,Perano, P., Hoffman, D., Mena, P., Birch, D.and Birch1996). Through various mechanisms, the omega 3 fatty acids, especially DHA and EPA reduce chronic inflammation in the human body (Chapkin,Kim, Lupton and McMurray,2009). This is in contrast to omega -6 fatty acids such as linoleic acid and arachidonic acid that are converted to pro-inflammatory compounds (Patterson,Wall, Fitzgerald, Ross and Stanton,2012). Chronic inflammation

contributes to the development of major physiological disorders and diseases such as atherosclerosis, cardiovascular diseases, diabetes, Alzheimers disease, and cancers. Therefore the dietary ratio of omega-3 fatty acids (anti-inflammatory) to the omega-6 fatty acids (pro-inflammatory) is important for maintaining good health. Linoleic acid (omega-6) is the predominant polyunsaturated fatty acid in the readily available and affordable vegetable oils in Kenya, such as corn oil, soybean oil, and sunflower oil. Hence, production and consumption of linolenic acid may help in improving the omega-6 to omega-3 fatty acid ratio in favour of the omega-3s.

Both linseed oil and mucilage were found to reduce gastric ulcers in a rat model (Dugani,Auzzi, Naas and Megwez,2008). Linseed is the richest source of dietary lignan precursors, and lignans are important in the prevention of osteoporosis, cardiovascular diseases, and hormone-associated cancers such as breast, endometrium and prostate cancers (Bernnachia, 2014). Because of such benefits, it is not surprising that approximately 200 new food and personal care products were introduced in the US market containing linseed or linseed ingredients in 2005 (Morris, 2007) suggesting that linseed based products have high growth potentials in functional food industry.

2.4:World linseed production

From the centres of origin, the linseed has now spread to all the continents. However, as shown in Table 2.1, the bulk of linseed production is concentrated in just a few countries. Only six countries including Canada, China, Russia, Kazakhstan, India and Ethiopia produced more than 100,000 tonnes in 2013. In the same year, Kenya produced a meagre 1000 tonnes (FAOSTAT, 2013). In tropical Africa, linseed production is concentrated in the Ethiopian highlands, where linseed has been grown since time immemorial. It is the second most important oil crop after niger seed (*Guizotiaabyssinica* (L.f.) Cass.) in the Ethiopian highlands(Singh, Mridula, Rehal, & Barnwal, 2011).

Table 2.1:The world's top linseed producing countries in 2013.

Rank	Country	Production (tons)	Rank	Country	Production
	World	2,305,000			
1	Canada	712,000	21	Bangladesh	6,000
2	China	399,000	22	Uruguay	6,000
3	Russia	326,000	23	Romania	4,000
4	Kazakhstan	295,000	24	Pakistan	3,000
5	India	147,000	25	Afghanistan	3,000
6	Ethiopia	105,000	26	Tunisia	2,000
7	USA	85,000	27	New Zealand	2,000
8	UK	51,000	28	Czech	2,000
9	Ukraine	25,000	29	Poland	2,000
10	Sweden	23,000	30	Italy	2,000
11	Argentina	17,000	31	Netherlands	2,000
12	France	16,000	32	Chile	1,000
13	Brazil	10,000	33	Slovakia	1,000
14	Spain	9,000	34	Peru	1,000
15	Nepal	8,000	35	Kenya	1,000
16	Belgium	7,000	36	Uzbekistan	1,000
17	Belarus	7,000	37	Iran	1,000
18	Australia	7,000	38	Mexico	1,000
19	Egypt	7,000	39	Hungary	1,000
20	Germany	6,000	40	Austria	1,000

Source:http://faostat3.fao.org/home/index.html#DOWNLOAD**Downloaded on 15th October 2015.**

2.5: Linseed production in Kenya

Currently, linseed production in Kenya is barely documented, likely due to the very low level of its production. Riungu (1988) presented the history of linseed/flax in the country, which can be summarized as follows: cultivation of the fibre crop beganin 1920 with 9297 acresunder its production, which increased to 26475 acres in 1922, and

decreased to 14585 acres in 1923. By 1956, production of linseed for fiber had ceased. The reason for the end to flax cultivation for fiber is not given, but is likely due to competition from the cheaper cotton. *Plusia* (*Phytometra*) *orichalcea*, F., was cited as the only important insect pest of the flax, and this affected large farms more than smallholder farms, and it was a more serious problem at altitudes lower than 2000 ft(Megaw, 1939). Between 1922 and 1956,the flax research station at Kabete screened and evaluated the introduced germplasm. Trials were done in Kiambu, Gilgil, Kericho, Njoro, Eldama Ravine, and Nakuru, with Dutch, Russian, Japanese and Irish seeds. Even whenfibre for flax was abandoned in 1956, trials were continued with oil type cultivars at OlJoroOrok, Kitale and Nakuru. From the trials, Kinangop and OlJoroOrok were recommended for linseed production in 1959. However, by 1962, difficulties in harvesting during the wet season and lower yields compared to wheat made linseed unpopular in Kinangop. From 1959, introduction, screening and evaluation of varieties, mainly from Canada and Ethiopia, were conducted at the National Plant Breeding Station in Njoro.By 1976, there were 25 varieties under trial, and results over the years showed that they performed well in both yield and oil content. The oil content ranged between 38% and 40%, and the only diseases observed were *pasmo*, rust and seedling rot. Based on the field performance data, 10 varieties were recommended and released for commercialization. However, by 1988, there was no production, and this was suggested to be possibly due to lack of markets, promotion and reasonable prices (Riungu, 1988). Currently, the on-going work at the National Plant Breeding Station

Njoro (KALRO-Food Crops research Institute) is mainly on bulking and maintenance of available germplasm and released varieties.

2.6: Seedling establishment

The recommended plant population for linseed varies between 350-500 plants/m^2 (Freer, 1993; Flax Council of Canada, 1996). The general seedling establishment is slow and seedlings have poor competitive ability. Germination rate is usually 93-98% (Freer, 1993) but seedling emergence rates of 50-60 % are obtained under field conditions (Flax Council of Canada, 1996). Seedlings emergence ranged from 5 days (Lisson and Mendham, 2000) to 7 - 21 DAS (Hocking & Pinkerton, 1991). Germination and seedling emergence may be influenced by seed colour (Culbertson & Kommendahl, 1956), temperature (O'Connor & Gusta, 1994; Saeide & Rowland, 1999), seed sowing depth (O'Connor and Gusta, 1994; Couture,Di Tommaso, Asbil and Watson, 2004) and available soil moisture (Casa,Russell, Cascioand Rossini, 1999). O'Connor & Gusta (1994) showed that seed sown 4 cm deep took 33 % longer to emerge than seed sown at 2 cm. Poor emergence gave uneven crop development and asynchronous ripening (Freer, 1993; D' Antuono & Rossini, 1995). Linseed plants are vulnerable to drought during the establishment phase (Casa *et al.,* 1999*).*

2.7: Growth and development

The Flax Council of Canada (1996) has described the life cycle of the linseed plant as consisting of a 45- to 60-day vegetative period, a 15- to 25-day flowering period,and a maturation period of 30 to 40 days. Although there is a period of intense flowering, a small number of flowers may continue to appear right up to maturity. Maturity is delayed under cool, wet conditions. The crop lifecycle from seeding to maturity is usually 90 to 125 days, depending on overall environmental conditions. Drought, high temperature and disease can shorten the growth period and crop lifecycle. If ripening occurs under high soil moisture and fertility conditions, stems may remain green and new growth may result in a second period of intense flowering.

There are 12 documented distinct growth stages (GS) in the development of a linseed plantaccording to the Flax Council of Canada(1996).After germination, the cotyledons (two small seed leaves) emerge and the young seedling proceeds to first true leaf formation.Shortly after, a second pair of leaves unfold before the third and more pairs of true leaves and stem extension. The linseed plant has one main stem, but two or more branches (tillers) may develop from the base/near the base of the plant when plant density is low and/or high soil nitrogen levels. The basal branching is also prominent if the main stem of a young seedling is damaged due to loss of apical dominance. The main stem continues to extend and buds form at the top of the plant. Early branching of the main stem becomes obvious towards the top of the plant at about 30 cm up when the

first flower begins to open. The main stem and branches give rise to a multi-branched, irregular arrangement of flowers. The linseed plant has a short, branched taproot which may extend to a depth of more than 1 m with side branches.

New flowers open early each morning and petals are usually shed by mid-day. Colour of flower petalsmay be used to distinguishlinseed varieties, and ranges from white, light blue, blue, dark blue, pink, violet, to red-violet (Tammes, 1928; Dillman, 1936). The mostfrequent color is blue, followed by white.Flowering typically lasts from 15 to 25 days though a small amount of flowering may continue on late formed branches throughout the season under wet and fertile conditions.The ovary is the seed house known as a boll or capsule, which contains the developing seeds. Ripening of the boll begins 20 to 25 days after flowering. With complete seed set, the boll contains ten seeds, though an average of six to eight seeds per boll is usual. At physiological maturity, ripe seeds will rattle within the boll or capsule. Flax seeds are flat, oval and are pointed at one end (Flax council of Canada, 1996).

Plate 2.2:A capsule of Summit growing in a greenhouse at JKUAT

Plate2.3: Seeds of Summit harvested from JKUAT field

2.8: Effect of nitrogen nutrition on linseed growth and performance

Nitrogen is the most important nutrient in plant nutrition (Hofman & Cleemput, 2004) with linseed seed yield being highly related to nitrogen nutrition.The type and amount of fertilizer applied is among the most important agronomic factors in crop production (Perscom). The nitrogen nutrition status of plants is one of more important nutritional factors that determine the level of yield components (Dordas, 2010). However according to Grant,Dribnenki & Bailey (1999), too high rates of mineral fertilizers and particularly nitrogenous fertilizers can cause excessive branching of plants; which in turn promotes their lodging and hence reduction in yield. Indra (2005) reported significant increase in plant height, tillers per m^2, leaf numbers and weight, leaf area index, dry matter accumulation, grains/ear, grain weight/ear and 1000 grain weight of hybrid rice variety 'Proago 6207'. However, there have been inconsistent reports on findings regarding the effect of fertilizers (especially nitrogen) on linseed. For example, Hocking,Randalland DeMarco(1997) reported a non-significant effect of increasing nitrogen rates on seed yield, while Strašil & Vorlíček (2004) found the application of 60 kg N/ha to increase seed yield of linseed by 5.8% compared to the treatment without nitrogen fertilization. A study by Andruszczak,Gawlik-Dziki, Kraska, Kwiecińska-Poppe, Różyło and Pałys(2015) found that, regardless of cultivar and row spacing, linseed fertilized with nitrogen at rates of 60 and 80 kgN/ha, compared to a rate of 40 kgN/ha had a significantly higher plant density per unit area as well as better productivity per plant and per crop.Fertilizer application also affects the oil content and

fatty acid profile of linseed. The mineral fertilization up to 80 kgN/hasignificantly increased the content of α-linolenic acid in seeds of cvs. Szafir and Oliwin but the levelof oleic acid significantly decreased (Andruszczak*et al*., 2015). Increasing the level of mineral fertilizationof two cultivars of linseed led to an increase in the content of palmitic and oleic acids, whereas the proportion of α-linolenic acid decreased (Klimek-Kopyra,Zając and Rębilas, 2013).Tanwar,Zhang& Teixeira (2011) reporteda significant increase in seed yield with increasing NPK levels.However, they observed highest oil content with lowestdose of NPK level while progressive increase in oil yieldwas obtained with successive increment of NPK up to thehighest level, but increment beyond 90-45-45 kg NPK/hawas not significant. The reduction in oil content at highernitrogen levels is hypothesized to be due to conversion of morecarbohydrate into protein and therefore the amount of synthesizedcarbohydrates left for conversion into fats are relatively lowas compared to other nitrogen treated plants. Similar results have been reported by Singh,Pant, Korres, Nizami, Prasad& Murphy(2010).

2.9: Effect of water stress on linseed growth and performance

Water stress is considered one of the mostimportant factors limiting crop performance and crop yield in the world(Dutta,Ram Mohan Rao & Singh,1995). Kenya is one of the most water-scarce countries in Africa (Kandji, 2000; WRI, Kenya Ministry of Envinment and Natural Resources, Kenya Ministry of Planning and National Development, and International Livestock Research Institute,2007; Mango,Melesse,

McClain, Gann and Setegn, 2010). As a result of climate change, the long wet seasons have currently become unreliable and significantly reduced, with these trends being accompanied by extreme events, such as drought (Climate Change Profile Kenya, 2015).

Flax is adapted to many environments mainly in temperateclimates (Casa *et al*., 1999; Adugna and Labuschagne, 2003).Linseed is a shallow rooted crop that requires sufficient moisture during the growing season (Wood, 1997), and water stress would limitgrowth by hastening physiological maturity according to Couture *et al.,*(2002). Several reports have claimed that linseed plantsare susceptible to drought at flowering (Foster,Poone and Mackay,1998) andduring early seed development stages (Martin,Leonardand Stamp,1976; Green *et al.,* 1994; Flax Council ofCanada, 1996). Plants have adopted certain mechanisms to respond to water stresses.The impact of water stress on root cell development would likely impair nutrientuptake as well as cause detrimental effects on photosynthesis, essential for biomass accumulation and therefore on shoot and root elongation. Under water stressphotoinhibition occursdue to damage to the reaction center of photosystem II. The electron transport activity and the photosynthetic apparatus of linseed seedlings withcertain drought-resistance are damaged. Carbohydrates are the main osmotic adjustment substances and so areimportant indicators of drought tolerance (Rui, Hao & Gong,2012). Their increase inshoots of linseed seedlings with increasing water stress indicates that theymay help to regulate and maintainthe activity of physiological processes within the plant in a

highwater-stress environment by raising the osmoticpotential of the cells (Rui *et al*., 2012). Limited water conditions decrease uptake and translocation of nutrients (Shabbir, Ashraf, Waraich, Ahmad and Shahbaz, 2015).

Seedyield and seed yield components, plant height, time to reachharvestmaturity, oil content, and oil composition also depend on the temperature during plant development (Casa *et al*., 1999; Adugna & Labuschagne, 2003; Dybing, Evenson & Lay,1988). Experiments carried out in controlled conditions showedthat high temperatures (>30^{o}C) during the ripening phasereduce the number of seeds per capsule and the seed weightand decrease oil yield and quality (Dybing & Zimmerman, 1965).

CHAPTER THREE

EVALUATIONOF GENETIC VARIATION AMONG LINSEED (*Linumusitatissimum*L.)CULTIVARS IN KENYA USING SSR MARKERS

Abstract

Fingerprinting with molecular markers allows precise, objective and rapid varietal identification. It has been proven to be an efficient tool for crop germplasm characterization, collection and management. Microsatellites also called Simple Sequence Repeat (SSR) markers are most suitable for many applications because of the ease in handling, reproducibility, co-dominant inheritance and genome-wide coverage. Thirteen linseed cultivars (Jawhar, CI-1652, Norlea, S19/31, S19/12, Summit, CI-1525, Raja, S19/21, S/10/03, Concurrent, S25/61 and 7331 were characterized using 28 SSR markers. DNA extraction followed a modified CTAB protocol according to Allen *et al.,* (2006). PCR reactions were carried out in a Thermal Cycler (Applied Biosystems 2720, USA) and PCR products separated using 3% agarose gel electrophoresis stained with 1.5µl ethidium bromide at 100V for 30mins thenvisualized under UV light using UVP PhotDoc- It imaging system. Band profiles were scored only for distinct reproducible bands as present (1) or absent (0) for each SSR primer pair. Dissimilarity analysis and representation for windows (DARwin 6.0.0) software was used to construct a dendogramto cluster the genotypes. Two of the 28SSR markers (LU4 and LU5) did not show any amplification with any linseed cultivars tested. The rest showed amplifications with at least one of the tested samples forming one, two or even three

amplification bands. The sizes of the amplification products varied but their sizes were between 50 and 200bp. The tested linseed cultivars fell into three main clusters with most of the cultivars being in the second cluster while one genotype, S25/61 fell into its own cluster. Three Principal Components (PC) accounted for most of the variation with PC1 accounting for 49.6% of the total variation, PC2 11.23% and PC3 9.13% respectively. The total variation accounted for by the three was 69.97%. A total of 46 alleles were detectedat 26 loci using the microsatellite primer pairs. The number of alleles per locus ranged from 1 to 3, with an average of 1.769 alleles perlocus. The average polymorphic informationcontent (PIC) for theSSR markers was estimated to be 0.2033; LU9 and LU11 had values greater than 0.5 and are therefore recommended for use in diversity studies.

3.1: Introduction

The importance of genetic diversity in crop improvement has been recognized by breeders but the basic difficulty has been to know and to evaluate exact diversity and to identify genetically diverse genotypes for their use in hybridization programmes (Fulkar,Ghorpade, Maheshwari, Patil, Reddy& Chebrolu, 2007).

High-quality seeds and elite varieties play a crucial role in crop production. However, new cultivars normally arise from hybridizations between members of an elite group of genetically similar parentsand the amount of genetic variability among newly developed cultivars is likely to become even smaller (Rahman,Molla, Alam& Rahman, 2009). This

makes it more difficult to unambiguously distinguish cultivars from the others basedon morphological characteristics and isozyme electrophoresis patterns because of influences by environmental factors. On the other hand, fingerprinting with molecular markers allows precise, objective and rapid varietal identification, and it is an efficient tool for crop germplasm characterization, collection and management.

Among the molecular markers, microsatellites also called Simple Sequence Repeat (SSR) markers are most suitable for many applications because of their ease of handling, reproducibility, co-dominant inheritance and genome-wide coverage (McCouch *et al*., 1997).They are tandem repeats, of one to six nucleotides long DNA motifs, present in eukaryotic genomes.They are a class of repetitive DNAsequences usually 2.6 bp that are distributed throughout whole genome and are flanked by highly conserved region (Chambers & Avoy, 2000). They have been widely used for genetic analysis and varietal identification because they are abundant, co-dominantly inherent, highly polymorphic, reproducible, and easy to assay by PCR (Kuleung,Baenziger&Dweikat2004; Xie,Zhou, Wang, Zhang, Chen& Gao, 2011).These are valued in molecular breeding due to their desirable genetic attributes like hypervariability, wide genomic distribution, co-dominant inheritance, reproducibility, multi-allelic nature and chromosome specific location. These markers are suitable for paternity determination, mapping of useful genes, marker-assisted selection and for establishing evolutionary relationships (Parker,Fox, Langridge, Chalmers, Whan& Ganter,2002; Aitken Jacksonand McIntyre,2005).

Assessment of genetic variability is the first step in any crop improvement programme. Diversity analysis is an essential process for clear and sound identification of the genetic relatedness of the available genetic resources. It is also required for effective choice of parents for subsequent crossing and selection of the progenies.

There are different ways to evaluate linseed genetic variation, such as the use of morphological characteristics (Adugna, Viljoen, & Labuschagne, 2005;Adugna, Labuschagne & Viljoen 2006). Morphological characters tend to be more quantitative and are influenced by environmental conditions (Cloutier *et al*., 2009). Molecular characterization of linseed cultivars is useful in evaluating the diversity of Kenya's linseed germplasm. A better understanding of the genetic basis of phenotypic variability will improve the efficiency of linseed improvement for nitrogen and water-stress tolerance. This study therefore focused on characterizing13linseed cultivarsusing28 SSR markers.

3.2: Materials and Methods

3.2.1: Raising of seedlings for DNA extraction

Seeds of 13 linseed cultivars ((Jawhar, CI-1652, Norlea, S19/31, S19/12, Summit, CI-1525, Raja, S19/21, S/10/03, Concurrent, S25/61 and 7331) were obtained from KALRO-Njoro and used in this study.

Three seeds of each of the 13 linseed cultivars were sowed in pots in a greenhouse in JKUAT, Juja in June 2014. The seeds germinated within five to seven days after sowing. Two weeks after germination,the third leaf of each seedling and for each cultivar was harvested, put in well labelled polythene bags insertedinto an ice pack and quickly taken to the laboratory to preserve the leaf samplesand ensure that the quality of DNA to be extracted will be high.

3.2.2: DNA extraction

DNA extraction followed a modified cetyltrimethylammonium bromide (CTAB) protocol according to Allen *et al.,* (2006). About 0.4g of fresh leaves of each cultivar were weighed and ground in 3ml of CTAB extraction buffer using a pestle and mortar. The slurry was then transferred to 1.5ml microfuge tubes for incubation in a water bath at 65^{o}C for 30 minutes. This was followed by centrifugation at 13000 revolutions per minute (rpm) for 10 minutes. Approximately 750 µl of the supernatant were added to 750µl of chloroform:isoamylalcohol (CIA) (24:1), vortexed, andthen centrifuged for ten minutes at 13000rpm. Six hundred microlitres of the aqueous phase was then mixed with an equal volume of CIA and the process repeated twice. Finally the aqueous upper phase was drawn, mixed with an equal amount of ice-cold isopropanol and the mixture centrifuged at 13000rpm for ten minutes before carefully decanting the supernatant to leave the DNA pellet at the bottom of the tube. The pellet was washed twice with 500µl of 70% ethanol by centrifuging at 13000rpm for 5 minutes.

3.2.3: Quantification of DNA

A spectrophotometer was used in quantifying the amount of DNA present in the sample. A DNA sample was diluted 50X. Readings were taken at wavelengths of 260 nm and 280 nm. As an indication of nucleic acid purity, the OD_{260}/OD_{280} ratio was calculated. To determine the concentration of DNA in the original sample, the following calculation was performed:

DNA concentration = 50 µg/mL x OD_{260} x dilution factor

3.2.4: Storage of DNA

After air drying the DNA pellet for 20 minutes, it was re-suspended in 50µl de-ionized water and stored at -20^{o}C for later use.

Table 3.1:Names and characteristics of microsatellite markers used in this study

S.No	Primers	Sequence 5'--> 3'	Linkage Group	Ta (^{0}C)	Size(bp)
1	LU 1	F:TCATTCATCTCCTTCCACTAAAA R:TTGAAAGCCCTAGTAGACACCA	LG1*	58	146-179
2	LU 2	F:TCCGGACCCTTTCAATATCA R:AACTACCGCCGGTGATGA	***	58	125-150
3	LU 3	F: GCTCGTGATCTCCTTCATCC R: AAAACCACGTCCAGATGCTC	LG3*	58	153-173
4	LU 6	F:CCCCATTTCTACCATCTCCTT R:CAACAGCGGAACTGATGAAA	LG6*	58	125-140
5	LU 7	F:CATCCAACAAAGGGTGGTG R:GGAACAAAGGGTAGCCATGA	LG7*	58	134-154
6	LU 8	F:TCCCGTAATATTCTATGTTCTTCC R:TGAGTTGGACCTTACAAGACTCA	LG8*	58	181-221
7	LU 9	F:TTGCGTGATTATCTGCTTCG R:ATGGCAGGTTCTGCTGTTTC	***	58	123-175
8	LU 10	F:GCCTAAAGCTGATGCGTTTC R:TGTCAGGCTCCTTCTTTTGC	***	58	141-148
9	LU 11	F:ATGGCAGGTTCTGCTGTTTC R:TTGCGTGATTATCTGCTTCG	LG11*	58	100-150
10	LU 13	F: AAGATGACGTCGGTGGTGAT R: CGGAACCTTCCATTTTCCTC	LG3**	58	95-100
11	LU 14	F: GCTTGCGAGAAGAAGGAGAA R: TCACCAAAGGCATTCACAAA	LG17**	58	141-150
12	LU 15	F:TGGACGACGATGAAGATGAA R:CCGCCGGGTACACTACTACT	***	58	114-120
13	LU 16	F: TTATTCTTGCCTGCCAATCG R: TCCAGCTCTTGCTCGTTCTT	***	58	153-167
14	LU 18	F:AGAGGCGGAGGGCATTAC R:TTGGAGAGTTGGAATCGAGA	***	58	140-150
15	LU 20	F:TTCAACCAGGCAAATTTCAA R:CAAGAAGAGGCCCAGAATTG	***	58	122-125
16	LU 21	F:AAGGGTGGTGGTGGGAAC R:GTTGGGGTGAAGAGGAACAA	***	58	135-150
17	LU 22	F:GATGGGGTTGAAGCCAGTAG R:CCCACCCCATCTATCATTTG	***	58	132-148
18	LU 24	F:ATGGCAGGTTCTGCTGTTTC R:TTGCGTGATTATCTGCTTCG	***	58	95-138
19	LU 25	F:TCTACAGAGTTCAATTCCCGTAA R:GTTGGACCTTACAAGACTCACTG	***	58	200-250
20	LU 27	F:GTTTGAGAAGAGGGCATCCA R:GTTGGGGTGAAGAGGAACAA	***	58	152-173
21	LU 29	F:GGGCAGTGATTGATTGGTTT R:GGCGGCAATTGCTACATT	***	58	102-115
22	LU 31	F:TCTTTGTTTGGTGCCAAAGTT R:TTCATGATCTCACCTAACCTGA	***	58	102-117
23	LU 32	F:ACGCGTAAACTTTCCGTTTC R: ATAATGTCGGCTGCTTCTGC	***	58	120-125
24	LU 33	F:TTCTCCATCATCTCACATCCA R: CCAAATCAGAATGTGCGTGT	***	58	149-155
25	LU 34	F:GGAAGAATTGGAAGAGGAAGG R:CCTTCTCCCATGATCAAACAA	***	58	110-130
26	LU 35	F: CCAACGGATCATCCTCTAGC R: GGACAGAAAGGGGAAAGGAA	***	58	143-149

Ta: annealing temperature; * According to Santosh *et al.,* (2015); ** According to Cloutier *et al*., (2011); *** Linkage group not known.

3.2.5: Polymerase chain reaction (PCR) for SSR markers

Three microlitres of genomic DNA was used as a template for SSR amplification for each sample in a total volume of 12.5µL per reaction. Each reaction had 2.5µL of 5*PCR buffer (supplied with the lit; Bioline), 0.25µL forward primer, 0.25µL reverse primer, 0.15µL Taq polymerase and 6.35µL double distilled sterile water. PCRs were performed in a Thermal Cycler (Applied Biosystems 2720, USA)with an initial denaturation at 95 °C for 3 min, followed by 32 cycles of 95 °C for 30 s, annealing at 58 °C for 30 s, extension at 72 °C for 30 s followed by a final extension of 72 °C for 3 min.

The amplified PCR products were separated using 3% agarose gel electrophoresis stained with 1.5µl ethidium bromide at 100V for 30mins and visualized under UV light using UVP PhotDoc- It imaging system. Tris-Borate EDTA (TBE) buffer was used to prepare and run the gel.

3.2.6: Data analysis

The band profiles were scored only for distinct reproducible bands as present (1) or absent (0) for each SSR primer pair and this data used for the subsequent analysis. Principal component analysis and biplot display were done using XLSTAT 2014.5.03. To calculate PIC values, the data was transposed into co-dominant type then analysed using CERVUS 3.0.7. Dissimilarity analysis and representation for windows (DARwin 6.0.0) software was used to construct a dendogramto cluster the genotypes.

3.3: Results

Among the 28 SSR markers used, two of them (LU4 and LU5) did not show any amplification with any linseed cultivar tested. Six of the SSR markers were monomorphic and showed only one band in all the cultivars. The rest of the SSR markers showed amplifications with at least one of the tested samples forming two or even three amplification bands. The sizes of the amplification products varied but their sizes were between 50 and 200bp.

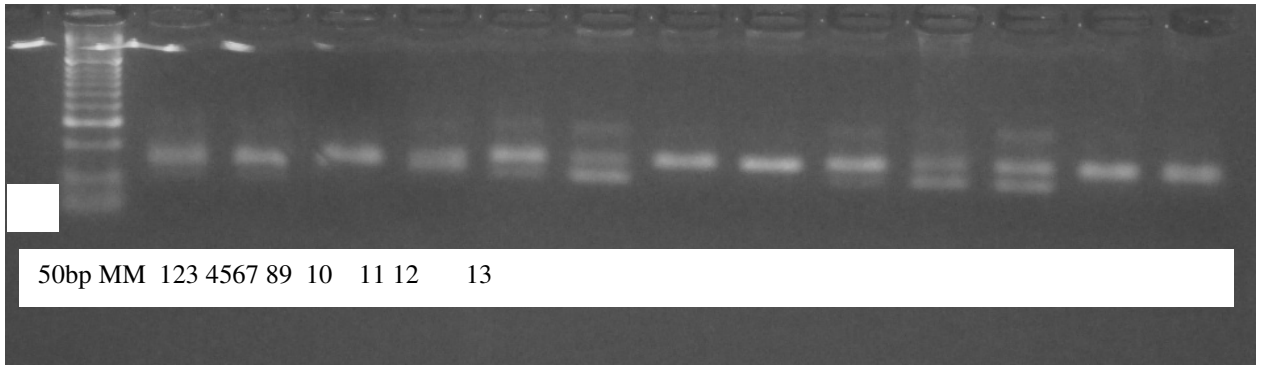


Figure 3.1: **Molecular profiles of 13 linseed cultivars obtained with microsatellite marker LU9. The numbers of lanes 2 to 14 correspond to the linseed cultivars: Lane 1- 50 bp DNA ladder, lane 2- Jawhar, lane 3- C1-1652, lane 4- Norlea, lane 5- S19/31, lane 6- S19/12, lane 7- Summit, lane 8- C1-1525, lane 9- Raja, lane 10- S19/21, lane 11- S/10/03, lane 12- Concurrent, lane 13- S25/61 and lane 14- 7331.**

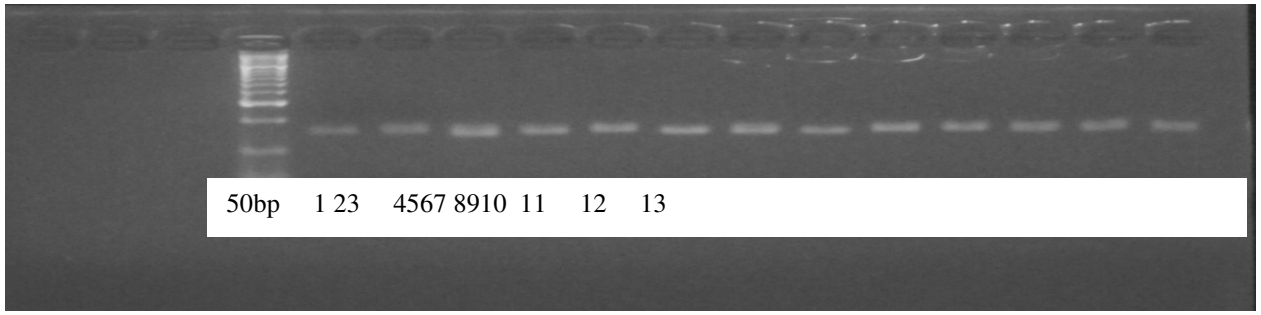


Figure 3.2: **Molecular profiles of 13 linseed cultivars obtained with microsatellite marker LU11. The numbers of lanes 2 to 14 correspond to the linseed cultivars: Lane 1- 50 bp DNA ladder, lane 2- Jawhar, lane 3- C1-1652, lane 4- Norlea, lane 5- S19/31, lane 6- S19/12, lane 7- Summit, lane 8- C1-1525, lane 9- Raja, lane 10- S19/21, lane 11- S/10/03, lane 12- Concurrent, lane 13- S25/61 and lane 14- 7331.**

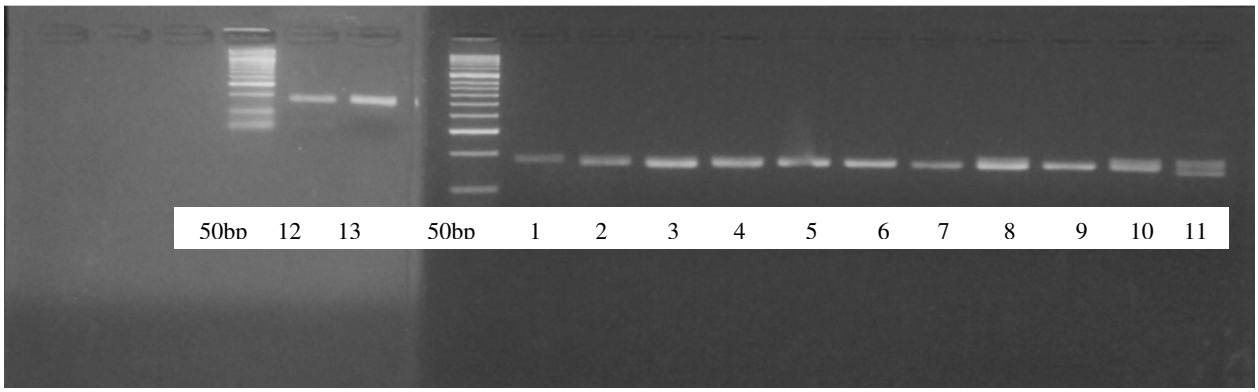
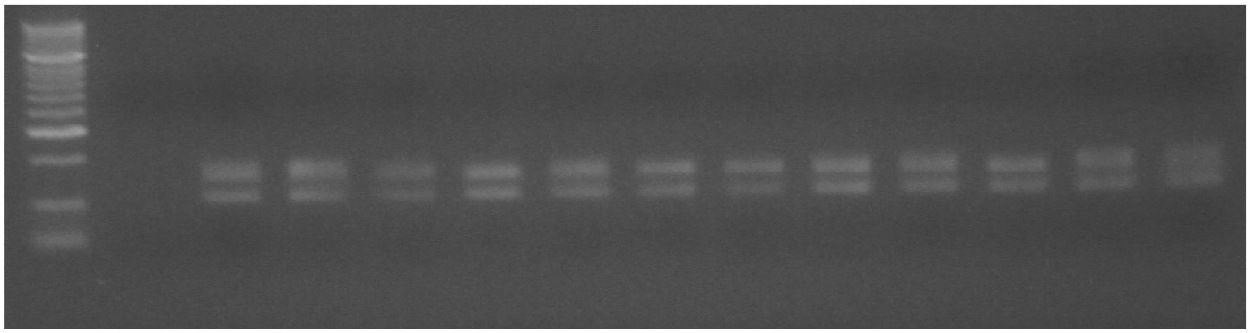


Figure 3.3: **Molecular profiles of 13 linseed cultivars obtained with microsatellite marker LU27. The numbers of lanes 2 to 14 correspond to the linseed cultivars: Lane 1- 50 bp DNA ladder, lane 2- Jawhar, lane 3- C1-1652, lane 4- Norlea, lane 5- S19/31, lane 6- S19/12, lane 7- Summit, lane 8- C1-1525, lane 9- Raja, lane 10- S19/21, lane 11- S/10/03, lane 12- Concurrent, lane 13- S25/61 and lane 14- 7331.**



50bp MM 123 456723 4567 8 9 10 11 12 13

Figure 3.4: **Molecular profiles of 13 linseed cultivars obtained with microsatellite marker LU21 The numbers of lanes 2 to 14 correspond to the linseed cultivars: Lane 1- 50 bp DNA ladder, lane 2- Jawhar, lane 3- C1-1652, lane 4- Norlea, lane 5- S19/31, lane 6- S19/12, lane 7- Summit, lane 8- C1-1525, lane 9- Raja, lane 10- S19/21, lane 11- S/10/03, lane 12- Concurrent, lane 13- S25/61 and lane 14- 7331.**

3.3.1: Allelic frequency and PIC

The sixteen SSR markers generated a total of 46 alleles which were used to estimate genetic diversity among the thirteen linseed cultivars. The number of alleles revealed by each marker ranged from 2 (12 markers) to 3 (4 markers). The frequency of the major allele ranged from 26 (LU29-LU35) to 6 (LU9). The PIC value for the SSR markers ranged from 0.1319 (LU27 and LU32) to 0.5361 (LU11) with a mean of 0.2033. Two markers (LU9 and LU11) revealed PIC values of more than 0.50(Table 3.2).

Table 3.2:Major allele frequency, number of alleles identified and polymorphic information content of 28SSR markers in this study.

Marker	Major allele (bp)	Total number alleles	PIC
LU1	100	2	0.3680
LU2	125	2	0.1411
LU6	125	2	0.3353
LU7	125	2	0.1319
LU8	150	3	0.4918
LU9	150	3	0.5186
LU10	50	2	0.3318
LU11	150	3	0.5361
LU15	100	2	0.3613
LU16	125	2	0.3735
LU20	100	2	0.3613
LU21	150	3	0.4042
LU24	100	2	0.3750
LU25	150	2	0.2920
LU27	150	2	0.1319
LU32	50	2	0.1319
Mean		**1.769 alleles/loci**	**0.2033**

3.3.2: Cluster analysis

Sixteen out of the 26SSR markers were polymorphic. The thirteen linseed cultivars were clustered into three main clusters. The first cluster had only one cultivar S25/61.Seven cultivars were grouped into the second cluster while cluster three had five

cultivars. All tested cultivars were distinctly different and none resembled the other completely.

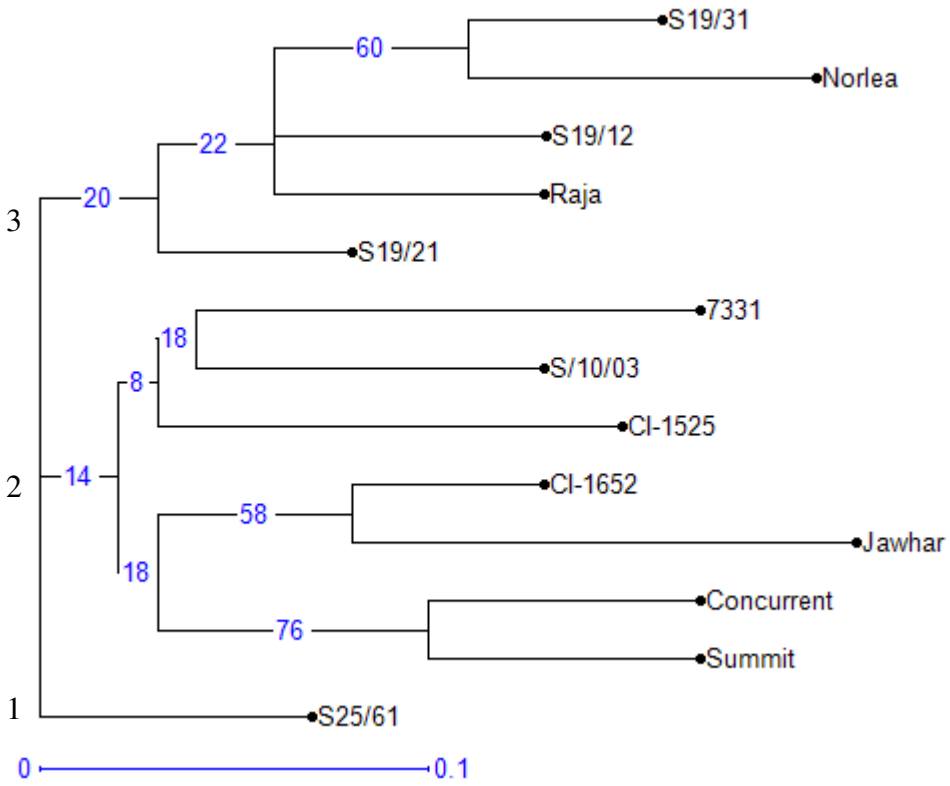


Figure 3.5: Dendrogram showing genetic relationship among thirteen linseed genotypes based on 28 SSR markers

3.3.3: Principal component analysis

The first three principal components (PC 1, PC 2 and PC 3) accounted for 69.97 % of the total variation (Table 3.3). Eigenvalues greater than one is considered significant. The Eigenvectors decreased significantly from principal component 1 from 49.62% to

11.23% to 9.13 for principal components 2 and 3 respectively. This means that after principal component 3, the rest of the principal components did not describe much variation. Thus, only the first three eigenvalues were considered. The first PC with a value of 49.62 % revealed the most variation among the genotypes, showing a high degree of correlation among the markers used in the study. All in all, the PCA analysis performed under this study shows that SSR markers are a useful tool in segregating linseed genotypes.

Table 3.3: Eigenvectors, percentage variation, eigenvalues and cumulative variance of thirteen linseed genotypes.

Eigenvectors			
Genotype	**PC1**	**PC2**	**PC3**
Jawhar	0.181	-0.491	0.486
CI-1652	0.281	-0.209	0.358
Norlea	0.218	0.446	0.392
S19/31	0.255	0.223	0.495
S19/12	0.291	0.328	-0.071
Summit	0.262	-0.284	-0.140
CI-1525	0.302	-0.136	-0.196
Raja	0.300	0.281	-0.125
S19/21	0.324	0.190	-0.180
S/10/03	0.305	-0.091	-0.199
Concurrent	0.277	-0.361	-0.069
S25/61	0.324	0.040	-0.142
7331	0.249	-0.069	-0.248
Eigenvalue	**6.450**	**1.460**	**1.186**
% Variation	49.617	11.230	9.127
% Cumulative variance	49.617	60.847	69.974

3.3.4: Biplot display

The genotypic diversity among the linseed genotypes in this study was also shown ina PCA biplot (Figure 3.6). The display pattern indicated that linseed cultivars were spread across all the four quadrants of the biplot. S19/12, Raja, S19/21 and S25/61 were found on the positive left quadrant. This means that they have a close genetic relationship amongst themselves. Three other linseed genotypes Norlea, S19/31 and CI-1652 clustered together in the lower left quadrant. The clustering too shows a close relationship genetically. In the upper right quadrant clustered 7331, S/10/03 and CI-1525 while Summit, Concurrent and Jawhar clustered in the lower right quadrant. Those genotypes that clustered in one quadrant have closer relationship compared to those that clustered in different quadrants.

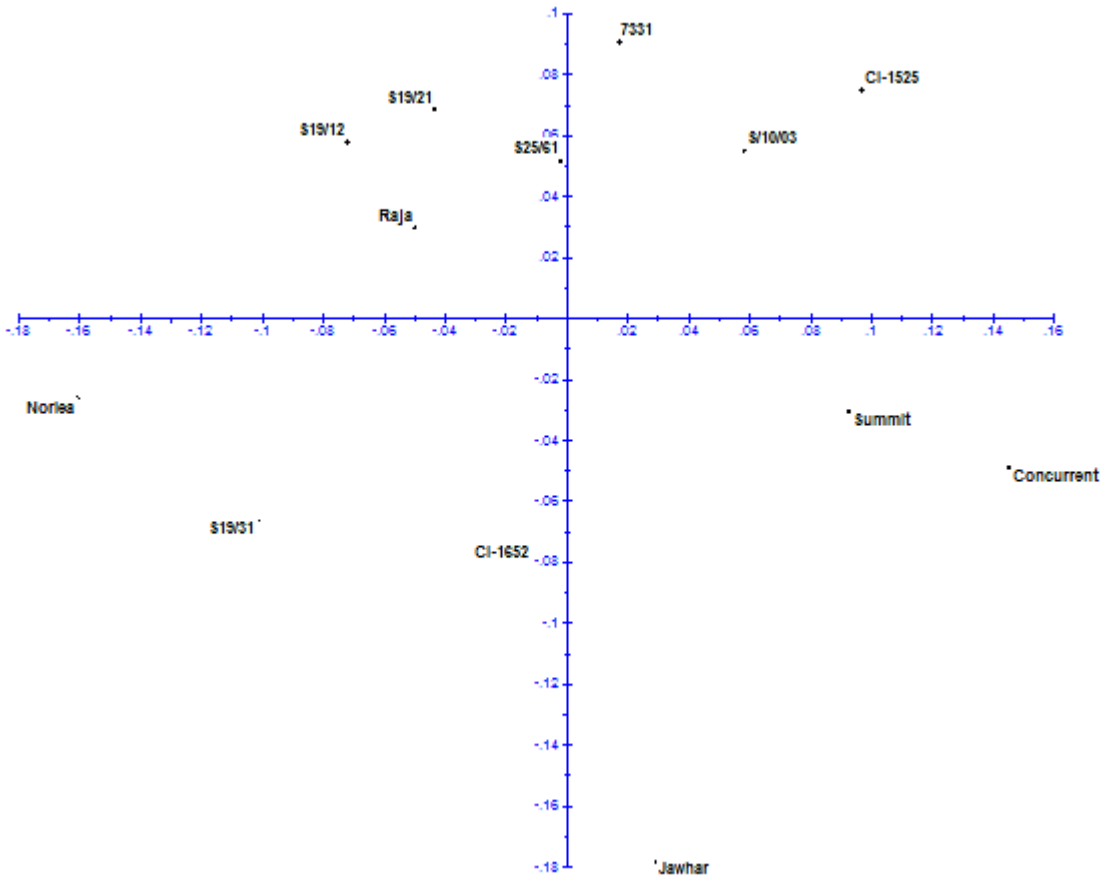


Figure 3.6: Principal component biplot accounting for genetic variation in 13 linseed genotypes using 28 SSR markers

3.4: Discussion

Genetic diversity assessment has potential uses in evolution, breeding and conservation of genetic resources (Wu,Fiser, Kuile,Sali & Muller, 1999). Genetic diversity

studiesoflinseed based on molecular markers is a crucial measure for unambiguous and quick identification of similar or closely-related cultivars.

The close/distant association between certain genotypes could significantly dictate the kind and type of genetic crosses. Geneticists could exploit this information by crossing genotypes with high values since this signifies distant relationship.

Earlier researchers have reported that SSRs show veryhigh level of polymorphism in plants (Powell,Maachray& Proven, 1996). In the present study, 13 linseed genotypes representing a sample of linseed banked at the KALRO-Food Crops Research Institute were surveyed using 28 SSRmarkers. These results prove that data generated from a set of 28 SSR markers were highly informative and the 13 linseed cultivars were successfully distinguished. The averagenumber of alleles per SSR marker in this study was 1.769,lower than the 2.1 alleles per primer pair obtained fromSSR marker analysis of 4 linseed cultivars under cultivation in Chhattisgarh state of India (Vikas,Verma, Xalxo, Saxena, Mehta and Verulkar, 2014). Thisrelatively small number of detected alleles is most likely due to the polymorphism of SSR markers used in this study, the diversity of tested linseed germplasmand the sensitivity of DNA fragmentseparation systems. However, since the SSR markersselected were evenly distributed along the linseed genome,then the genetic relationships revealed by this study are representativeand real. The association between numberof alleles per locus and PIC

means that either of the two estimators isuseful for determining the value of a marker for use in diversitystudies.

The abundance ofalleles at SSR loci reflects the overallgenetic diversity inside a population. This will influence thegenetic distance between the study population from other populations, based on dissimilaritymatrices. Primers that had three alleles showed a higher PIC value than those with 2 meaning that these were better placed to tell the differences even between the more closely related linseed genotypes. As such, LU8, LU9, LU11 and LU21 would be best for revealing the diversity between the studied linseed cultivars.

Cultivar relationships as revealed by UPGMA clustering (Figure 3.6) is a probable reflectionof the tendency of cultivars toassociate with geographic location of origin.For instance, Riungu (1988) documented that earlier introductions of linseed to Kenya were of Dutch, Russian, Japanese and Irish origin. S19/12, Raja, S19/21 and S25/61 which fell on the positive left quadrant supposedly have a close genetic relationship amongst themselves, which could indicate that they probably were introduced to Kenya from a similar geographical location; or were probably adapted to similar growth conditions. Norlea, S19/31 and CI-1652 clustered together in the lower left quadrantwhile 7331, S/10/03 and CI-1525 clustered in the upper right quadrant while Summit, Concurrent and Jawhar clustered in the lower right quadrant. Those genotypes that clustered in one

quadrant have closer relationship compared to those that clustered in different quadrants and are therefore likely to express similar traits.

It is common knowledge that molecular diversity could beused to select parents for hybridization. Further, geneticdistance is useful for predicting yield potential and vigour of intra-subspecific hybrids (Xiao,Li, Yuan, McCouch& Tanksley, 1996), and a positive correlationhas been found between genetic distances determined bymolecular markers and heterosis in other crops(Yu,Hu, Zhao&Guo, 2005; Riaz,Li, Quresh, Swati and Quiros, 2001; Diers,McVetty& Osborn, 1996; Ali,Copeland, Elias& Kelley,1995). The same could be true for linseed. Results from this study have indicated high genetic distance between the 1st and the 3rdclusters. Cultivars in these clusters could represent potential candidates for use in a breeding programme.

3.5: Conclusions and Recommendations

1. Sixteen SSR markers were found to be polymorphic among the thirteen linseed cultivars while 10 were monomorphic. The 16 polymorphic markers are good candidates for use in linseed diversity studies.
2. The sixteen SSR markers generated a total of 46 alleles, with the number of alleles revealed by each marker ranging from 2-3. Two SSR markers (LU9 and LU11) revealed PIC values of more than 0.50 and could be recommended for future studies on linseed diversity.

3. The thirteen linseed cultivars fell into three major clusters with one of the clusters having only one cultivar. The second cluster was the largest having seven cultivars.

CHAPTER FOUR

THE GROWTH AND SEED YIELD OF FIVE LINSEED (Linum usitatissimumL.) CULTIVARS AS INFLUENCED BY NITROGEN FERTILIZATION IN TWO AGRO-ECOLOGICAL LOCATIONS

Abstract

Linseed is an excellent source of the health-promoting Omega-3 fatty acid, alpha linolenic acid. Currently, there is negligible production of thiscropin Kenya. This study aimed to determine the effect of different levels of nitrogen fertilizer on growth and yield of five linseedcultivarsin Juja and Njoro, Kenya.Five linseed cultivars namely Summit, S19/21, Raja, Jawhar andS19/12 were evaluated for two seasons namely, February-June and July-December 2012 at 0, 75 and 150 kgN/ha fertilizer levels. Data were recorded on number of leaves, plant height, number of tillers, dry weight, number of heads and seedyield.Data analysis revealed that there were no significant interactions between the cultivars and nitrogen. The number of leaves and plant heightdid not differ significantly in the tested cultivars in both sites. Raja had the most biomass accumulated in both sites. Application of nitrogen did not cause significant variations in production of leaves, plant height, number of tillers and dryweight in both sites and seasons. Raja yielded higherhead than the other cultivars in both seasons and sites ($P<0.05$). In addition to Raja, Summit too gave high seed yield in Njoro during the February-June season. The highest headyield was obtained at 75 kgN/ha during the February-June season while it was 150 kgN/haduring the July-December season. In Njoro, more heads

were formed with addition of nitrogen. Since nitrogen fertilization up to 150 kgN/ha did not result in significantdifferences in growth of the tested linseed cultivars, Summit, S19/21, Raja, Jawhar and S19/12 can therefore be consideredmoderate consumers of nitrogen. Raja yielded more seedthan to the other four cultivars and can therefore be recommended for adoption in linseed seed production in Juja, Njoro and other similar agro-ecological zones, as well as for futureresearch and breeding programs.

4.1:Introduction

Linseed (*Linumusitatissimum* L.) has many uses as an ingredient in the production of a variety of industrial products including linoleum, paints, and inks (Chauhan *et al.,* 2009; Singh *et al.,*2011). More importantly, it is emerging as an important ingredient for functional foods and nutraceuticals and as an ingredient for animal feeds targeting functional animal food products (Singh *et al.,* 2011; Bernachia,Preti& Vinci, 2014). Consumption of linseed may reduce the risk of major chronic diseases such as atherosclerosis, cardiovascular diseases, ulcers and certain types of cancer. In Kenya, linseed is currently sold expensively in health food shops, where only a small fraction of the population can access it. This may be largely due to the limited production of this crop in the country, despite the fact that research from the 1920s to 1970s showed that linseed farming is viable in the country (Riungu, 1988).

A few reasons have been suggested for the failure of linseed cultivation to become a major commercial activity in the country (Riungu, 1988). In the past, linseed was

mainly cultivated in the country for fiber and for industrial oil. The cultivation of linseed for fibre was abandoned in 1956 perhaps due to competition from the cheaper cotton. The desertion of cultivation of linseed for oil in Kinangop was suggested to have been due to poorer yield than other crops especially wheat. At that time, the concept of functional foods and nutraceuticals had not gained ground, and products such as wheat may have been considered to be of more value than linseed.

In order to increase linseed production in the country, it is necessary to target not only the limited agro-ecological regions that were previously recommended for its production, based on limited research, but also other zones, especially where other high value crops such as wheat are currently not grown. This will require evaluation of the performance of different cultivars in such areas, since previous research has shown that different cultivars perform differently in different environments (Alem & Dessalegn, 2014). In addition proper management practices tailored for the different environments need to be established, including the proper application of mineral fertilizers.

Nitrogen fertilizer normally plays a key role in crop yields (Cheema,Malik, Hussain, Shah and Basra, 2001), since nitrogen is a component of proteins, chlorophyll, energy transfer compounds such as adenosine triphosphate (ATP), and nucleic acids such as DNA and RNA (Wright,Smith & Woodroffe, 1988; Frink,Waggoner & Ausubel1999).According to Hocking & Pinkerton (1991), seed yield of linseed is highly related to N and water availability and, when water availability is not limited, N

deficiency is the main cause of seed yield reduction. Where N is applied, the rate required to obtain maximum crop yield varies greatly with site and other environmental factors, moreso the soil water level (Grant,Dribnenki & Bailey, 1999). Information on recommended N rates for linseed is limited, but a range of 20 – 85 kgN/ha has suggested (Diepenbrock and Porksen, 1993; Freer, 1993; Hocking,Randall& DeMarco,1997*).* Soil tests from Njoro which is traditionally a linseed growing region gave a 0.01% N (table 4.2) which according to Okalebo,Gathua and Woomer,2002 is classified as low for crop production. The cost of most nitrogen-containing fertilizers in Kenya is high.

In this chapter, the growth performance and seed yield of five linseed cultivars in two agro-ecological locations and the influence of nitrogen fertilizer on these attributes is reported.

4.2: Materials and Methods

4.2.1:Experimental site

Field experiments were conducted in two sites representing two agro-ecological zones.One site was at the research fields of JomoKenyatta University of Agriculture andTechnology (JKUAT) –JujaCampus.Juja stands at an altitude of 1416 m abovesea level (asl), (1° 10' S, 37° 7' E) in Kiambu County,Kenya, approximately 35 km north of NairobiCity.Juja's climate is tropical savannahwith average temperature being 19.6°C

and the average rainfall of 799mm per annum. Soils are high in lixisols. The Kenya Agricultural and Livestock Research Organisation (KALRO-Food Crops research Institute) – Njoro was selected as the second site. The site lies at an altitude of 2423m asl (-0^o 29'S, 35^o 56'E) in Nakuru County, Kenya, about 20 km south west of Nakuru town. Its climate is warm and temperate with an average temperature of 16.1°C andaverage rainfall of 937mm annually.Njoro has a fertile mollic andosol.

4.2.2: Experimental design

Experiments were laid out as split plots inrandomized complete block design with threeN-fertilizer levels{0Kg N/ha (control), 75KgN/ha and 150Kg N/ha} as main plots, and fivelinseed cultivars {Summit (V1), S19/21 (V2),Raja (V3), Jawhar (V4) and S19/12 (V5)} assub-plots in two seasons (February-June 2012season and July-December 2012 season). The five cultivars were chosen based on their levels of oil contents from a preliminary study.

4.2.3: Field layout

The field was laid out as shown below, where N1 is 0 kgN/ha, N2 is 75kgN/ha and N3 150 kgN/ha. V1 is Summit, V2 is S19/21, V3 is Raja, V4 is Jawhar and V5 is S19/12.

Table 4.1: **Field layout for experiments in Juja and Njoro.**

N3V4	N3V3	N3V2	N3V5	N3V1
N1V1	N1V5	N1V4	N1V2	N1V3
N2V1	N2V2	N2V5	N2V3	N2V4
N1V1	N1V3	N1V2	N1V4	N1V5
N3V4	N3V1	N3V3	N3V5	N3V2
N2V5	N2V2	N2V1	N2V4	N2V3
N2V3	N2V2	N2V5	N2V4	N2V1
N3V4	N3V3	N3V1	N3V5	N3V2
N1V2	N1V4	N1V3	N1V1	N1V5

4.2.4:Land preparation and planting

Before setting up the field experiments, soil testswere conducted to determine initial levels ofsoil pH, electrical conductivity and % nitrogenaccording to the standard procedure as documented in Okalebo*et al*., (2002). A minimum-maximum thermometer was mounted in the field to monitor temperature. Landwas ploughed to a fine tilth and plotsmeasuring 3mx 3m prepared. Drills of 20cmapart and 2cm deep were made along each ofthe plots. Seeds were evenly spread along thedrills at approximately 650 seeds/$m^2$and then covered with a thin layer of soil.Overhead irrigation was done to ensure the soil remained moist in cases where there was no rainfall. Germinated seedlings were kept weed free by manually uprooting and/or hoeing.Application of N-fertilizer was done as calcium ammonium nitrate ((CAN (26% N))topdress and

commenced 30 days afterplanting and was done in three equal splits,being 2 weeks apart from each other.

4.2.5: Data collection

Starting from the 21stday after planting and at weekly intervals for nine weeks, data were recorded from the central branch of a randomly selected destructively harvested plant in each plot. Plant height (cm) was determined by measuring the length from the ground level to the tip of the central stem. Number of primary branches (tillers) was the count of all branches emanating from the central stem. Determination of number of leavesinvolved counting all the leaves from the base to the tip of the central stem. For determination of dry matter accumulation, the plants were then wrapped in paper to hold individual samples together and put in oven. They were dried at105±1^{o}C for 24hrs then weighed to determinedry matter accumulation.

At maturity, number of capsules/plantwas estimated from counts on five sample-plants/plots. Capsules were removed by hand, seed manually extracted and cleaned and the total number of seeds/capsule recorded.The plants were manually harvested then left to completely dry out in the sun for three days. Seed was extracted manually by threshing, winnowed to remove dirty and plant residue then weighed to determine total seed weight per plot from which seed weight per plant was estimated.

4.2.5: Data analyses

Data was subjected to analysis of variance (ANOVA) using PROC GLM in SAS 9.1.3 portable version to identify main effects and interactions in response to N; the means were separated using LSD procedure at the 0.05 level of significance and graphs plotted using SigmaPlot 12.0.

4.3: Results

Juja received 176 and 119 mm rainfall during the February-June and July-December seasonswhile that received during the two seasons in Njoro were 171 and 146mm respectively. The average temperature in Juja ranged between 16.5-20.2 ^{o}C while the range was 15.4-18.3^{o}C in Njoro (Appendix 2).

Initial soil tests were as follows:

Table 4.2: **Initial soil pH, Ec and nitrogen levels**

Site	**pH**	**Ec (µmol/cm)**	**%N**
Juja	6.2	0.12	0.1
Njoro	5.94	0.54	0.1

4.3.1: Vegetative growth

There were no significant cultivar-nitrogen interactions and therefore the results presented and for the main factors (Appendix 3). Results for the two sites were analysed separately. Similarly, results for the seasons were analysed separately.

4.3.1.1: Number of leaves

In Juja, there were no significantvariations in production of leaves by cultivars inFebruary-June 2012 as well as in July-December 2012 (Figure 4.1a, b). Application of nitrogendid not result in significant variations inproduction of leaves in both seasons(Figure 4.1c,d). Similarly in Njoro, there were no significantvariations in production of leaves by cultivars inFebruary-June 2012 as well as in July-December 2012 (Figure 4.2a, b). Application of nitrogendid not result in significant variations inproduction of leaves in both seasons(Figure 4.2c,d). In both seasons, the control gave the least number of leaves while 150 kgN/ha gave the most.

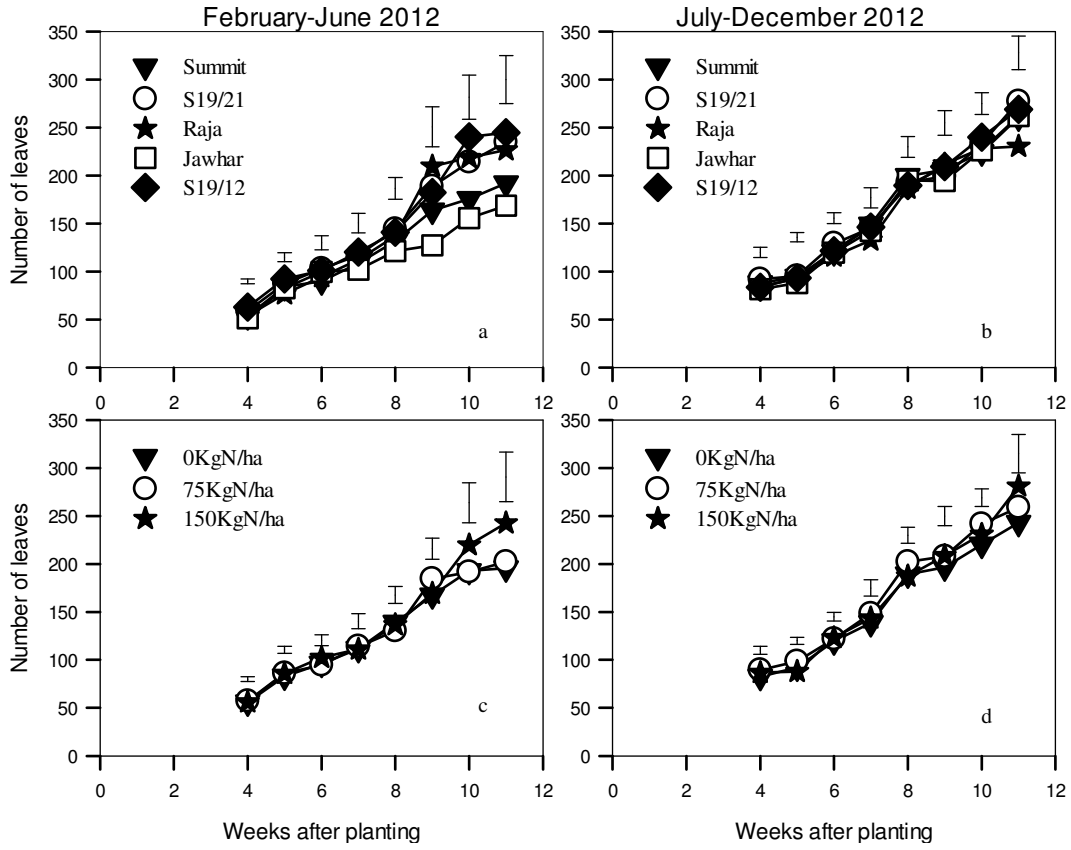


Figure 4.1: Number of leaves of linseed as influenced by cultivars and nitrogen fertilizer levels during the February-June and July-December seasons 2012 in Juja.

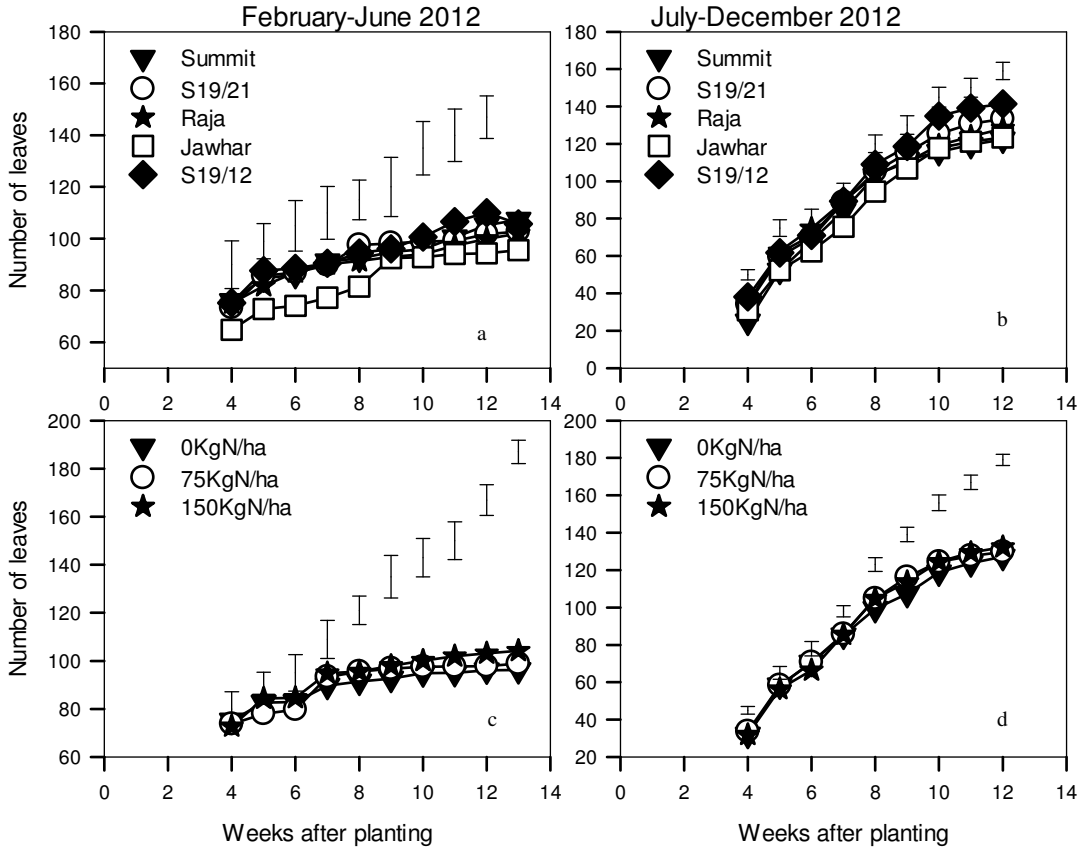


Figure 4.2:Number of leaves of linseed as influenced bycultivars and nitrogen fertilizer levels during the February-June and July-December seasons 2012 in Njoro.

4.3.1.2: Number of tillers

Cultivars varied in production of tillers inboth seasons in both Juja(Figure 4.3) and Njoro (Figure 4.4). In Juja, Raja tillered more in both seasons producing 14 and 6tillers at the eleventh week in the February-Juneand July-December seasons respectivelycompared to a range of 6-8 and 2-4 in the othercultivars. In Njoro, Raja

produced the highest number of tillers during the February-June 2012 season while it was Summit that tillered most during the July-December season. Raja produced 28 tillers during the February-June season compared to 13 produced by the other cultivars. On the other hand, Summit produced 11 tillers during the July-December season in comparison to the other cultivarswhose highest numbers during the season were 6 tillers. In both sites, cultivars tillered more during theFebruary-June season than in July-December season (Figures 4.3a, b and 4.4a, b). Tillering was notsignificantly affected by the amounts ofnitrogen fertilizer applied in either Juja or Njoro (Figures 4.3c, d and 4.4c, d). Nitrogen triggered production of more tillers during the February-June season compared to the July-December season.

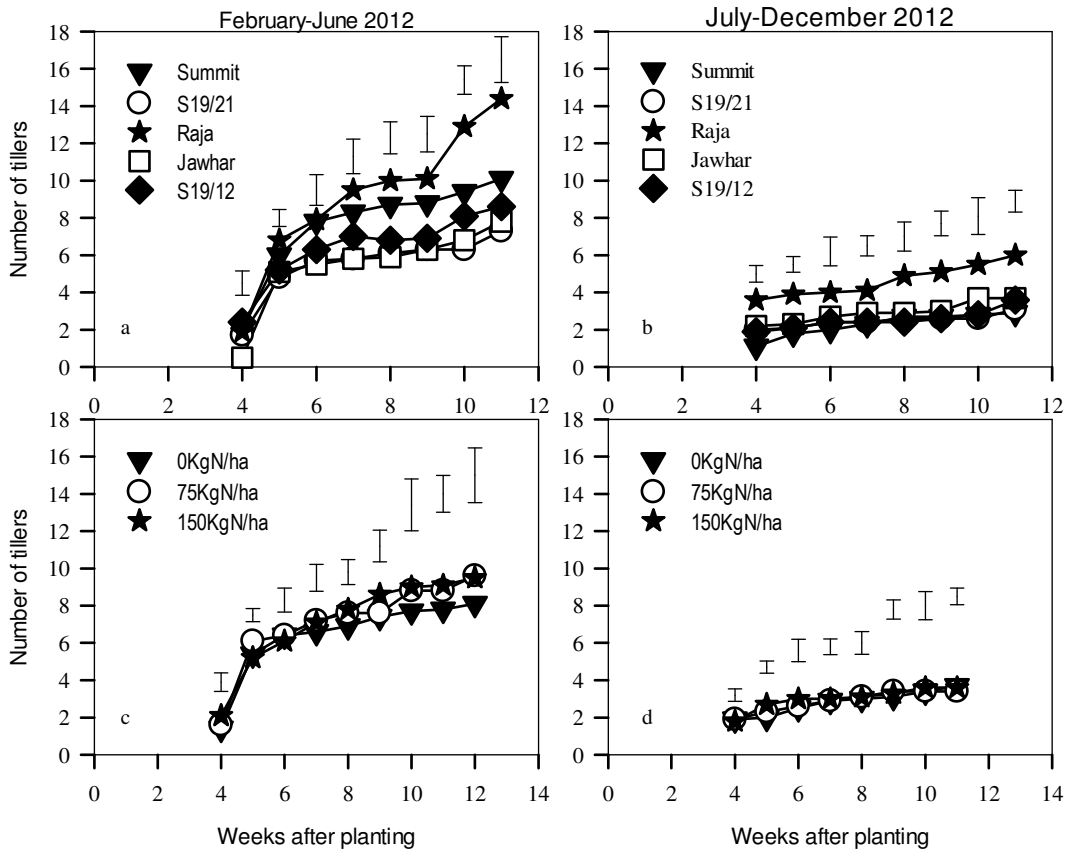


Figure 4.3: Production of tillers by linseed as influenced by cultivars and nitrogen fertilizer levels during the February-June and July-December seasons 2012 in Juja.

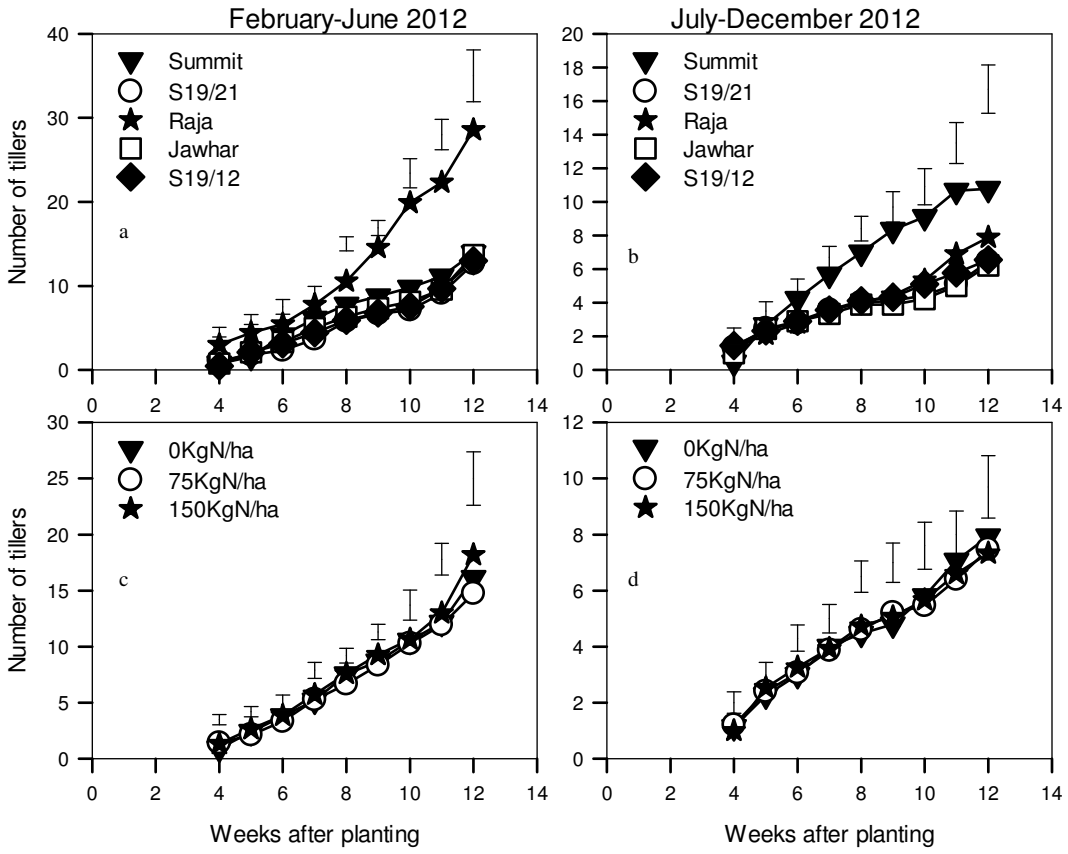


Figure 4.4:Production of tillers by linseed as influenced bycultivars and nitrogen fertilizer levels during the February-June and July-December seasons 2012 in Njoro.

4.3.1.3: Plant height

There were no significant differences in the height attained by the linseed cultivars in both seasons in both Juja (Figure 4.5a, b) and Njoro (Figure 4.6a, b). In both sites, increase in height across the cultivarswas more rapid during the July-December season compared to the February-June seasonApplication of nitrogen fertilizer at

differentlevels did not lead to significant differences in the heights attained byplants in both sites during the two experimental seasons (Figures 4.5c,d and 4.6c, d).The initial height increase was slow andsimilar till the ninth week after which the increase in height for the plants under treatment was much more rapid.

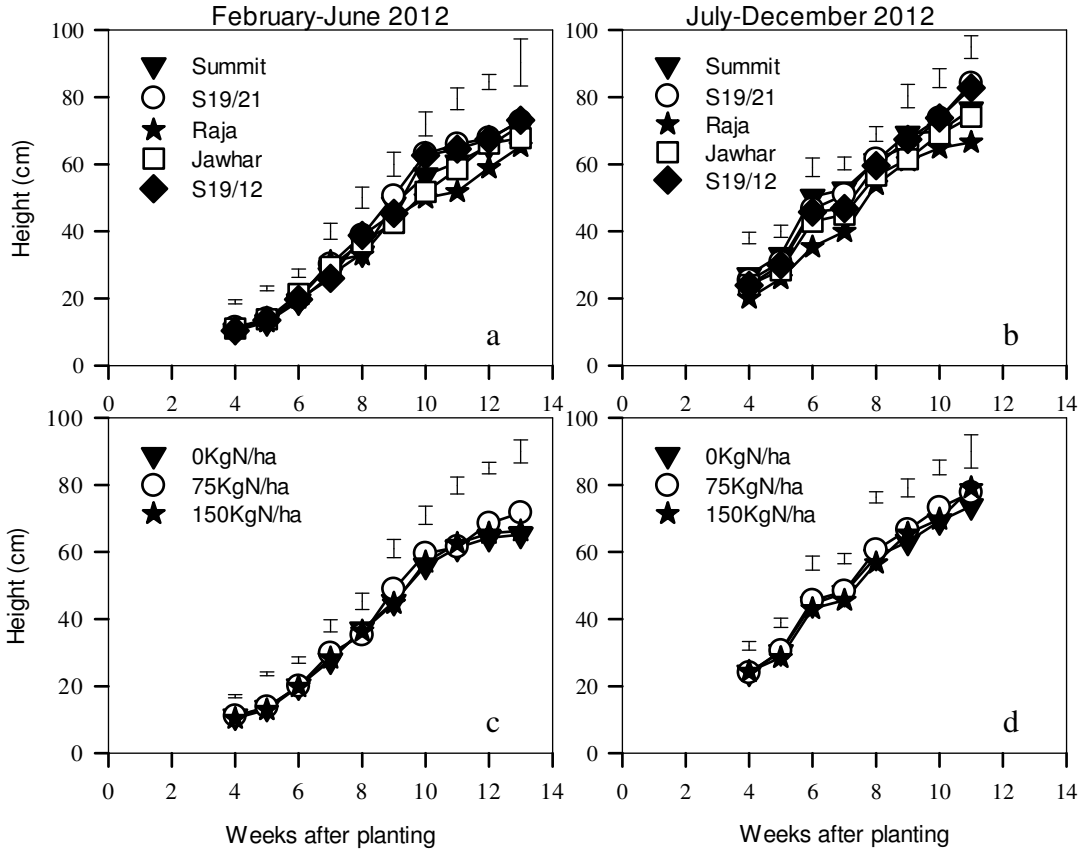


Figure 4.5: **Height of linseed as influenced by cultivars and nitrogen fertilizer levels during the February-June and July-December seasons 2012 in Juja.**

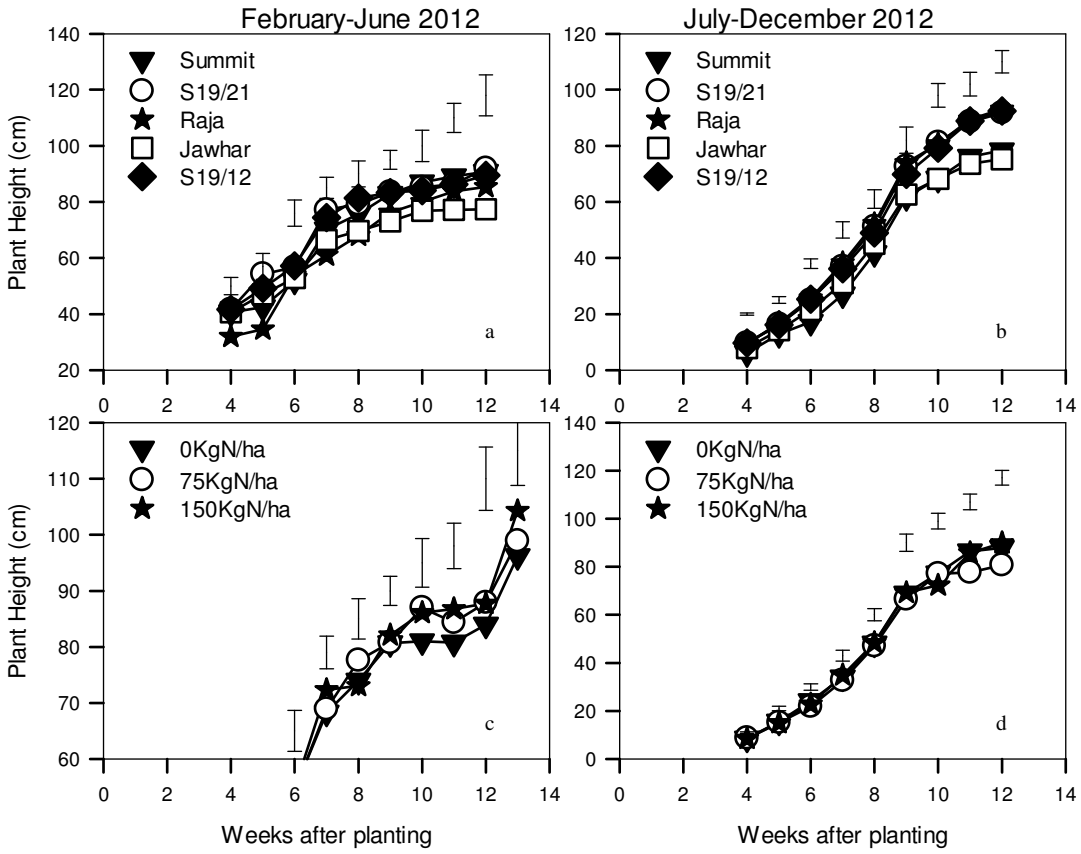


Figure 4.6: Height **of linseed as influenced bycultivars and nitrogen fertilizer levels during the February-June and July-December seasons 2012 in Njoro.**

4.3.1.4: Dry Matter

In Juja, dry matter accumulation was significantly higher in Raja than all theother cultivars in both seasons (Figure 4.7a, b). However, in Njoro, dry matter accumulation wassignificantlyhigher in Raja and Summit than in theother cultivarsin the February-June season (Figure 4.8a). During the July-December season, it was Summit which accumulated much higher dry weight than the rest of the cultivars(Figure 4.8b).

Drymatter accumulation in the different cultivarsduring the February-June season ranged from0-11.4g while it ranged from 0-7.1g during theJuly- December season. In both Juja and Njoro, the initial increase inbiomass was slow at the beginning but rosesharply from the 7th week in both seasons. Additionally, there were no significant differences indry weight upon addition of thedifferent N-fertilizer levels in both seasons (Figures 4.7c,d and 4.8c, d), only difference occurring towards the end of the experiment. The rate of increase was slow at first andincreased with time. A sharp increase in bothseasons however occurred at around the 10thweek after the lastsplit of fertilizer had beenadded.

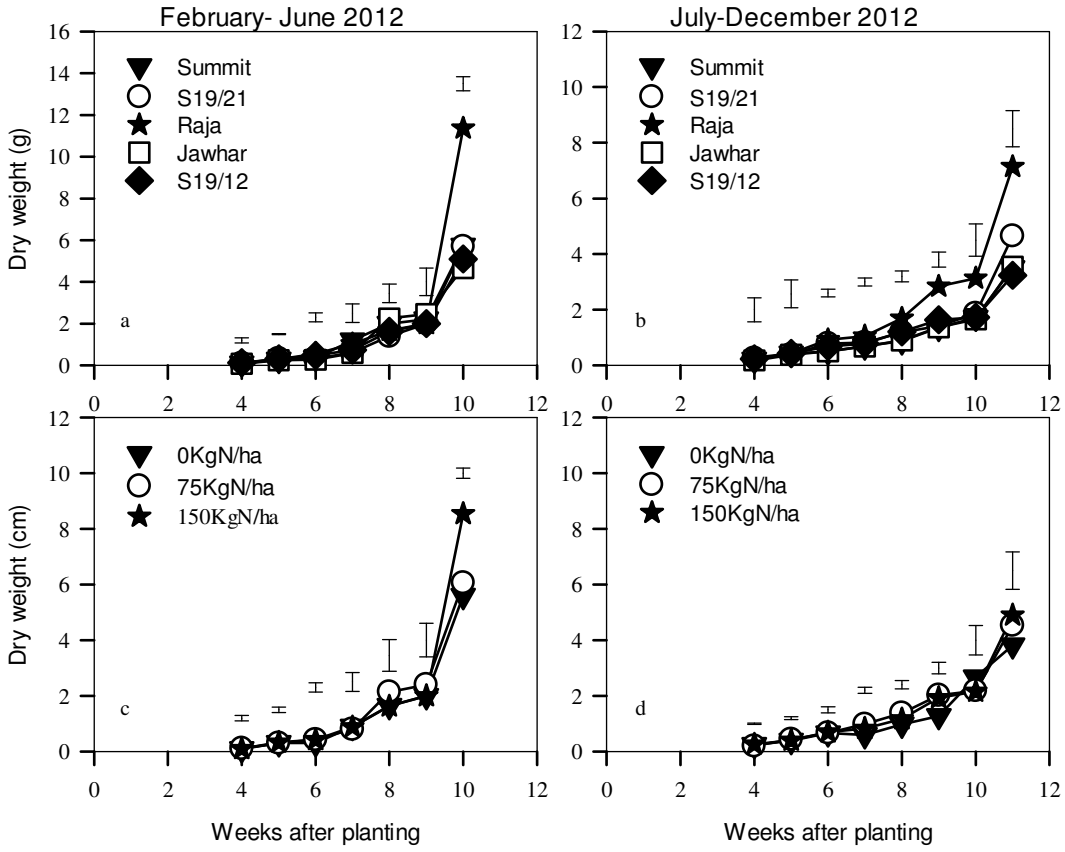


Figure 4.7:Dry weight of linseed as influenced by cultivars and nitrogen fertilizer levels during the February-June and July-December seasons 2012 in Juja.

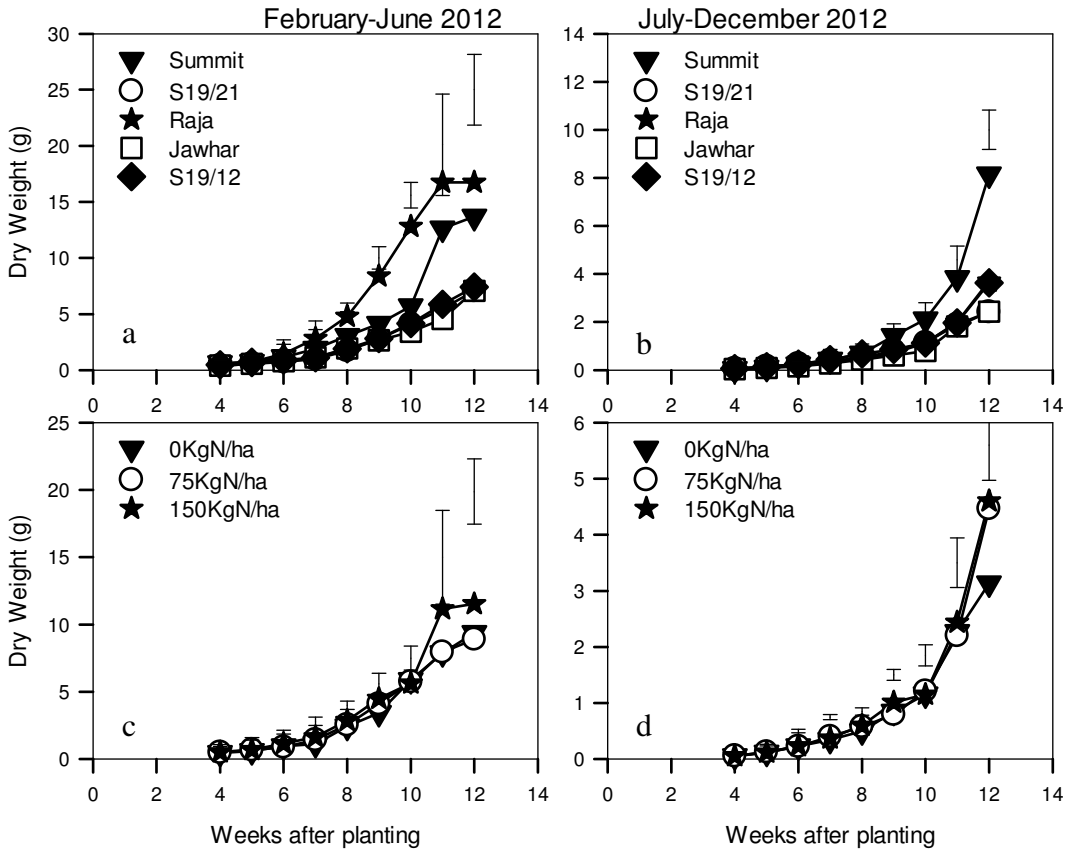


Figure 4.8: Dry weight of linseed as influenced bycultivars and nitrogen fertilizer levels during the February-June and July-December seasons 2012 in Njoro.

4.3.1.5: Linear relationships

There were significant linearrelationships between increase in number ofleaves and dry matter accumulation during the July-December season in Juja (Figure 4.9 July-December 2012) and in both seasons in Njoro (Figure 4.10). Thisrelationship was however missing during theFebruary-June season (Figure 4.9 February-June 2012) in

Juja. For thedifferent cultivarsand seasons, a unit increasein number of leaves was accompanied by anincrease in dry weight.

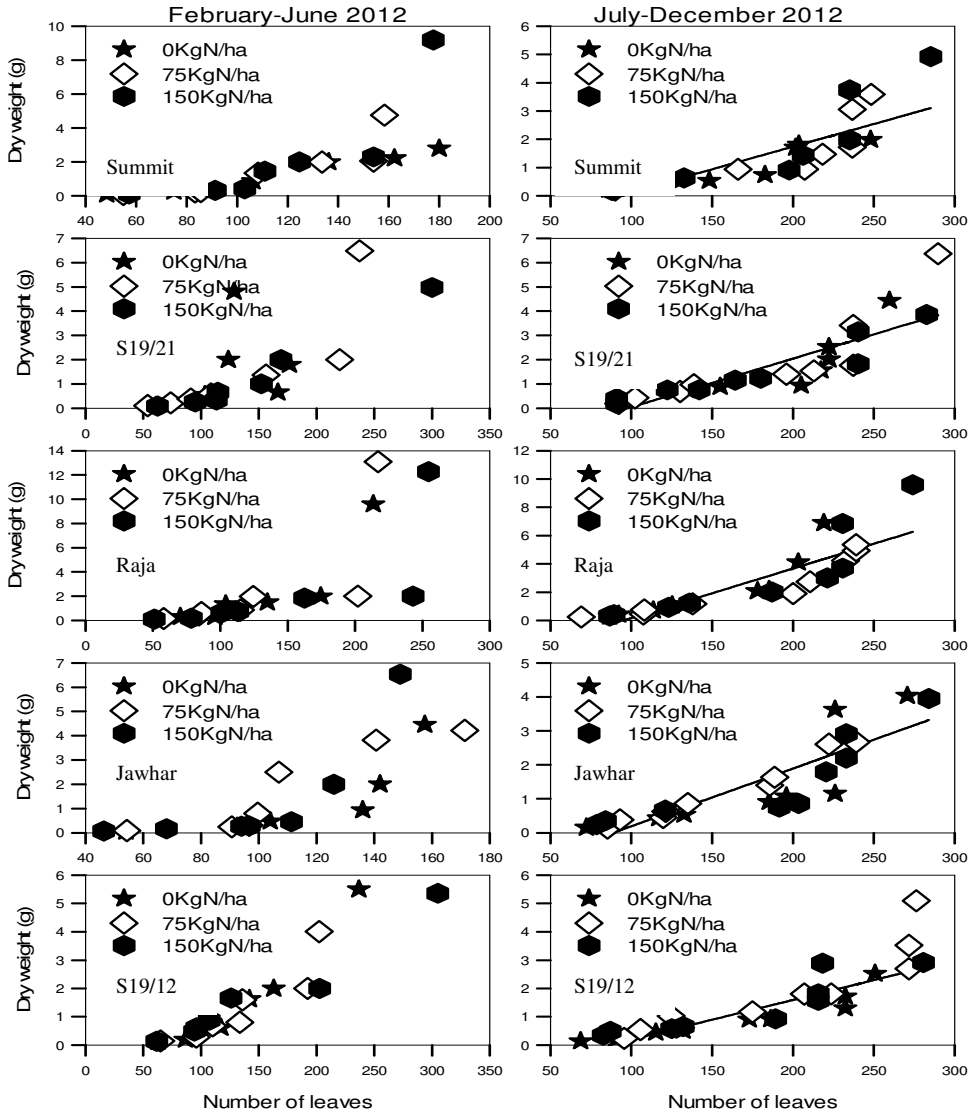


Figure 4.9:Relationship between number of leaves and dry weight accumulation in linseed during the February-June and July-December seasons 2012 in Juja.

This was at varied proportions which depended on the cultivars as shown in table 4.3 (Juja), and on season where they were higher during the July-December season than during the February-June season (Table 4.4).

Table 4.3: The slope and intercepts and their standard errors for the linear functions in Figure 4.9

Cultivar	**February-June**					**July-December**				
-	Slope	Std err	Intercept	Std err	R^2	Slope	Std err	Intercept	Std err	R^2
Summit	19.4	0.0	36.2	0.01	0.8	0.02	-	1.5	-	0.01
S19/21	33.4	0.0	84.9	0.05	0.7	0.02	-	2.0	-	0.01
Raja	10.7	0.0	101.7	0.01	0.6	0.04	-	3.3	-	0.00
Jawhar	17.2	0.0	81.6	0.02	0.8	0.02	-	1.5	-	0.01
S19/12	36.0	0.0	81.7	0.06	0.8	0.02	-	1.2	-	0.01

Table 4.4: The slope and intercepts and their standard errors for the linear functions in Figure 4.10

Cultivar	**February-June**					**July-December**				
-	Slope	Std err	Intercept	Std err	R^2	Slope	Std err	Intercept	Std err	R^2
Summit	0.4	0.07	29.5	7.36	0.8	0.05	-	2.8	-	0.43
S19/21	0.2	-	15.0	-	0.8	0.02	-	1.0	-	0.70
Raja	0.6	-	49.8	-	0.8	0.02	-	1.6	-	0.52
Jawhar	0.2	-	12.0	-	0.8	0.02	-	1.0	-	0.62
S19/12	0.1	-	9.3	-	0.7	0.02	-	1.4	-	0.60

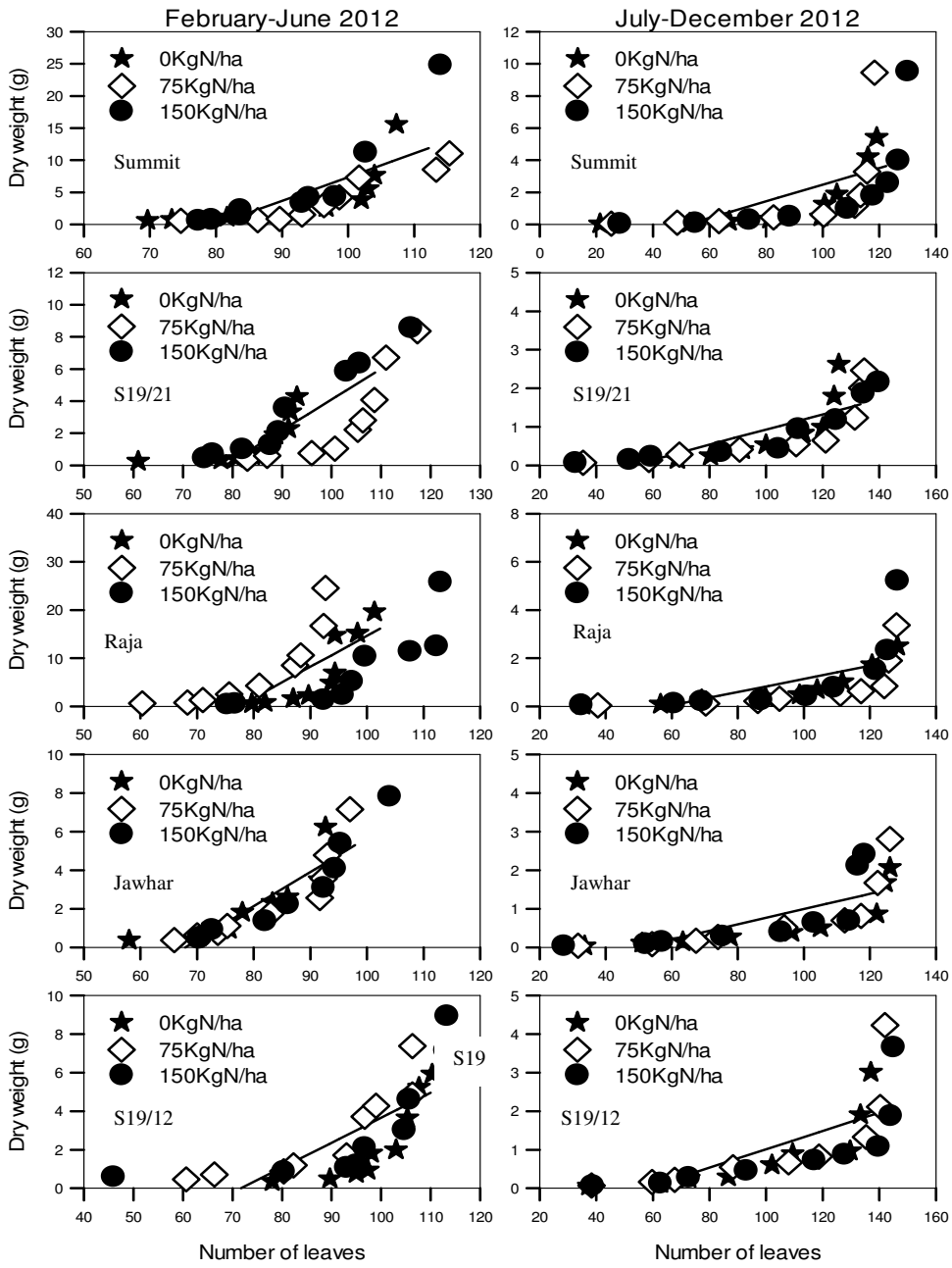


Figure 4.10: Relationship between leaves and dry weight accumulation in linseed during the February-June and July-December seasons 2012 in Njoro.

There was nosignificant relationship between tillering ability anddry matter accumulation in all cultivarsin Jujaduring the February-June season, (Figure 4.11 February-June 2012). However, a significant relationshipexisted during the July-December season as shown in figure 4.11 (July-December 2012) during when anincrease in number of tillers was accompaniedby an increase in dry weight at proportions thatdepended on the cultivars as shown in Table 4.5.A unit increase in tillers resulted in 1.1, 2.5,1.9, 1.7 and 1.7 times increase in dry matter accumulation for Summit, S19/21, Raja, Jawharand S19/12 respectively. In Njoro, an increase in number of tillers resulted in a linear increase in dry weights for all the cultivarsduring the two seasons (Figure 4.12). During these two seasons, anincrease in number of tillers wasaccompaniedby an increase in dry weight at proportions that depended on the cultivar as shown in Table 4.6.A unit increase in tillers resulted in 0.5-1.1 times increase in dry matteraccumulation for the cultivarsduring the February-June season. The rate of increase was lower, at between 0.5 and 0.6 times, during the July-December season. During the February-June season, the greatest increase in dry weight per unit increase in tillers was with Summit while it was Summit and S19/12 during the July-December season.

Table 4.5: The slope and intercepts and their standard errors for the linear functions in Figure4.11.

Cultivar	February-June					July-December				
	Slope	Std err	Intercept	Std err	R^2	Slope	Std err	Intercept	Std err	R^2
Summit	0.50	-	2.1	0.0	0.6	1.1	-	1.2	0.0	0.7
S19/21	0.7	-	2.2	0.04	0.3	2.5	-	4.6	0.0	0.9
Raja	0.5	-	2.0	0.0	0.5	1.9	-	6.5	0.0	0.9
Jawhar	0.6	0.0	1.32	0.0	0.4	1.7	-	3.6	0.0	0.9
S19/12	0.7	0.0	2.49	0.0	0.4	1.7	-	3.0	0.0	0.7

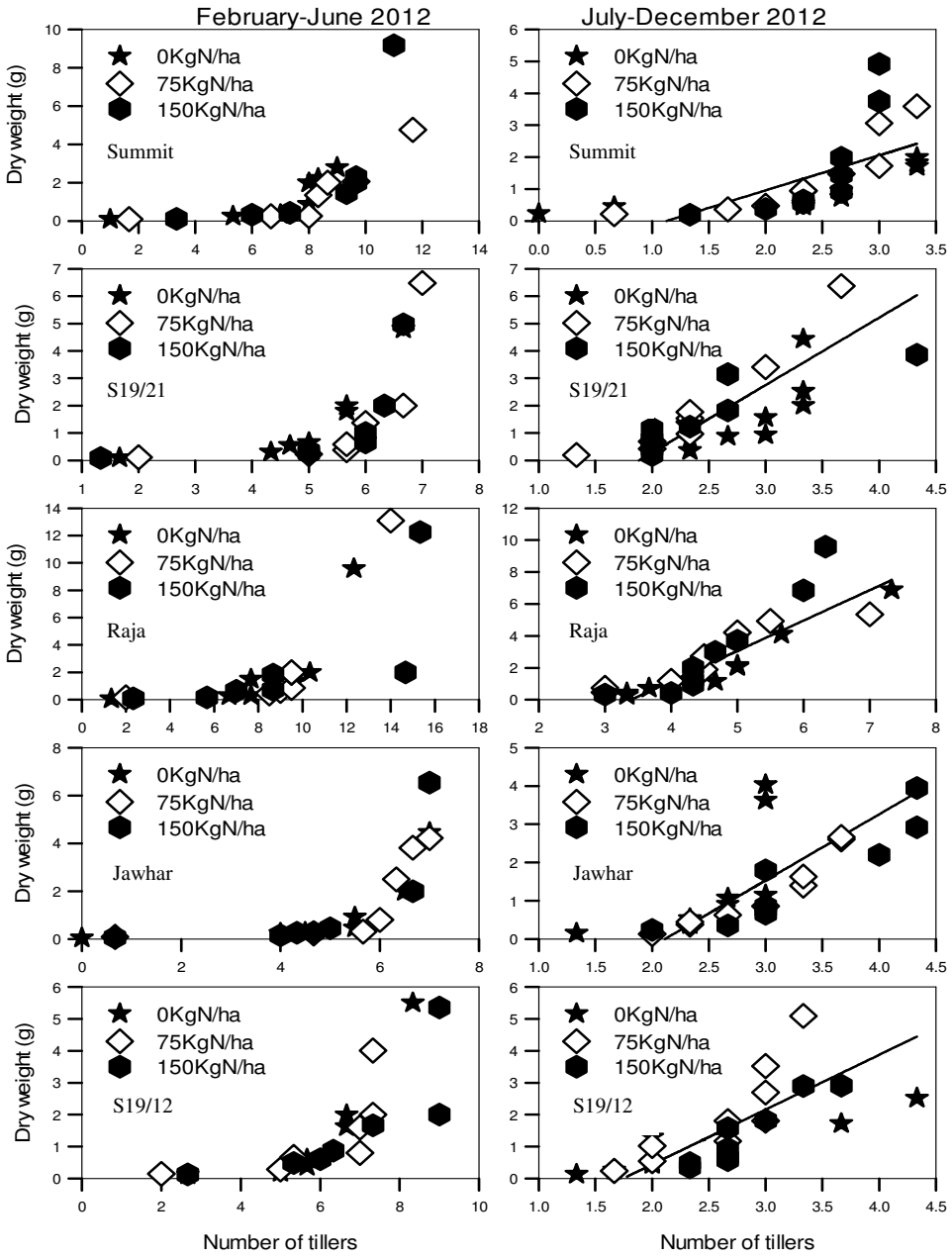


Figure 4.11:Relationship between number of tillers and dry weight accumulation in linseed during the February-June and July-December seasons 2012 in Juja.

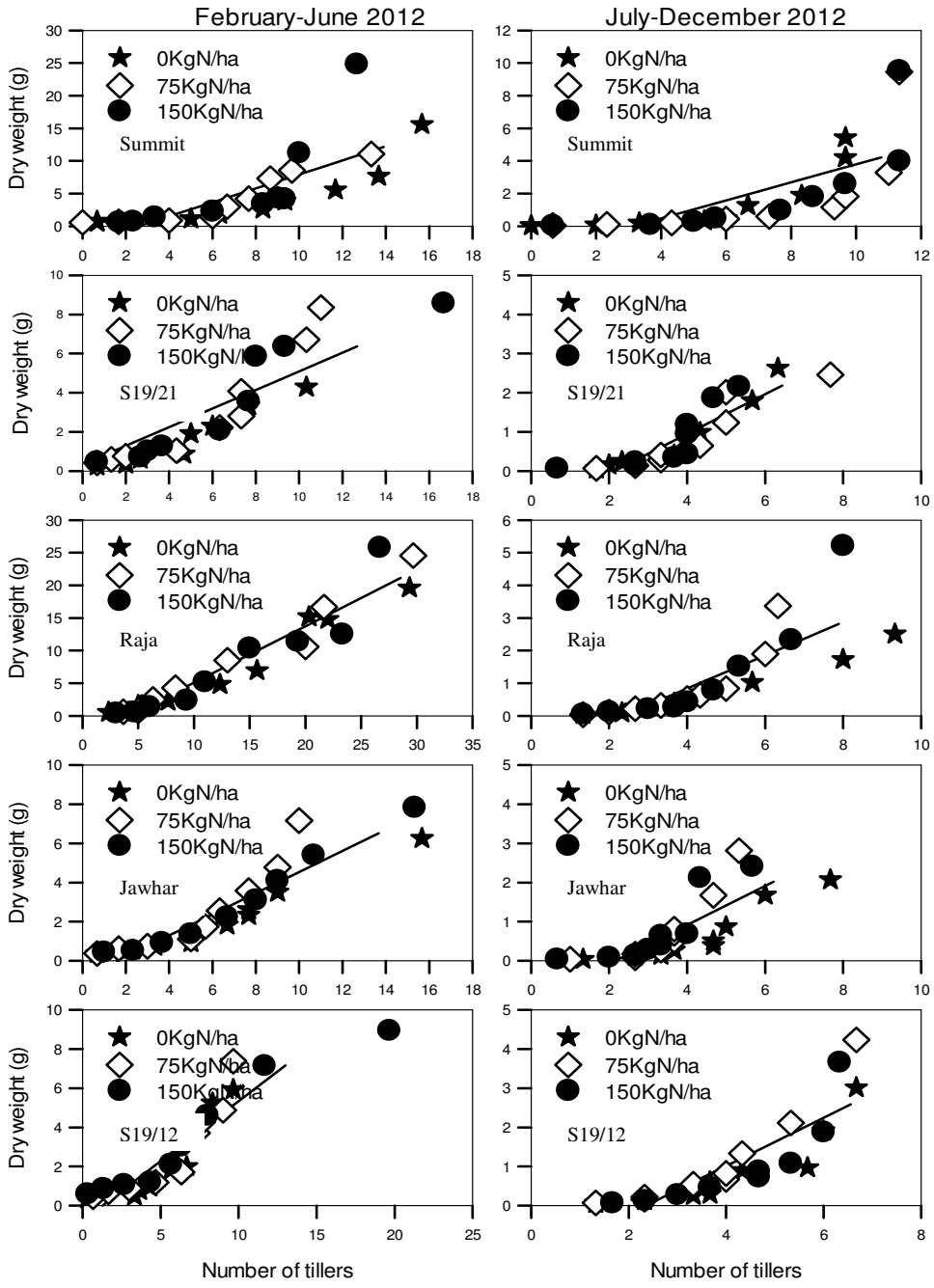


Figure 4.12:Relationship between number of tillers and dry weight accumulation in linseed during the February-June and July-December seasons 2012 in Njoro.

Table 4.6: The slope and intercepts and their standard errors for the linear functions in Figure 4.12

Cultivar	February-June					July-December				
-	Slope	Std err	Intercept	Std err	R^2	Slope	Std err	Intercept	Std err	R^2
Summit	1.1	-	2.8	0.0002	0.8	0.6	-	1.7	-	0.6
S19/21	0.5	-	0.3	0.0002	0.9	0.5	-	1.1	-	0.9
Raja	0.9	-	3.5	0.0002	1.0	0.5	-	1.2	-	0.8
Jawhar	0.5	-	0.9	0.0002	0.9	0.5	-	1.1	-	0.8
S19/12	0.6	-	0.8	0.0002	0.9	0.6	-	1.4	-	0.8

In Juja, plant height showed a significant linearrelationship with number of leaves. In Njoro on the other hand, though there was a linear relationship between plant height and number of leaves, this relationship was not significant. In Juja, there was an increase by 2.2-3.3 and 3.2-3.6leaves per unit increase in height in theFebruary-June (Figure 4.13 February-June 2012) and July-December2012seasons(Figure 4.13 July-December 2012), respectively, as shown in table 4.7.On the other hand, there was an increase of0.04-0.8 and 0.8-1.0 leaves per unit increase in height in the February-June and July-December 2012 seasons in Njoro respectively (Figure 4.14,Table 4.8). This increasedepended on the cultivar. In both Juja and Njoro, the rate of increase in leaves per unit increase in height was higher during the July-December seasonthan during the February-June season. In both sites and for all the cultivars, nitrogen did not significantly influence the relationship.

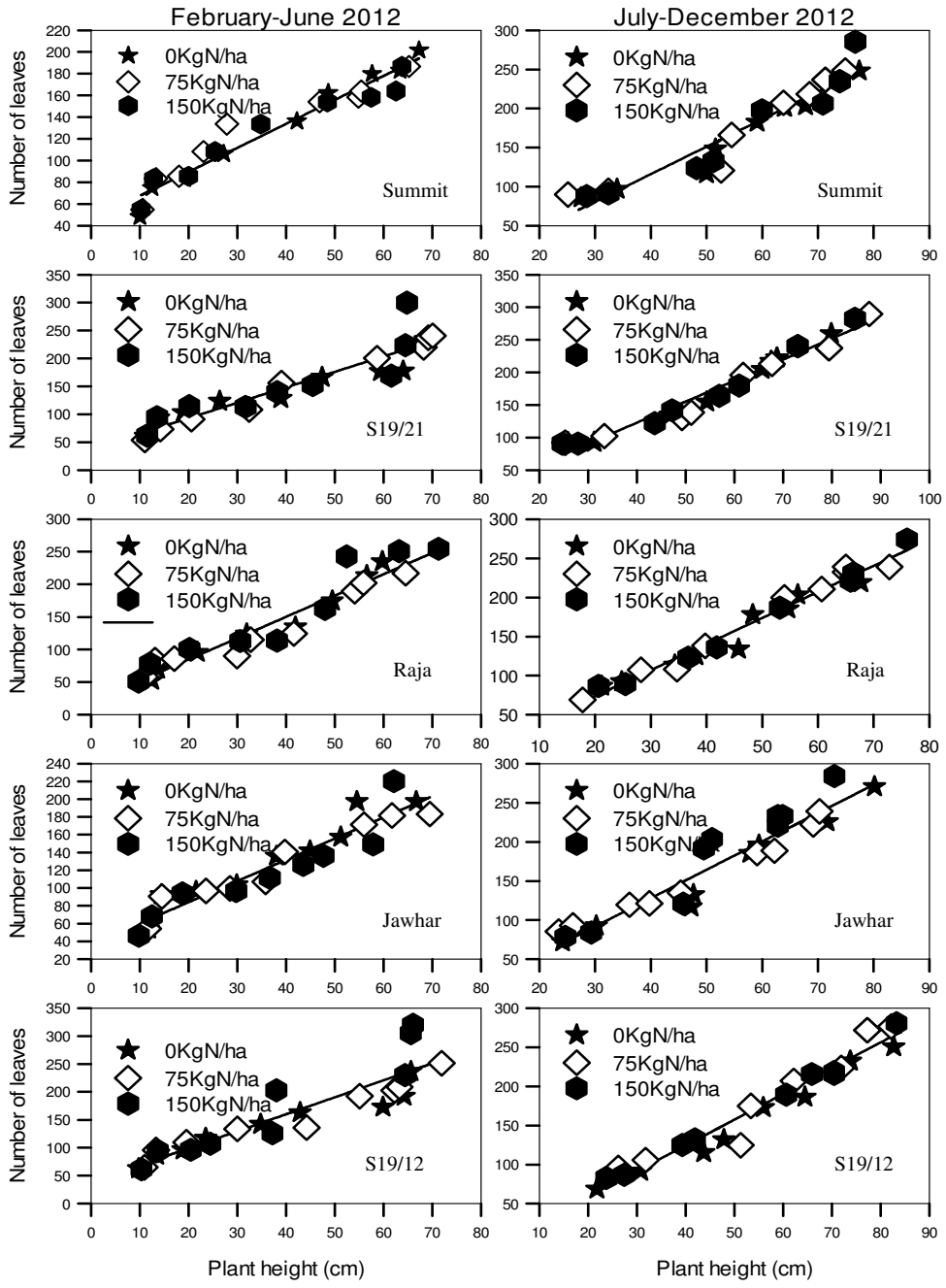


Figure 4.13:Relationship between number of leaves and plant height in linseed during the February-June and July-December seasons 2012 in Juja.

Table 4.7: The slope and intercepts and their standard errors for the linear functions in Figure 4.13.

Cultivar	February-June					July-December				
	Slope	Std err	Intercept	Std err	R^2	Slope	Std err	Intercept	Std err	R^2
Summit	2.2	-	45.4	-	0.8	3.5	-	24.6	-	0.6
S19/21	2.4	-	36.3	-	0.7	3.2	-	6.2	0.001	0.6
Raja	3.3	-	19.3	-	0.6	3.3	-	6.9	0.001	0.9
Jawhar	2.4	-	36.2	-	0.9	3.6	-	17.0	0.001	0.9
S19/12	3.0	-	38.3	-	0.9	3.3	-	7.3	0.001	0.8

Table 4.8: The slope and intercepts and their standard errors for the linear functions in Figure 4.14

Cultivar	February-June					July-December				
-	Slope	Std err	Intercept	Std err	R^2	Slope	Std err	Intercept	Std err	R^2
Summit	0.1	0.003	93.6	3	0.9	0.8	-	28.0	-	0.9
S19/21	0.2	-	78.2	-	0.9	0.9	-	32.3	-	0.9
Raja	0.6	-	51.9	-	1.0	1.0	-	38.9	-	0.9
Jawhar	0.1	-	79.2	-	0.01	0.8	-	25.6	-	0.9
S19/12	0.8	-	34.0	-	0.9	0.9	-	34.6	-	0.9

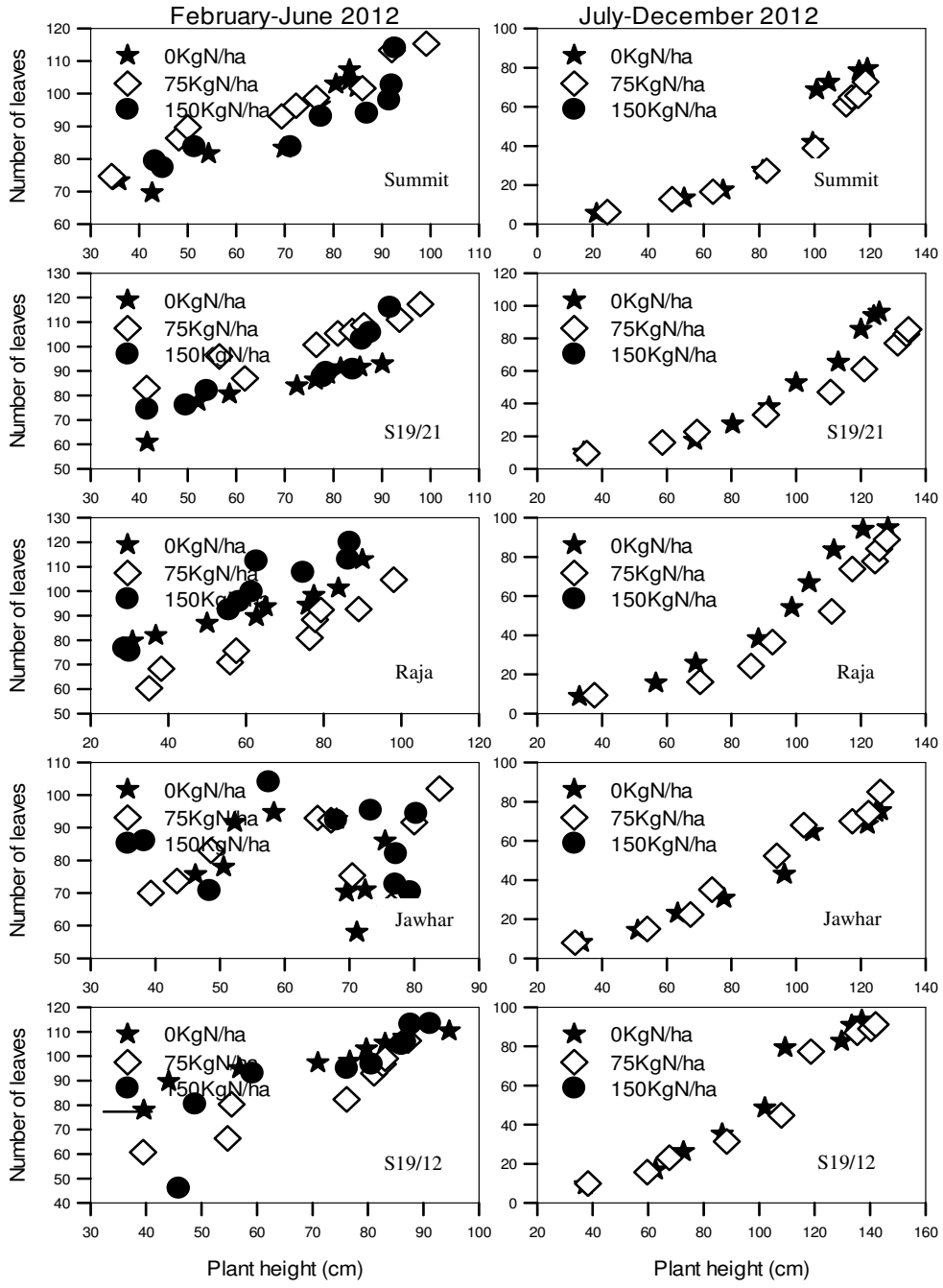


Figure 4.14: Relationship between number of leaves and plant height in linseed during the February-June and July-December seasons 2012 in Njoro.

In the two seasons and sites, there were linear relationships between the height of the linseed plants andincrease in dry weight (Figure 4.15 and 4.16). As plant heightincreased so did the dry weight although the proportion of increase depended on the cultivar(Table 4.9 and 4.10). In Juja, during the February-June 2012 season,a unit increase in height resulted in a 0.1-0.2 timesincrease in the dry matter of Summit, S19/21,Raja, Jawhar and S19/12 (Figure 4.15 February-December 2012).Consequently, an increase in a single leafresulted in a 0.05-0.1 times increase in dry weight forSummit, S19/21, Raja, Jawhar and S19/12during the July-December season (Figure 4.15 July-December 2012).Though the relationship for Raja during theFebruary-June season was statistically significant, this relationship did not appearlinearly real.In Njoro, a unit increase in height resulted in a 0.1-0.3 timesincrease in the dry matter for Summit, S19/21,Raja, Jawhar and S19/12 during the February-June season (Figure 4.16 February-June 2012, Table 4.10). On the other hand, a unit increase in heightresulted in a 0.02-0.07 times increase in dry weight forSummit, S19/21, Raja, Jawhar and S19/12during the July-December season (Figure 4.16 July-December 2012, Table 4.10). During both seasons, the greatest increase in dry weight per unit increase in height was by Raja. In both Juja and Njoro, the relationships were independent of the level of N-fertilizeradded.

Table 4.9: The slope and intercepts and their standard errors for the linear functions in Figure 4.15.

Cultivar	February-June					July-December				
	Slope	Std err	Intercept	Std err	R^2	Slope	Std err	Intercept	Std err	R^2
Summit	0.1	-	1.3	0.0002	0.9	0.1	-	1.7	-	0.9
S19/21	0.1	-	1.4	0.0002	1.0	0.1	-	2.0	-	1.0
Raja	0.2	-	3.5	0.0003	0.9	0.1	-	3.1	-	1.0
Jawhar	0.1	-	1.8	0.0002	0.9	0.1	-	1.8	-	1.0
S19/12	0.1	-	1.1	0.0002	0.9	0.1	-	1.3	-	1.0

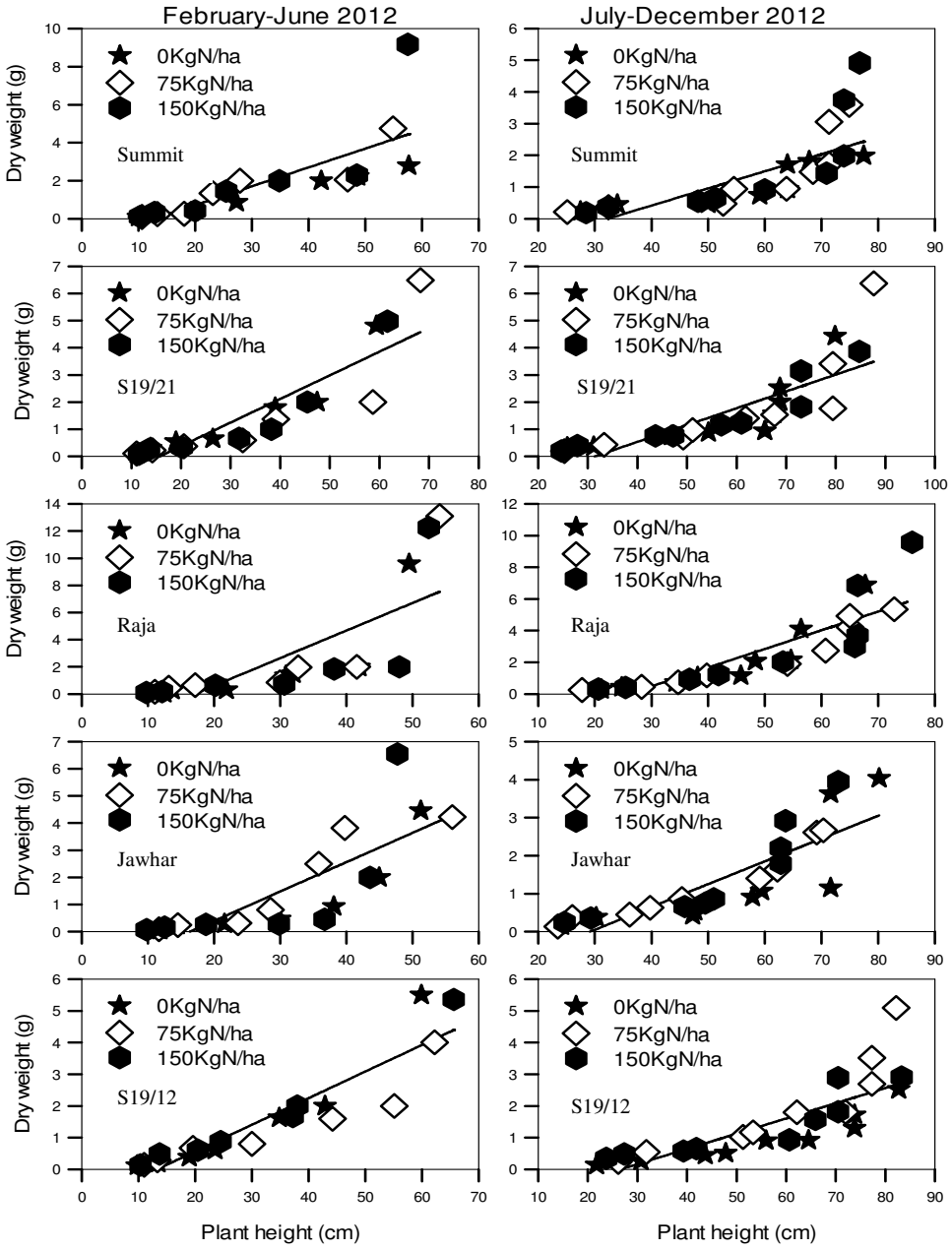


Figure 4.15:Relationship between dry weight and plant height in linseed during the February-June and July-December seasons 2012 in Juja.

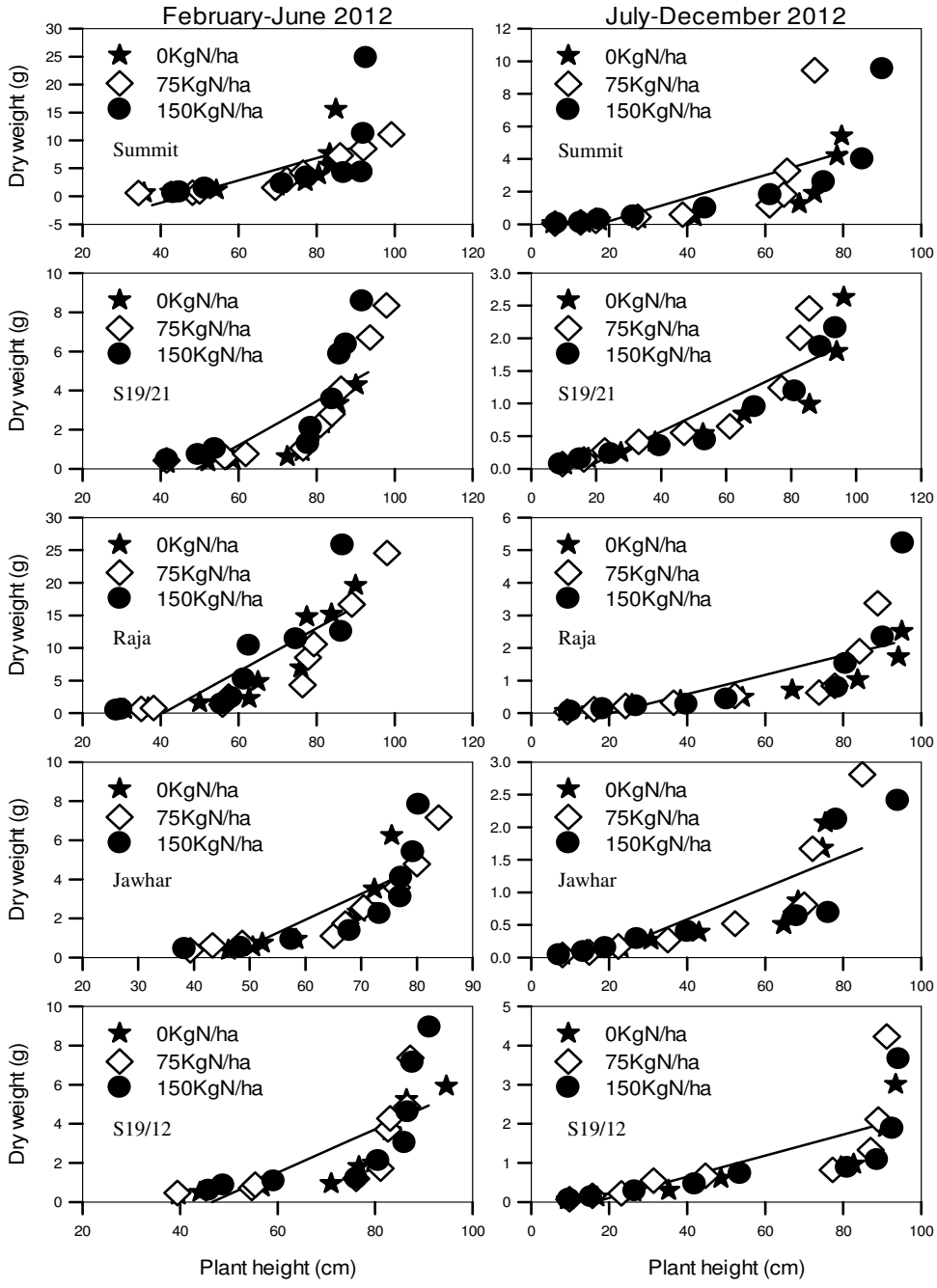


Figure 4.16:Relationship between dry weight and plant height in linseed during the February-June and July-December seasons 2012 in Njoro.

Table 4.10: The slope and intercepts and their standard errors for the linear functions in Figure 4.16

Cultivar	February-June					July-December				
-	Slope	Std err	Intercept	Std err	R^2	Slope	Std err	Intercept	Std err	R^2
Summit	0.2	-	9.2	-	0.6	0.07	-	1.2	-	0.6
S19/21	0.1	-	5.5	-	0.7	0.02	-	0.4	-	0.8
Raja	0.3	-	13.5	-	0.8	0.03	-	0.6	-	0.6
Jawhar	0.1	-	6.0	-	0.7	0.02	-	0.4	-	0.7
S19/12	0.1	-	5.2	-	0.6	0.03	-	0.4	-	0.6

4.3.2: Reproductive growth

Summit and Raja produced significantly highercapsules in the February-June season and July-December season in Juja respectively (Table 4.11); the two produced significantly higher capsules in both seasons in Njoro (Table 4.11). In theJuly-December season in Juja, Summit gave the lowest.S19/21, Jawhar and S19/12 did notsignificantly differ significantly in head yield (P>0.05) (Table 4.11). In both seasons in Juja, production of capsules wassignificantly increased by addition of nitrogen fertilization at 75 kgN/ha and 150 kgN/ha. In Njoro on the other hand, Summit and Raja produced significantly higher numbers of capsules than the rest of the cultivars during the two seasons. In general, the cultivarsproduced more capsules during the July-December season than the February-June season. In both seasons in Njoro, S19/12 produced the least heads. Application of nitrogen at 75 and 150 kgN/ha significantly increased the number of capsules produced though the two nitrogen fertilizer rates did not differ in the number of heads they initiated.

Table 4.11: Production of capsules as influenced by linseed cultivars and different nitrogen fertilizer levels during the February-June and July-December 2012 seasons.

Cultivar	**Number of capsules per plant**	
	February-June 2012	**July-December 2012**
Summit	211a	82d
S19/21	103b	135b
Raja	163b	232a
Jawhar	106b	132b
S19/12	111b	105c
LSD ($p \leq 0.05$)	65.7	20.2
Nitrogen (Kg/ha)	**Number of capsules per plant**	
	February-June 2012	**July-December 2012**
0	119b	133b
75	150a	137a
150	146a	140a
LSD ($p \leq 0.05$)	50.8	3.1

Table 4.12: Production of capsules as influenced by linseed cultivars and different nitrogen fertilizer levels during the February-June and July-December 2012 seasons in Njoro.

Cultivar	**Number of capsules per plant**	
	February-June 2012	**July-December 2012**
Summit	167a	173a
S19/21	108c	147b
Raja	159a	208a
Jawhar	131ab	107b
S19/12	71c	96b
LSD($p \leq 0.05$)	47.6	52
Nitrogen (Kg/ha)	**Number of capsules per plant**	
	February-June 2012	**July-December 2012**
0	138b	78b
75	159a	139a
150	157a	164a
LSD($p \leq 0.05$)	2.8	42.4

Seed yield perplant shows that Raja yielded significantlyhigher seed ($P<0.05$) than the other cultivarsinboth seasons and sites (Table 4.13 and 4.14).In the two seasonsin Juja, theyield per plant between Summit, S19/21,Jawhar and S19/12 did not vary significantly. Inthe February-June season, the control (0 kgN/ha)gave a significantly higher seed yield while inthe June-December season, seed yield for thedifferent rates of N application was notsignificantly different (Table 4.13). In Njoro, while the yield per plant for the Summit, S19/21, Jawhar and S19/12 did not differ during the July-December season, S19/21, Jawhar and S19/12 gave lower yield than Summit in the

February-June season (Table 4.14). Raja and Summit yielded more seed per plant during the February-June season than during the July-December season. The opposite is true for the rest of the cultivars. Nitrogen did not influence plant yield during the February-June season. It however led to increased yield per plant during the following season where the lowest seed yield was given by the control.

Table 4.13: The yield per plant of five linseed cultivars as influenced by nitrogen fertilizer levels during the February-June and July-December 2012 seasons in Juja.

Cultivar	**Average seed yield (g/plant)**	
	February-June 2012	**July-December 2012**
Summit	8.938b	0.1493b
S19/21	1.513b	0.3038b
Raja	21.383a	3.2929a
Jawhar	0.837b	0.4200b
S19/12	1.343b	0.2366b
LSD($p \leq 0.05$)	11.9	0.6
Nitrogen (Kg/ ((Kg/ha)	**Average yield (g/plant)**	
	February-June 2012	**July-December 2012**
0	9.400a	0.8533a
75	4.047b	0.8101a
150	5.075b	0.9781a
LSD($p \leq 0.05$)	2.2	0.5

***Any two means not sharing a letter common in a column differ significantly at 5% probability level according to the lsd test.*

Table 4.14: The yield per plant of five linseed cultivars as influenced by nitrogen fertilizer levels during the February-June and July-December 2012 seasons in Njoro.

Cultivar	Average yield (g/plant)	
	February-June 2012	**July-December 2012**
Summit	2.41b	0.587b
S19/21	0.19c	1.002b
Raja	5.94a	4.790a
Jawhar	0.11c	0.324b
S19/12	0.19c	0.691b
LSD(p≤0.05)	1.9	2.3
Nitrogen (Kg/ ((Kg/ha)	**Average yield (g/plant)**	
	February-June 2012	**July-December 2012**
0	2.31a	0.529b
75	1.34a	0.802a
150	1.30a	0.801a
LSD(p≤0.05)	1.2	0.2

***Any two means not sharing a letter common in a column differ significantly at 5% probability level according to the LSD test.*

4.5: Discussion

The average rainfall received in both Juja and Njoro during the two seasons was below the expected level.Average temperatures were within the expected limits for the two sites. Linseed cultivars produced similar number of leaves at both sites and in all seasons.The cultivars significantly differed in the number of tillers produced, with Raja producing the most side shoots in Juja during both seasons and in Njoro in the February-June season. Summit produced the most tillers in Njoro during the July-

December season and was closely followed by Raja. The difference in number of tillers per plant among the linseed cultivars may be attributed to genetic factors.

Depending on the location, different cultivars reached varied heights. For instance, S19/12 emerged the tallest cultivar in Juja in both seasons, Raja was the shortest cultivar in Juja in both seasons, while Jawhar was the shortest cultivar in Njoro. These differences in height were however not significantly different which could indicate non significant cultivar-nitrogen interactions. Mohamed (2012) reported significant differences in heights of linseed cultivars Belinka, Strain and Hera. The cultivars used in the present study are however different from Belinka, Strain and Hera.

The pattern of dry matter accumulation during the experimental period followed that typical of many cultivated species, with exponential, linear and decreasing phases (Gardner,Pearce and Mitchell,1985). These phases were clearly visible and pronounced during both seasons of the experiment. During the early period of growth, all the treatments accumulated smaller weights but differences were visible. However, when the plants were well established in their specific environments they made the necessary adjustments to enable full exploitation of the available resources. The momentum of accumulation was maximal during the main growth period, and it declined at maturity. This later reduction in dry matter accumulation may be the result of senescence of leaves, complete dieback of basal branches and lodging during the maturity period, which was caused by heavy rains and especially during the July-December season.

Cultivars varied in biomass accumulation in both seasons: Raja accumulated much more biomass than the other cultivars in both seasons in Juja, and in the February-June season in Njoro, while Summit accumulated much more biomass during the July-December season in Njoro. Production of dry matter among cultivars has been found to vary significantly by previous researchers (Turner, 1991; Lisson & Mendham, 2000; Easson & Molloy, 2000; Couture,Asbil, DiTommaso& Watson, 2002).

Total dry matter accumulation has been shown to be a function of assimilating organs and the photosynthetic capacity of the leaf canopy (Bisco & Gallangher, 1977; Diepenbrock and Porksen, 1992). During the June -December season in Juja, an increase in number of leaves led to a linear increase in the dry weight, a relationship that did not exist during the February-June season. In Njoro, the relationship was linear in both seasons. This is perhaps due to increased sites of photosynthetic assimilates production. The higher the number of leaves, the bigger the photosynthetic surface area; translating into higher photosynthetic assimilates and hence biomass.

The significant linear relationship between plant height and dry weights could have resulted from the contribution of both the elongated stem as well as the leaves growing on the elongated stem since the longer the stem, the more the nodes, internodes and leaves.

Cultivars differed in production of capsules per plant. For example, in Juja, Raja and Summit produced the highest numbers in the February-June season and Raja in the

July-December season. Summit produced the least heads per plant during the July-December season. On the other hand, Raja had the most heads while Summit had the least in both seasons in Njoro. Similar genotype-dependent differences in the number of capsules produced by linseed has been reported before. For example, Kurt (1996) compared eight linseed cultivars and found that there were significant differences among cultivars for number of capsules per plant. Muhammad, Bismillahi Khan, (2005) reported that number of capsules per plant significantly differed among genotypes, P14-80-79-52, Randkat, PB-180, P16-80-99, Royal-4, LS-30, T-5 and Carlos-80. Linola produced significantly higher number of capsules per plant than linseed (Ali,Cheema, Sattar and Saleem, 2011) pointing to genotype dependence in this trait. The difference in cultivar' production of heads was expected going by the proven genetic differences using the 28 SSR markers (Chapter 3).

Nitrogen fertilizer plays a vital role in enhancing crop yields (Cheema *et al.,* 2001) and plays the most important role in building the protein structure (Frink *et al*., 1999). It is also an important component of chlorophyll structure, energy transfer molecules such as ATP, as well as nucleic acids such as DNA. Generally, a high rate of N increases leaf area development, improves leaf area development after flowering and increases overall crop assimilation, thus contributing to increased seed yield (Wright *et al.,* 1988). However, at least in tomatoes, excess nitrogen is associated with toxicity, reduced yields and lowered crop quality (Frias-Moreno, 2014; Upendra,Bharat and & Rahman,2009).

In the present study, the influence of nitrogen application at 75 and 150 kgN/ha did not significantly influence the number of leaves on the five linseed cultivars. Similarly, the number of tillers per plant was not significantly affected by the two levels of nitrogen fertilizer application.This is contrary to the findings of Ali *et al.,* (2011) that Linseed cv. Chandni produced significantly higher number of tillers at 100 kgN/ha than at 0, 50 and 75 kgN/ha. Moreover, working with linola and linseed at 0, 25, 50, 75 and 100 kgN/ha, they found that maximum number of tillers per plant (4.93) was produced at 75 kgN/ha while the least (2.13) was by linseed at 0 kgN/ha. Similarly, Hocking (1995) found that linola plants under adequate N-supply (140 kgN/ha) produced about 3.7 tillers per plant against 0.8 for the most N-stressed and concluded that nitrogen stress reduced the number of tillers (secondary basal stems) and fruiting branches per plant and an adequate supply of N is required throughout the growth of linola crop. Even in cultivars of other crops such as wheat and rice, a significant effect of nitrogen application on tillering has been reported (Ali,Ahmad, Ali & Hassan,2005; Ali, 2011). The fact that tillering in the present study showed no significant response to N may thus be due to genetic differences with the cultivars used in previous studies.

The rate of nitrogen application was found not to significantly affect plant height in both sites during both seasons, which is different from the results reported by Ali*et al*., (2011), probably due to differences in cultivars used. Also, the linseed cultivars used could low nitrogen users and perharps the initial nitrogen levels in the soil were sufficient.The level of nitrogen fertilizer supplied had no significant effect on dry

weight in the five tested linseed cultivars. These results contradict those reported by other researchers. For instance, Sattar,Cheema, Wahid, Saleem&Hassan,(2011) and Leleu (2000) observed that plant dry weight of shoot of canola increased with increasing nitrogen levels.

Erhart,Feichtinger and Hart (2007) found non-significant differences in the growth parameters of plants under different nitrogen application rates, and attributed this to potential migration of nitrogen from treatment plots to control plots. However, such an explanation may not be satisfactory in the present study because(i)visiblecolour differences betweenthe control (no N fertilizer application) and treatment plots were observed, indicating differences in uptake of nitrogen by these plants (data not shown) since nitrogen affects leaf colour (Bergmann, 1992)(Appendix 1),and, (ii) nitrogen application significantly affected the number of capsules and seed yield. Thus, as already suggested above, linseed generally showed limited response to nitrogen fertilizer in terms of leaf and tiller numbers or dry weight more likely to be attributed to the genetic make-up of the plant, and how the plant allocates nutrient resources.

In both Juja and Njoro, nitrogen at 75 kg/ha and 150 kg/ha produced significantly higher numbers of capsules per plant than the control plants in both seasons. This is consistent with results obtained by Rahimi,Zarei &Arminian(2011) who applied 100 kgN/ha or 150 kgN/ha. In an experiment involving two varieties (chandni and linola) and five nitrogen levels (0, 25, 50, 75 and 100 kgN/ha), the results revealed that number

of capsules per plant was among the parameters significantly affected by different nitrogen rates with N level (75 kgN/ha) producing maximum value compared to the control. Indeed, it has also been reported that increase in the number of capsules is the most important effect of N fertilization in linseed (Hocking & Pinckerton, 1991; Khajani,Irannezhad andMajidian, 2012).

The effect of nitrogen on seed yield (g) was largely dissimilar to the effect of nitrogen on the number of capsules. In the July-December season in Njoro, application of nitrogen at 75 kg/ha led to a significant increase in seed yield as compared to control plots but the difference between N application at 75 kg/ha and 150 kg/ha was not significant in the July-December season in Njoro. This is in agreement with the previous suggestion that N fertilizer requirements for linseed range between 20-80KgN\ha (Diepenbrock and Porksen, 1993; Freer, 1993; Hocking *et al.,*1997), and the results that N increases seed yield of linseed (El-Nagdy,Dalia, Eman & Gelan,2010;Upadhyay,Tiwari and Kumar, 2012; Guleria& Singh, 1984). On the other hand, both levels of N application resulted in significantly lower seed yield in both seasons in Juja, and during the February-June season in Njoro. Emam & Dewdar (2015) reported related results that the number of capsules is not necessarily proportional to the seed yield per plant, since the higher number of capsules may be associated with lower number of seeds per capsule and less seed weight. It has also been previously reported that number of seeds per capsule is not as sensitive to N as the number of capsules per plant (Khajani *et al*., 2012).In addition, lodging was observed in N fertilized plots and

this could have contributed to reduced seed yields. Koshta & Battawar (1981) reported that more than 60 kgN/ha caused extensive crop lodging with rain and reduced yield. Similarly, Hocking *et al*., (1997) reported that when water was not limiting, lodging was a problem in linseed at high N rates. Similar effect of application of high nitrogen levels on linseed cultivars was reported by Sharief,EL-Hindi, EL-Moursy& Seadh,(2005) and Sakandar,Cheema, Wahid, Sattar& Saleem, (2011).

The significant effect of 75 kg/ha nitrogen on the number of capsules and seed yield but not number of leaves, height and tillers indicates that linseed preferentially allocates resources towards seed development, which is desirable. This might be related to preferential transport of resources to the seeds. Sugar transport proteins are required for the cell-to –cell and long distance distribution of sugars throughout the plant, and higher plants have two families of these transporters, namely monosaccharide transporters and disaccharide transporter, which are controlled at various levels during plant development and when the normal environment is disturbed (Williams *et al*., 2000).

4.6: Conclusions and Recommendations

From the study, it can be concluded that:

- Plant height and number of leaves were not significantly different among the five linseed cultivars.Significant differences however existed in production of tillers, dry weights and capsules where Raja and Summitproduced significantly

more tillers, dry weight and capsules than the other cultivars. Raja yielded much more seed than the other cultivars, producing more than double the amount of seed produced by other cultivars.

- Although most of the cultivars had higher head formation in the July-December season than the February-June season, the opposite was true for the seed yield by most of the cultivars. In the February-June season, better seed yields were obtained in Juja than Njoro, while the opposite was true in the July-December season.
- There was non-significant increase in linseed's number of leaves, tillers, and height and seed yield with application of 75 and 150 kgN/ha. However, nitrogen application significantly increased the number of capsules formed seed.

Thus, with the right cultivar, it would not be necessaryfor farmers in Juja and Njoro linseed growing any of the five linseed cultivars for seed production to apply nitrogen at 0-150 kgN/ha.

It is recommended that:

1. Raja could be adopted for seed production in Juja and Njoro.
2. More trials be conducted using other linseed cultivars,in moreagro-ecological zones and mineral nutrients such as Phosphorus and Potassium.

CHAPTER FIVE

OIL CONTENT AND FATTY ACID COMPOSITION OF FIVE LINSEED CULTIVARS AS INFLUENCED BY LOCATION AND NITROGEN FERTILIZATION

Abstract

Linseed oil is rich in the health promoting fatty acids, alpha-linolenic acid (omega-3) and oleic acid (omega-9), and is increasingly being used as a nutraceutical. Despite the benefits of linseed oil, there is limited linseed production in Kenya today, necessitating an increase in its production while ensuring a high content of the two fatty acids. This study aimed to determine the oil content and fatty acid profiles of five linseed cultivars grown in a relatively warm location, Juja, with that grown in a cool location, Njoro, in two seasons (February-June and July-December 2012); as well as the effect of nitrogen fertilizer application on linseed oil contents and fatty acid profiles. Mean oil content was significantly higher ($p < 0.05$) in Juja than in Njoro (34.8% versus 32.5 %, respectively). Mean oleic acid content was significantly higher in Juja (24.2 %) than Njoro (19.0 %), while alpha-linolenic acid was significantly higher in Njoro (48.5%) than Juja (44.2%). There were inter-varietal differences in both oil content and fatty acid profiles, and the significance of these differences were both season and location-dependent. Nitrogen fertilizer application had minimal effect on oil contents and fatty acid profiles. In conclusion therefore, good quality linseed can be produced in the two agro-ecological locations.

5.1: Introduction

The linseed plant (*Linumusitatissimum*L.) is widely cultivated for fibre and oil (Bayrak,Kiralan, Ipek, Arslan, Cosge&Khawar,2010). Linseed has a high oil content of about 20-40%, and α-linolenic acid, anω-3 fatty acid, makes up 30-60% of its total fatty acid content (Green & Marshall, 1981; Bean & Leeson, 2001; Pali & Mehta, 2014). Other major unsaturated fatty acids in linseed oil are oleic acid (ω-9) which accounts for up-to 30% and linoleic acid (ω-6) which accounts for up-to 20% of the fatty acids (Green & Marshall, 1981; Bean & Leeson, 2001; Pali & Mehta, 2014). While ω-6 fatty acids such as linoleic acid and arachidonic acid are converted in human tissues to pro-inflammatory metabolites that promote the development of physiological disorders such as atherosclerosis, cancer and diabetes, the ω-3 fatty acids are converted to anti-inflammatory products with various health benefits (Deckelbaum& Torrejon, 2012). Oleic acid also has such health benefits as increasing the levels of anti-aging metabolites (Enot *et al.*, 2015), a hypotensive effect, activity against breast, bladder, prostate and oesophageal cancers (Moon,Jeong, Park, Kim, Min& Choi, 2014), and the ability to promote adaptability of cells to oxidative stress (Haeiwa,Fujita, Yasukazu and Miwa, 2014). Modern diets tend to have a high ratio of ω-6 to ω-3 fatty acids, and this has been suggested to contribute to the present rise in non-communicable diseases (Patterson,Wall, Fitzgerald, Ross& Stanton, 2012), which were previously considered to affect only high income countries, but are now a heavy burden on low income countries as well (Kankeu,Saksena,Xu &Evans, 2013). Hence there is a need to increase the

amounts of health-promoting ω-3 fatty acids and oleic acid (ω-9) in the diet, and this may be partly achieved by increasing the production and consumption of linseed oil.

In Kenya, linseed production is limited to high altitude areas with cool climate, where there is pressure for land to grow other high value crops such as tea, coffee and wheat. Thus there is a need to explore linseed production in other agro-ecological zones. This requires, among others, selection of cultivarsthat are suited for different agro-ecological zones, and determination of the effects of various agronomic conditions and practices on linseed oil quality. For linseed, two key quality parameters are oil content and fatty acid profiles, and there is literature indicating that the latter is particularly affected by environmental conditions (Lanna,Quadros &Bomfim,2005).

The objective of this study was to (i) determine and compare the oil contents and fatty acid profiles of linseeds of five different cultivarsgrown in two different agro-ecological zones, (ii) determine the effect of season on the oil content and fatty acid profiles of the linseed cultivars and (iii) determine the effect of nitrogen fertilizer application on the oil contents and fatty acid profiles of the linseed cultivars.

5.2: Materials and Methods

5.2.1: Cultivation of linseeds

Five linseed cultivarsnamely Summit, Raja, S19/21, Jahwar, S19/12 were obtained from the Kenya Agricultural Research Station, Njoro. They were grown in the farm of Jomo

Kenyatta University of Agriculture and Technology, which is located in Juja (altitude of 1416 m above sea level, (1° 10' S, 37° 7' E), and Njoro (altitude of 2423m above sea level (-0^{o} 29'S, 35^{o} 56'E latitude). The experiments were laid out as split plots in randomized complete block design with three N-fertilizer levels{0 kg N/ha (control), 75 kg N/ha and 150 kg N/ha} as main plots, and five linseed cultivars{Summit (V1), S19/21 (V2),Raja (V3), Jawhar (V4) and S19/12 (V5)} as sub-plots in two seasons (February-June 2012 and July-December 2012). Land was ploughed to a fine tilth and plots measuring 3mx 3m prepared. Drills of 20cm apart and 2cm deep were made along each of the plots. Seeds were then evenly spread along the drills and covered with a thin layer of soil. Emerged seedlings were kept weed free. Application of treatment N-fertilizer was done as CAN (26% N) and commenced 30 days after planting and was done in three equal splits, being 2 weeks apart from each other. After maturity, the seeds were harvested and dried out in the sun before extracting seeds by threshing. Seeds from each plot were bulked and weighed to determine total plot yield as described in Chapter 4. A sample of 5g seed weight was drawn from each bulked seed per plot. This was ground into fine powder then used for determination of oil content.

5.2.2: Determination of oil content and fatty acid profiles

Oil content was determined by the AOAC method (1996). Fatty acids profiles were determined by gas chromatography. In brief, fatty acids were converted to their methyl esters by the acid-catalysedmethanolysis process. Ten miligrams of the extracted oil

was refluxed with 4ml methanolicHCl (consisting of 1.5 ml of 8% HCl in 7.5 ml of methanol) for one and a half hours. After cooling, the methyl esters were extracted with hexane, and the hexane layer dried over anhydrous sodium sulfate. A fifth of a microlitre of the extract was injected into a 9A Shimadzu gas chromatograph fitted with a 3m, 15% DEGS column and a FID detector. The carrier gas was nitrogen flowing at the speed of 20 m/s. The injection port and column were maintained at 170°C while the detector temperature was 240^{o}C.

5.2.3: Data collection

Qualitative interpretationof chromatograms was done through comparison of retention times of sample fatty acid methyl esters (FAMES) with those of FAME standards. Proportionate quantification of the fatty acids was based on peak areas.

5.2.4:Data analysis

Data on percent oil content and proportions of fatty acids was subjected to analysis of variance (ANOVA) using PROC GLM in SAS 9.1.3 portable version and the means were separated using LSD procedure at the 0.05 level of significance. Levels of significance between the means of seasons as well as sites were determined using paired t-test procedure in SAS. Graphs were plotted using SigmaPlot 12.0 while tables were inserted in word.

5.3: Results

There were no significant cultivar-nitrogen interactions and therefore the results presented and for the main factors (Appendix 3). Results for the two sites were analysed separately. Similarly, results for the seasons were analysed separately.

5.3.1:Monthly temperatures in Juja and Njoro

Table 5.1 shows the average monthly temperatures in the two locations during the study period.Njoro had lower temperatures than Juja during the entire study period. In both locations, the average temperatures were higher in the February-June season than in the July–December season. However, the monthly temperatures were higher at the beginning of the February-June season and decreased towards the end of the season. On the other hand, the July–December temperatures were lower at the beginning of the season and increased towards the end. Hence the temperatures during the seed fillingstage were comparable in both seasons (late April-early June and late October – earlyDecember).

Table 5.1: Mean monthly temperatures in Juja and Njoro during the study period

Month	Njoro (^{o}C)	Juja (^{o}C)	Mean Temp. by season
February	17.9	19.7	
March	18.3	20.2	
April	18.1	20.1	
May	17.1	18.9	
June	15.9	17.3	Mean season 1=18.5±1 ^{o}C
July	15.4	16.5	

August	15.5	16.7	
September	16.3	18.3	
October	17.1	19.5	
November	17.0	19.2	
December	17.1	19.0	Mean season 2=17.3±1 ^{o}C

5.3.2: Oil content of five linseed cultivarsin two seasons, two locations

During the February-June 2012 season in Juja, oil contents of the five linseed cultivars ranged from 33.7% in Summit to 38.5% in Raja (Figure 5.1a). However, the inter-varietal differences in oil content were not significant ($p > 0.05$). In the July-December 2012 season, the oil contents reduced, ranging from 32.7 % in Raja to 36.3 % in S19/21 (Figure 5.1b). In the July-December season, S19/21 produced significantly higher ($p<0.05$)oil content than the other cultivars (Figure5.1b).The fact that Raja had the highest oil content in the February-July season and the lowest in the July-December season indicates a greater sensitivity of this cultivar to environmental conditions. Similarly to Juja, the oil contents in Njoro were higher during the February-June season (31.3 -38.4%) than the July-December season (28.0 % -31.3%) (Figure5.1c and 5.1d). However, in this location, inter-cultivardifferences in oil contents were significant in both seasons ($p<0.05$). S19/21 had the highest oil contents in both seasons, while the least oil content was produced by Jahwar (31.3 %) and Raja (28.6 %) in the two seasons, respectively. The average oil content of the five cultivarsin the two seasons was slightly higher in Juja than Njoro, and this difference was significant (Table 5.2).

However, inter-season differences in oil content were not significantin both Juja and Njoro (Table 5.3).

5.3.3: Effects of nitrogen fertilizer on oil contents

In the February-June season in Juja, application of N fertilizer at 75 kg/ha led to a significantly higher oil content ($p < 0.05$) than the control (0 kg/ha) or application of the fertilizer at 150 kg/ha (Figure 5.2a). However, there was no significant difference in oil content between the control and the 150 kg/ha treatment. In the July –December season, the control gave significantly higher oil content ($p< 0.05$) than the 75 kg/ha or 150 kg/ha treatments (Figure 5.2b). In Njoro, the control (0 kgN/ha) gave significantly higher oil content ($p<0.05$) during the February-June season than the 75 kgN/ha treatment (Figure 5.2c). The highest N level (150 kgN/ha) gave higher oil content (Figure 5.2d) during the July-December 2012 season.However, this oil content was not significantlyhigher than the oil containedin seeds cultivated under 0 and 75 kgN/ha (Figure 5.2d).

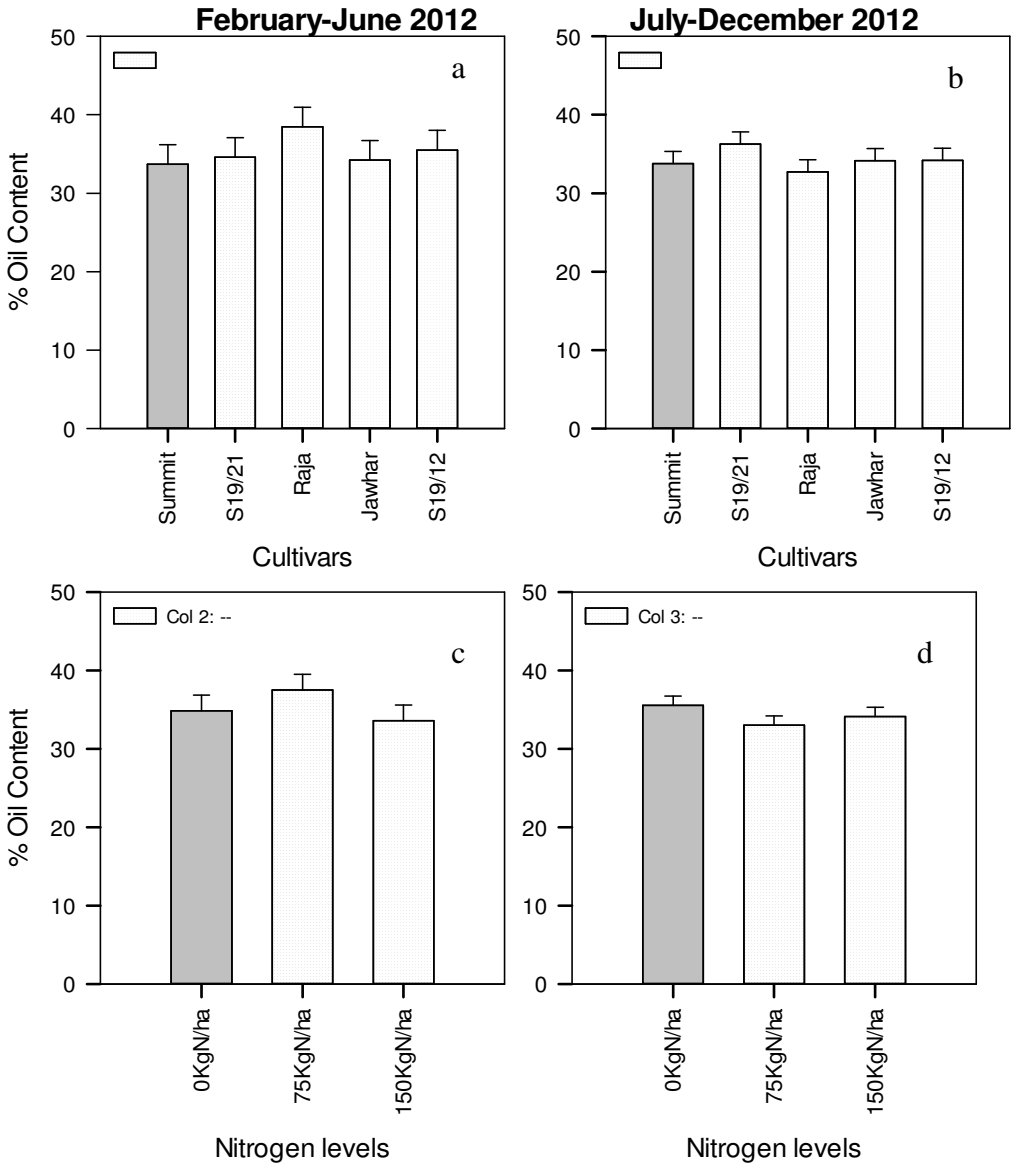


Figure 5.1: Oil content from five linseed cultivars grown in Juja (a,b) under three nitrogen levels (c,d) in two different seasons

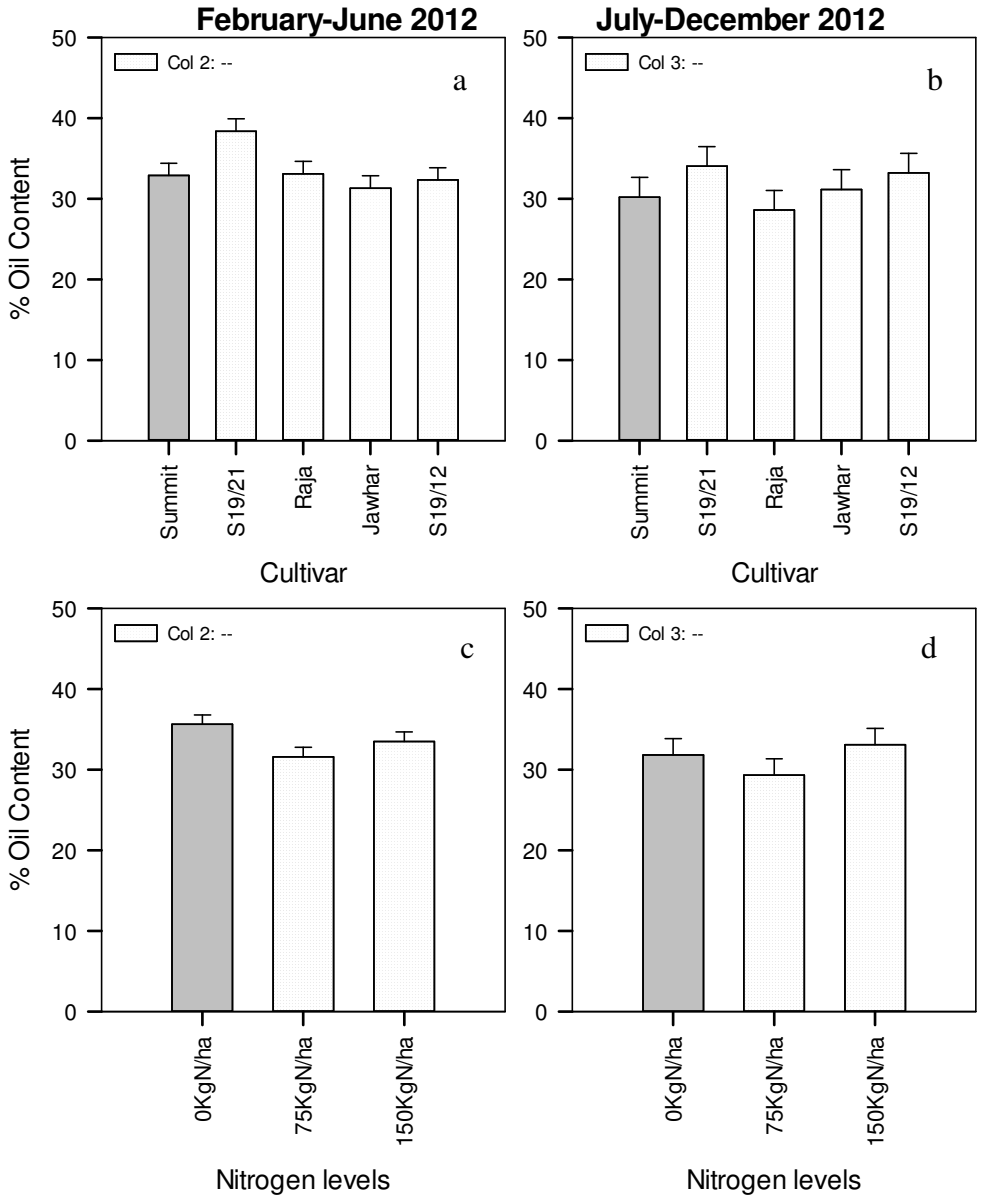


Figure 5.2: Oil content from five linseed cultivars grown in Njoro (a, b) under three nitrogen levels (c,d) in two different seasons

Table 5.2:Comparison of the mean oil contents (%), and proportions (%) of oleic acid and alpha-linolenic acid (ALA) in linseed produced in Juja and Njoro

Site	Parameter	Mean	Std dev	Std error	T statistic	Prob>t
Juja	Oil content	34.752	1.6272	0.5146	2.824	0.0199
Njoro		32.507	2.6172	0.8276		
Juja	Oleic acid	24.219	1.4186	0.4486	8.391	<.0001
Njoro		19.003	2.1082	0.6667		
Juja	ALA	44.235	2.9901	0.9455	-3.904	0.0036
Njoro		48.486	1.8527	0.5859		

Table 5.3: Comparison of mean oil contents (%) and proportions (%) of oleic acid and alpha-linolenic acid (ALA) in linseed grown in the February-June 2012 season and July-December 2012 season in Juja and Njoro.

Site	Parameter	Season	Mean	Std dev	Std	T	Prob>t
Juja	Oil content	1	35.292	1.8895	0.845	0.858	0.4391
		2	34.212	1.2876	0.5758		
	Oleic acid	1	23.884	1.2392	0.5542	-1.299	0.2637
		2	24.554	1.6468	0.7365		
	ALA (%)	1	45.174	1.705	0.7625	0.790	0.4737
		2	43.376	3.9197	1.753		
Njoro	Oil content	1	33.584	2.7576	1.2333	1.973	0.1197
		2	31.43	2.2154	0.9907		
	Oleic acid	1	17.684	2.2082	0.9875	-2.812	0.0482
		2	20.322	0.8805	0.3938		
	ALA	1	49.816	1.2601	0.5635	3.120	0.0355
		2	47.156	1.3087	0.5853		

5.3.4:Fatty acid profiles of five linseed cultivarsin two locations, two seasons

The major fatty acids in the seeds of the five cultivarswere palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2), and α-linolenic (18:3) (Tables 5.4and 5.5).

Table 5.4:Fatty acid (%) profiles of five linseed cultivars grown in Juja during February-June and July-December 2012.

		Fatty acid % and ω3:ω6 ratio						
Season	Cultivar	C16:0	C18:0	C18:1	C18:2	C18:3	ω3:ω6	ω3+ω9
1	**Summit**	8.71a	2.31a	23.32a	18.18a	45.23a	2.5:1	68.55
	S19/21	9.03a	1.85a	24.05a	19.32a	42.61a	2.2:1	66.66
	Raja	7.81a	1.27a	22.06a	18.10a	46.91a	2.6:1	68.97
	Jawhar	7.85a	1.49a	25.02a	17.13a	46.36a	2.7:1	71.38
	S19/12	8.40a	1.84a	24.97a	17.66a	44.76a	2.5:1	69.73
	Mean	8.36	1.75	23.88	18.01	45.17	2.5:1	69.06
	LSD	1.7	1.7	3.9	2.6	5.7		
Season	**Summit**	8.15a	1.70a	24.01a	18.27a	45.71a	2.5:1	69.72
	S19/21	7.13a	1.98a	23.17a	17.31a	48.22a	2.8:1	71.39
2	**Raja**	8.81a	2.42a	23.34a	19.08a	43.98a	2.3:1	67.32
	Jawhar	8.81a	2.58a	27.18b	19.30a	40.46a	2.1:1	67.64
	S19/12	8.88a	2.62a	25.07b	17.62a	38.51b	2.2:1	63.58
	Mean	8.36	2.26	24.55	18.32	43.37	2.4:1	67.93
	LSD	2.6	1.8	6.3	4.1	11.6		

*****_Values with same alphabet in each season are not significantly different along the column while values followed by different alphabets are significantly different at 5%probability level._

Table 5.5:Fatty acid (%) profiles of five linseed cultivarsgrown in Njoro during February-June and July-December 2012

		Fatty acid % and ω3:ω6 ratio						
Season 1	**Cultiv**	**C16:0**	**C18:0**	**C18:1**	**C18:2**	**C18:3**	**α3:α6**	**ω3 + ω9**
	Summi	9.55a	2.7a	13.88b	16.22b	51.3a	3.2:1	65.18
	S19/21	8.18a	2.07a	18ab	17.84ab	51.05a	2.9:1	69.05
	Raja	9.35a	2.95a	18.51a	17.92ab	49.0a	2.7:1	67.51
	Jawha	8.57a	2.21a	19.61a	18.78a	48.59a	2.6:1	68.2
	S19/12	8.9a	2.28a	18.42a	18.33ab	49.14a	2.7:1	67.56
	Mean	8.91	2.44	17.68	17.82	49.82	2.8:1	67.5
	LSD	2.2	1.5	5.0	2.3	5.4		
Season 2	**Summi**	9.49a	3.76a	20.17a	19.39a	45.75a	2.4:1	65.92
	S19/21	8.72a	3.51a	19.11a	18.75a	48.07a	2.7:1	67.18
	Raja	7.94a	3.45a	20.96a	18.16a	47.11a	2.6:1	68.07
	Jawha	9.09a	3.08a	21.37a	18.62a	46.03a	2.5:1	67.4
	S19/12	8.27a	3.17a	20.00a	17.57a	48.82a	2.8:1	68.82
	Mean	8.70	3.39	20.3	18.50	47.16	2.6:1	67.49
	LSD	2.6	1.1	2.6	2.3	6.5		

*****_Values with same alphabet in each season are not significantly different along thecolumn while values followed by different alphabets are significantly different at 5%probability level using LSD._

During the February-June season in Juja, the linseed cultivarsdid not significantly differ in their fatty acid composition (Table 5.4). However, in the July-December season, Jahwar and S19/12 had significantly higher oleic acid (18:1) content than the other cultivars(Table 18). In Njoro, there were significant inter-cultivardifferences in oleic acid (18:1) and linoleic acid (18:2) contents during the February–June season (Table 5.5). However, there were no significant differences in their fatty acid composition in the July-December Season (Table 5.5).

In both locations, there was a slightly higher average oleic acid content in the July-December season than the February-June season, while the opposite was true for α-linolenic acid, with the inter-season differences in these fatty acids being significant in Njoro but not in Juja (Table 5.3).

In both locations, the mean ω3:ω-6 ratio was higher in the February-June season than the July-December season (Tables 5.4 and 5.5), which is due to the higher amounts of α-linolenic acid contents in the former season than the latter. While the ω-3:ω-6 ratio was higher in Njoro than inJuja in both seasons, the combined percentage of α-linolenic acid and oleic acid (ω3 + ω9) was higher in Juja than Njoro.Since the amounts of α-linolenic acid and oleic acid in both sites falls within the healthy range, linseed produced in both locations can be considered to be of good value for use as a functional food.

5.3.5: Effect of nitrogen fertilizer application on fatty acid composition of linseed cultivars

As shown in Table 5.6, nitrogen application did not significantly affect the fatty acid profiles of the linseed oil in Juja in both seasons. Similar results were obtained in Njoro (data not shown).

Table 5.6: Effectof fertilizer application on fatty acid profiles of linseed in Juja

		Fatty acid (%) and ω3:ω6 ratio						
	KgN/ha	**C16:0**	**C18:0**	**C18:1**	**C18:2**	**C18:3**	**ω3:ω6**	**ω3 + ω9**
Season	**0**	8.71a	1.73a	23.04	18.6a	45.48a	2.4:1	68.52
	75	9.03a	1.37a	24.40	17.91	44.41a	2.5:1	65.81
1	**150**	7.81a	2.16a	24.22	17.72	45.64a	2.6:1	69.86
	LSD	1.3	1.3	3.0	2.0	4.4		
Season	**0**	7.85a	2.37a	24.43	17.4a	43.98a	2.5:1	68.41
	75	8.40a	2.45a	26.11	18.21	42.83a	2.4:1	68.94
2	**150**	8.71a	2.22a	25.13	19.34	43.32a	2.2:1	68.45
	LSD	2.0	1.4	4.9	3.2	9.0		

Values with same alphabet in each season are not significantly different along the column while values followed by different alphabets are significantly different at 5%probability levelusing LSD.

5.4: Discussion

Some studies on the oil contents of linseed cultivarsgrown under different agro-ecological environments have reported mean values ranging from 23- 45.7 % (Diederichsen and Fu 2008, Bayrak*et al.,* 2010; El-Beltagi*et al.,* 2007; El-Beltagi, Salama and El-Hariri,2011; Green & Marshall, 1981). The oil contents of the five cultivarsin the present study fell within this range (Figures 5.1 and 5.2). Canvin (1965) found that linseed grown under lower temperatures gave higher oil contents than that grown in higher temperatures. Because Njoro had lower average temperatures than Juja, oil contents would be expected to be higher at the former location than the latter. However, this was not the case (Table 5.1), showing that some other factor besides temperature also contributes to the oil content. Genotypic differences shown in Chapter 3 could be used to explain these differences. In both locations, the temperatures during seed filling were comparable in the two seasons, yet oil contents were higher in the February-June season than the July-December season, even if the inter-season differences were insignificant. The reason for this is not clear, but it might be linked to the fact that in the February-June season, the daily temperatures during seed filling period (late April-early June) were on a declining trend while those in the July-December season (late October-early December) were more or less constant.

Berti*et al.,* (2009) reported that N fertilization up to 200KgN/ha in South Central Chile increased oil content of linseed. Similarly, Ibrahim (2009) reported that increasing N

fertilization of linseed from 107 -179Kg/ha led to increased oil content. On the other hand, Rahimi*et al.,* (2011) found that up to 50Kg/ha of N fertilizer had no effect on linseed oil content, while 100Kg/ha and above reduced oil content. In the present study, the effect of nitrogen treatment was not only dependent on the rate of application but also on the location and the season. For example, during the February-June season, N application at 75KgN/ha increased oil content in Juja, but reduced oil content in Njoro. In both locations and seasons, there was no benefit of N application at 150KgN/ha.

Wide variations in the relative proportions of the five main fatty acids in linseed have been reported (Green & Marshall, 1981; Bean & Leeson, 2001; Pali & Mehta, 2014), with such values as 1.3 -7.6 % stearic acid, 5.0-9.9 % palmitic acid, 10.4-20.9 % linoleic, 13.3-35% oleic and 33.1 to 63.1% alpha-linolenic acids. Similar levels of these fatty acids were found in the present study (Tables 5.4 and 5.5).

The major linseed fatty acids of interest for improving human health are α-linolenic acid and oleic acid. Significantly higher α-linolenic acid contents were obtained in Njoro than Juja, while the opposite was true for oleic acid (Table 5.2). This may be attributed to the lower temperatures in Njoro than Juja in accordance with previous findings of Canvin (1965) and Gallardo,Milisich, Drago & González(2014) that higher temperatures result in higher oleic acid and lower linolenic acid contents of linseed. Similar results were obtained for rapeseed by Deng &Scarth (1998). The production of higher amounts of α-linolenic acid under lower temperatures may be explained by the

need for greater unsaturation of membrane phospholipids, which is important for maintaining membrane fluidity (Lanna *et al.*, 2005). Oleic acid is a precursor of α-linolenic acid through the successive actions of oleate desaturase and linoleate desaturase (Rabiei,Tahmasebi & Vannozzi, 2007), and this may explain the negative relationship between oleic acid and α-linolenic acid.

The result that the February-June season gave higher oil content (*vide supra*) and also gave higher α-linolenic acid content is related to the finding of Pali & Mehta (2014) that oil content in linseed was positively related with α-linolenic acid content. The same phenomenon manifests in the result that in Juja, Raja cultivarhad the highest oil content and highest α-linolenic acid content in the February-June season, while S19/21 had the highest oil content and highest linolenic acid content in the July-December season (Table 5.4).

5.5: Conclusions and Recommendations

1. From the results obtained in this study, it can be concluded that linseed grown in Juja and Njoro had acceptable oil content (32.5–34.8%) and fatty acid composition, which was largely polyunsaturated (ALA ranged between 44.5-48.5%).
2. The oil contents of the different cultivars were influenced to different extents by the seasons. Therefore, selection of a cultivar for production of oil should be

made with due consideration of the season. S19/21 produced considerably high oil in both locations and seasons.

3. Some inter-cultivar differences in fatty acid profiles were observed, but whether these differences were significant depended both on the fatty acid, the location and the season.
4. Nitrogen fertilizer application was found not to lead to much improvement, if any, in the content and quality of linseed oil in both locations tested.

It is therefore recommended that, considering the cost of nitrogen fertilizer, linseed farmers in Juja and Njoro may not need to add nitrogen to produce good quality linseed oils and in good amounts.It is also recommendedthat similar work be done in more agro-ecological regions to determine the potential for production of linseed as a functional food. Further work involving other mineral nutrients such as potassium needs to be conducted so as to establish their effect on linseed oil content and fatty acid profiles.

CHAPTER SIX

EFFECT OF WATER DEFICIT STRESS ON GROWTH OF LINSEED (*Linum usitatissimum* L.) CULTIVARS

Abstract

Linseed (*Linum usitatissimum* L.) is an annual oil crop that accounts for approximately 1% of the world's oilseed supplies. It produces seeds that are rich in the health promoting ω-3 fatty acid, α-linolenic acid. In Kenya, linseed is grown in the Rift Valley and Western regions, places which often experience drought. This study was aimed at evaluating the effect of water deficit stress on growth of three linseed cultivars, S19/12, Summit and Raja, and to establish the extent of water deficit stress' tolerance in the three cultivars. A greenhouse pot experiment in a completely randomized design and three replicates was conducted at Jomo Kenyatta University of Agriculture and Technology, Kenya for two seasons (February-May and August-November 2014). The pots, containing media of known weights, were well watered until the fourth week. After this, watering was completely withheld to a half of the pots (stressed) while the other half (well watered control) was maintained at 90% field capacity. Destructive harvesting was done when the stressed pots were at 90%, 70%, 60%, 50%, 40% field capacities and at permanent wilting point. There were no significant differences in production of leaves, plant height, number of tillers and biomass between the three cultivars in both seasons. Subjecting the linseed cultivars to permanent wilting resulted in reduced production of leaves, growth in height, production of tillers and dry weight

by 20-40%. Decline in all growth parameters begun when 30-80% of available soil water had been used up. There existed linear relationships between the various evaluated growth parameters. These relationships were not influenced either by the water status of soil or the cultivars. Relative water content for the three linseed cultivars declined after 25-67% of available soil water had been used up. As such, the tested linseed cultivars can be said to sensitive to water deficit stress.

6.1: Introduction

Linseed (*Linumusitatissimum*L.) has been a major source of industrial oil for products likepaints, linoleum, polish, inks and cosmetic (Green & Marshall, 1984; Zhang *et al*., 2007). Currently,linseed is important as a functional food from the point of view ofits nutrition and pharmaceutical value, and its nutritious components include oil, protein, lignin, resolvable fiber, mineral andvitamins (Wu *et al*., 2008). Linseed is the best source of theω-3fatty acid, α- linolenic acid (ALA), which constitutes nearly 55% of its total fatty acids.Because of these beneficial properties of linseed, there is increased interest in its production in Kenya, where its cultivation is currently negligible. Hence there is a need to determine how various factors including abiotic stresses affect linseed production such as water stress.

One of the most common abiotic stresses in agriculture today is drought, which is becoming more frequent due to climate change (Rui*et al*., 2012). Droughtcan significantly influence plant performance and survival and can lead to major constraints

in plant functioning, including a series of morphological, physiological and metabolic changes (Fischer % Turner, 1978; Ludlow & Muchow, 1990). Drought affects photosynthesis directly and indirectly and consequently dry matter production, and its allocation to various plant organs (Mayaki,Teare& Stone,1976). It also reduces leaf expansion and production, and promotes senescence and abscission (Karamanos, 1980).

Although irrigation may be used to ensure proper crop production in cases of inadequate rainfall, the availability of water for irrigation may also be a problem, especially aggravated by the climate change. Thus, it is important to understand the response of crops to water stress and the critical levels of moisture that must be available for crops to grow, and also the cultivar differences in response to water stress.

Diepenbrock, Leonand Clasen (1995) reported high linseed genotype-environment interactions in Europe, with yields varying considerably between seasons and locations. Nematallahi and Saeidi (2011) found significant differences in the response of several linseed genotypes to water stress. Ten breeding lines and 5 landraces were evaluated under two irrigation regimes based on 70 and 140 mm evaporation class A pan. Significant differences ($P<0.01$) among the genotypes for water stress indices and seed yield were recorded. Chaharmahal landrace was the most tolerant genotype and the breeding line KO_{10} was the most sensitive. Chaharmahal landrace produced 1468 and 1335 Kg/ha and genotype KO_{10} 527 and 111 Kg/ha seed yield in the first and second irrigation treatments, respectively.

The degree of drought stress can be physiologically estimated by measuring the leaf relative water content (RWC), which can control the plant response to water deficit (Hunt,Rock and Nobel, 1987, Virginia *et al.*, 2012). RWC estimates the current water content of the sampled leaf tissue relative to the maximal water content it can hold at full turgidity,and represents a balance between water supply to the leaf and the transpiration rate (Lugojan &Ciulca, 2011). Normal values of RWC range between 98% in turgid and transpiring leaves to about 40% in severely desiccated leaves, and in most crop species such as winter wheat, the typical RWC at about wilting is around 60% to 70% (Lugojan &Ciulca, 2011), with a few exceptions. Leaf relative water content (RWC) is probably a more important indicator of water status than other water potential parameters under drought stress conditions (Lugojan &Ciulca, 2011).Maintenance of a high RWC is a drought resistance mechanism, and ithas been proposed as a selection criterion for drought tolerance in many crops (Teulat *et al.*, 1997).

The objective of this studytherefore was to evaluate the effect of water deficit stress on different growth parameters and the relative water content (RWC)of three linseedcultivars, namely Summit, S19/12 and Raja.

6.2: Materials and Methods

6.2.1: Experimental site

This study investigated the effect of water deficit stress on three linseed cultivars (Summit, S19/12 and Raja) grown in the greenhouse of the department of Horticulture at the Jomo Kenyatta University of Agriculture and Technology (JKUAT), Kenya.

6.2.2: Experimental design

The experiment was setup in a completely randomized design with two treatments i.e. well watered and stressed (no watering after start of treatment). The experiment was replicated thrice and was repeated twice (February-May and August-November 2014).

6.2.3: Sowing

Pots were weighed and then filled with 2 kg soil obtained from JKUAT farm. Ten seeds were sowed in each pot before thinning to one after establishment. Soil moisture content and field capacity for this soil were determined gravimetrically. Treatments were applied four weeks after sowing and comprised withholding water application to a half of the pots (stressed) until the plants attained permanent wilting point. The well-watered control was maintained at 90% soil field capacity (FC) throughout the experimental period.

6.2.4: Data collection

Destructive harvesting was done guided by the water levels on plants under stress; at 90%FC, 70%FC, 60%FC, 50%FC, 40%FC and at end point. Data on plant height, number of leaves, number of tillers, and dry weight was recorded. Relative water content (RWC) was determined according to Turner (1986) where fresh leaves were taken from each cultivar at each harvest and weighed immediately to record fresh weight (FW). They were thenplaced in distilled water for 4h andweighed again to record turgid weight (TW). These were then subjected to oven drying at 70°C for 24h to record dryweight (DW). The RWC was calculated using the equation:RWC = ((FW - DW)/(TW - DW)) × 100.

6.2.5: Data analysis

All data were subjected to analysis of variance (ANOVA) using PROC GLM in SAS 9.1.3 portable version and means separated using LSD procedure at the 0.05 level of significance and graphs plotted using Microsoft Excel and SigmaPlot 12.0. Fraction of available soil water (FASW) was determined as follows: FASW= 1-{(a-b)/a-c}, where a=weight of pot at 100% FC; b= current weight of potunder stress, c= weight at end point.

6.3: Results

6.3.1: Number of leaves

Production of leaves by well-watered and stressed plants became significantly different ($P<0.05$) from 40 days after planting (Figure6.1). The well-watered produced 92 and 100 leaves during the February-Mayand August-November 2014 seasons respectively (Figure 6.1a, b). The stressed plants produced 74 leaves during each of the two seasons. However, there were no significant differences in the three cultivars'production of leaves in response to moisture levels in both seasons (Figure 6.1c, d).

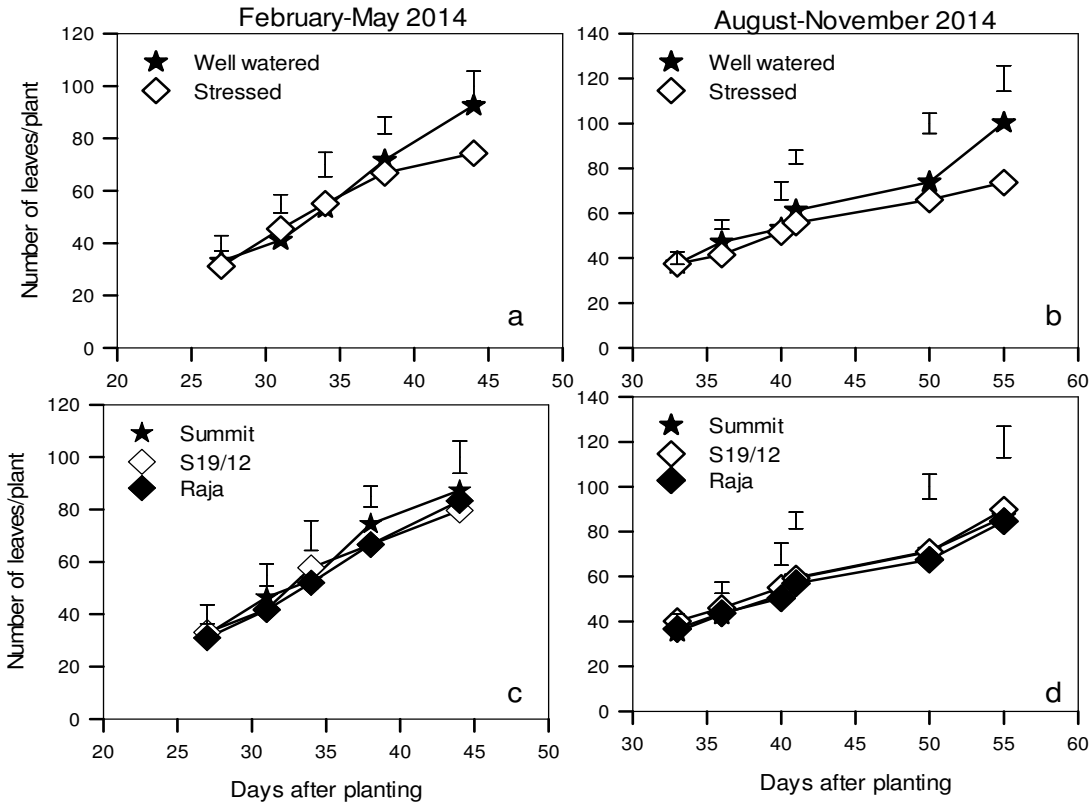


Figure 6.1:Production of leaves by linseed cultivars grown under water stress during the February-May and August-November seasons 2014.

6.3.2: Plant height

The well-watered and stressed plants did not vary significantly in height until after 40 days after planting. Well-watered plants grew to heights of 39cm while stressed plants reached 31cm (Figure 6.2a, b).Soil moisture level did not significantly influence plant height for the three tested linseed cultivars.(Figure 6.2c, d). Summit and Raja were

taller than S19/12 during the February-May season while all the cultivars attained similar heights of 34cm in the August-November season.

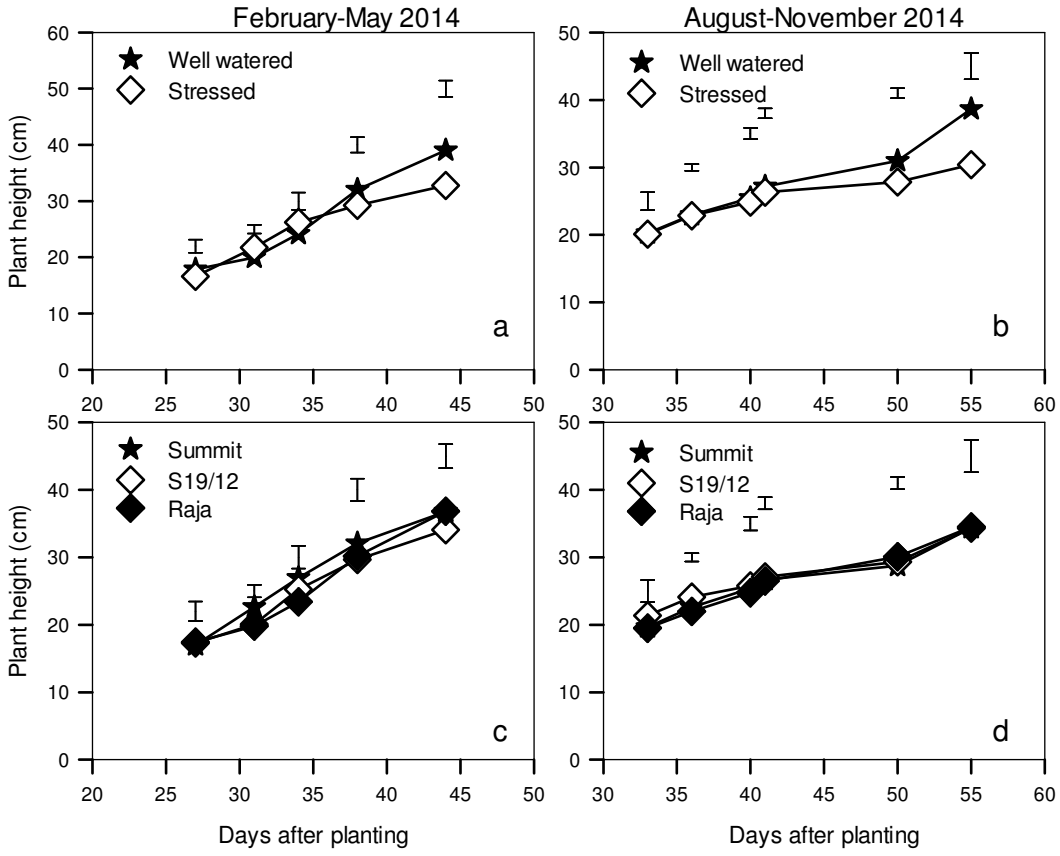


Figure 6.2:Effect of water stress on plant height during the February-May and August-November seasons 2014.

6.3.3: Number of tillers

In both February-May and August-November 2014 seasons, the number of tillers was higher in well-watered plants from 40 days after planting (Figure 6.3a, b). The

difference was significant only during the August-November months (P<0.05). The well-watered plants produced 4-5 tillers compared to 3-4 tillers in stressed plants.In both seasons, S19/12 produced the highest number of tillers with 4-5 tillers, while the least number of tillers was produced by Raja in both seasons with 2-3 tillers. These differences in the number of tillers among the three linseed cultivarswere however not significant (Figure 6.3c, d).

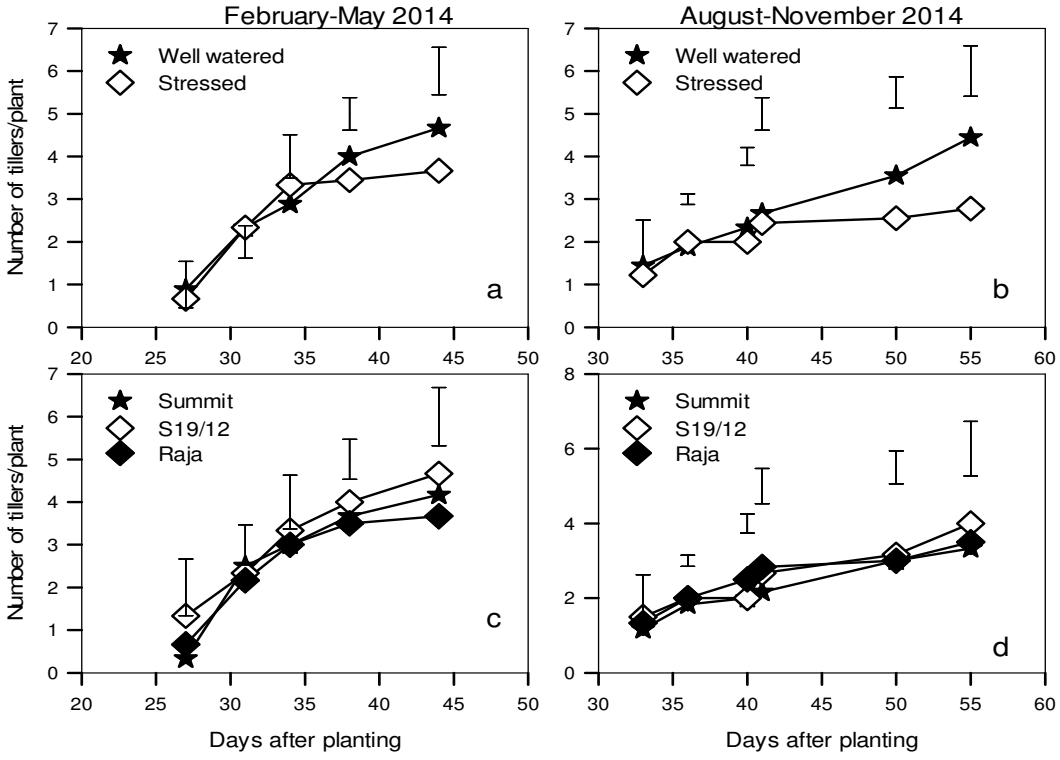


Figure 6.3:Mean number of tillers produced by linseed cultivars grown under water stress during the periods February-May and August-November, 2014.

6.3.4: Dry Weight

There was significant difference in dry weight between well-watered and stressed plants beyond 40 days after planting in both seasons (Figure 6.4). Wellwatered plants produced significantly higher dry weight ranging from 0.73-0.82 g/plant compared to 0.35-0.52 g/plant for stressed plants (Figure 6.4a, b). In the February-May 2014 season, S19/12 gave significantly higher dry weight (0.77g) (Figure 6.4c) compared to Raja (0.64g) and Summit (0.60g) ($P<0.005$) from 45 days after planting. However, in the August-November months, the three cultivars did not differ significantly in dry weight even at day 55, although S19/12 still gave higher dry weight than the other two cultivars(Figure 6.4d).

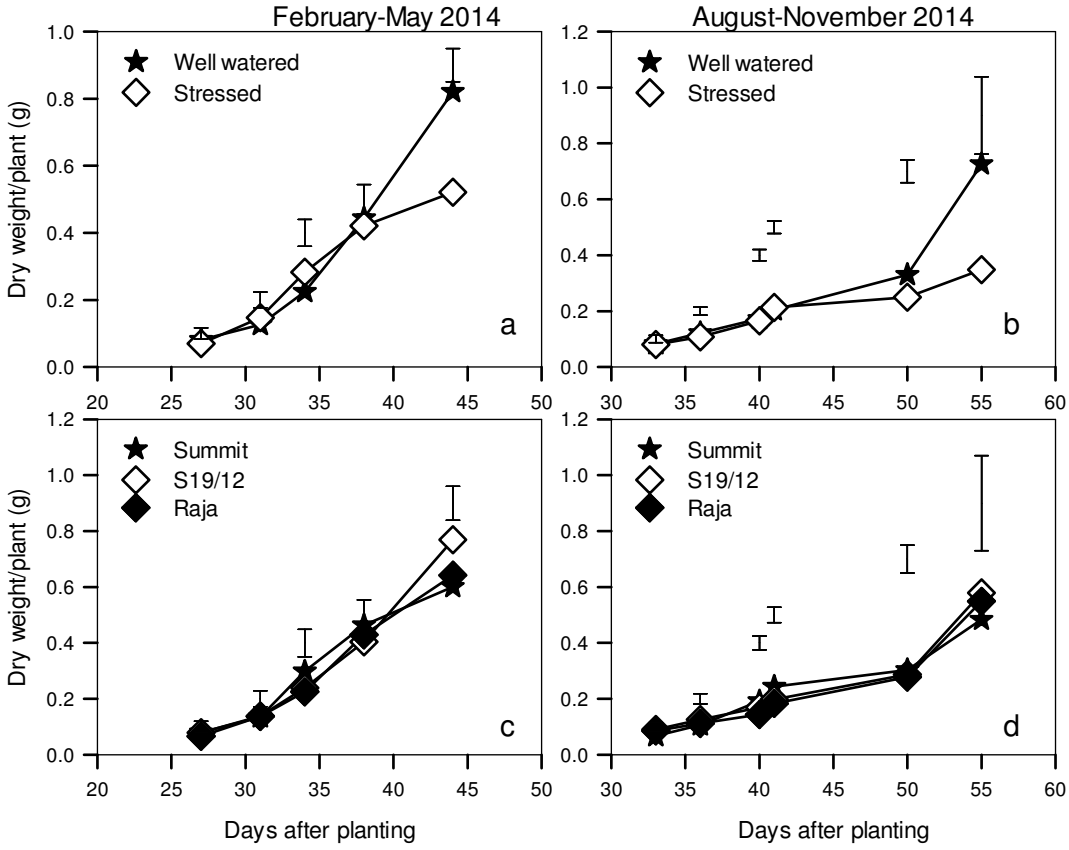


Figure 6.4:Dry weight accumulation by linseed grown under water stress during the February-May and August-November seasons 2014.

6.3.5: Growth ratios and relationships

Decline in production of leaves in both seasons started at fraction of available soil water (FASW) of 0.5 (Figure6.5a, b, Table 6.1). The decline was similar for the three cultivars. Production of leaves however ceased at a point when the ratio of leaves of the stressed plants to the well watered was 0.7-0.75.

During the February-May season, decline in plant height for the stressed plants begun at FASW of 0.56. This decline however started at 0.20 FASW during the August-November season (Table 6.1).Severe stress caused plants to cease increasing in height. In both seasons, this happened when the height of the stressed plants was 0.7-0.8 that of the well watered plants (Figure 6.5c, d).

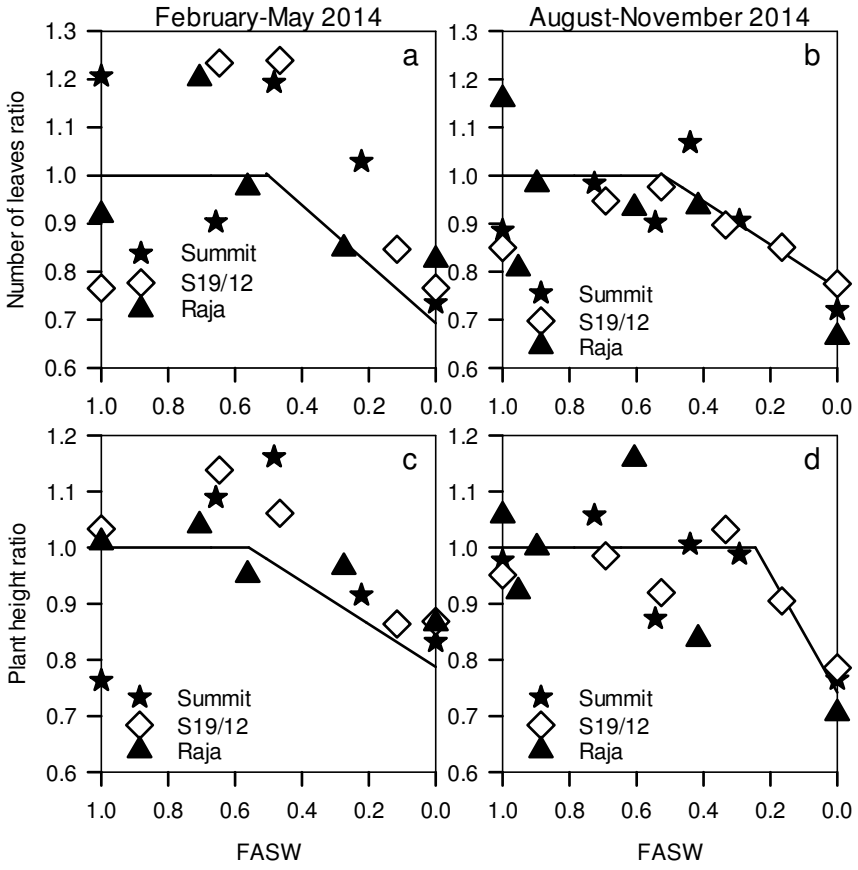


Figure 6.5:Ratio of number of leaves (a,b) and plant height (c,d) of stressed plants to well watered plants in response to changing FASW for linseed cultivarsgrown during the February-May and August-November periods, 2014.

Table 6.1: **Relationship between slope and critical points and their standard errorsfor the non-linear functions in Figures6.5, 6.8 and 6.10.**

	February-May				August-November			
	Slope	Std err	Critical point	Std err	Slope	Std err	Critical point	Std err
Leaves	0.29	0.0000	0.50	0.0000	0.72	0.000	0.52	0.0000
Height	0.38	0.0005	0.56	0.0004	1.08	0.462	0.20	0.0000
Dry weight	1.17	0.0006	0.43	0.0001	0.81	0.0007	0.62	0.0003
%RWC	1.66	0.0006	0.33	0.00008	1.26	0.0006	0.75	0.0002

For the three cultivars, increase in number of leaves resulted in a linear increase in dry weights (Figure6.6, Table 6.2). The rate of this increase was independent of the cultivarand was similar in both seasons. In the two seasons, a unit increase in leaf number resulted in 0.01 times increase in dry weight for all the cultivars.

Table 6.2: The slope and intercepts and their standard errors for the linear functions in Figure 6.6

Cultivar	February-June					July-December				
	Slope	Std err	Intercept	Std err	R^2	Slope	Std err	Intercept	Std err	R^2
S19/12	0.01	0.001	0.258	0.062	0.970	0.008	0.0006	0.228	0.034	0.981
Raja	0.014	0.003	0.437	0.156	0.897	0.01	0.0012	0.336	0.077	0.938
Summit	0.011	0.001	0.319	0.05	0.983	0.01	0.001	0.314	0.0079	0.927

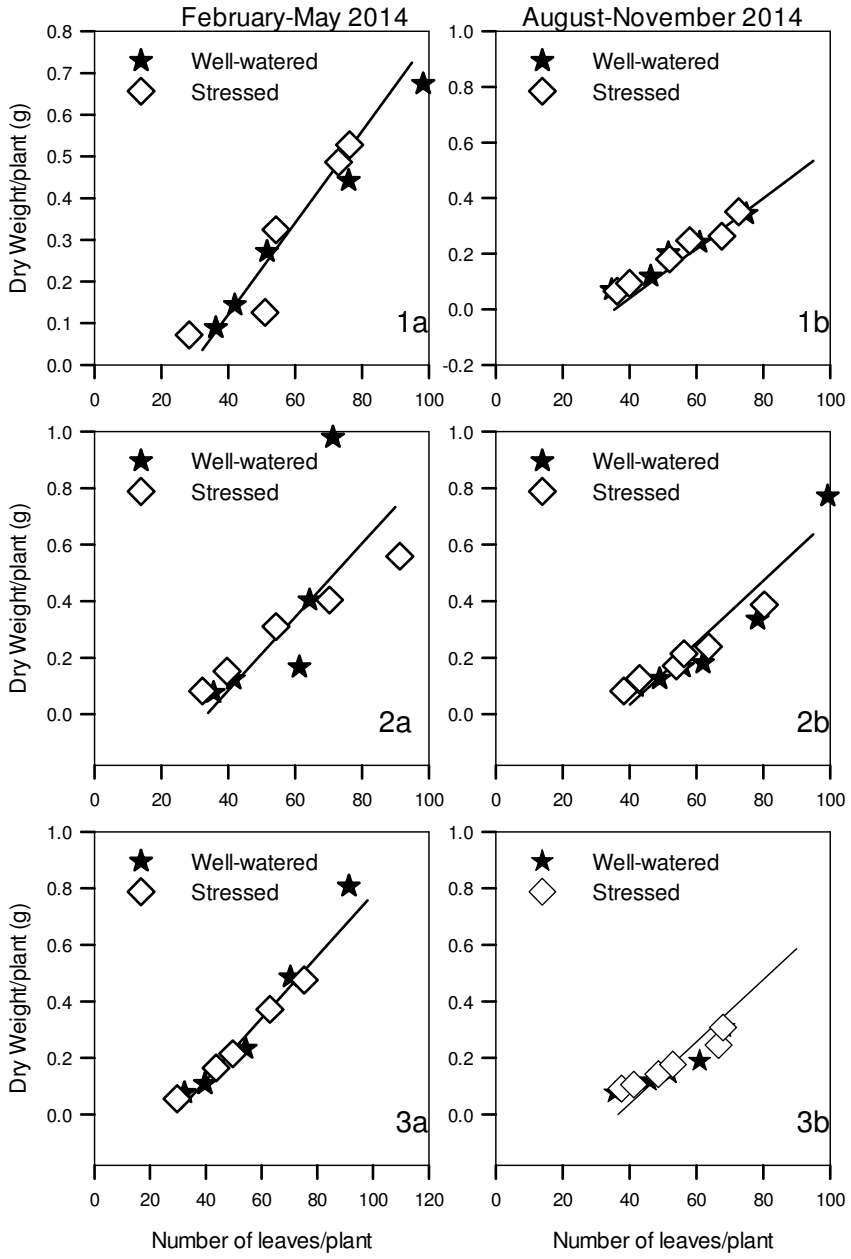


Figure 6.6:Relationship between number of leaves produced and dry weight accumulated by linseed cultivarsSummit (1a,1b), S19/12 (2a,2b) and Raja (3a,3b) grown during the February-May and August-November periods, 2014.

There was a linear relationship between increase in plant height and increase in plant dry weight for all the cultivarsin both seasons (Figure 6.7). A unit increase in plant height for S19/12 and Summit resulted in 0.028-0.030 times increase in dry weight during both seasons (Table 6.3). A unit increase in height for Raja produced 0.038 times increase in dry weight in both seasons. This increase was significantly higher than that for S19/12 and Summit.

Table 6.3: **The slope and intercepts and their standard errors for the linear functions in Figure 6.7**

Cultivar	**February-June**					**July-December**				
	Slope	Std err	Intercept	Std err	R^2	Slope	Std err	Intercept	Std err	R^2
S19/12	0.028	0.003	0.436	0.08	0.969	0.029	0.002	0.521	0.042	0.988
Raja	0.038	0.007	0.642	0.191	0.900	0.038	0.005	0.775	0.141	0.930
Summit	0.029	0.001	0.452	0.019	0.998	0.030	0.005	0.547	0.139	0.889

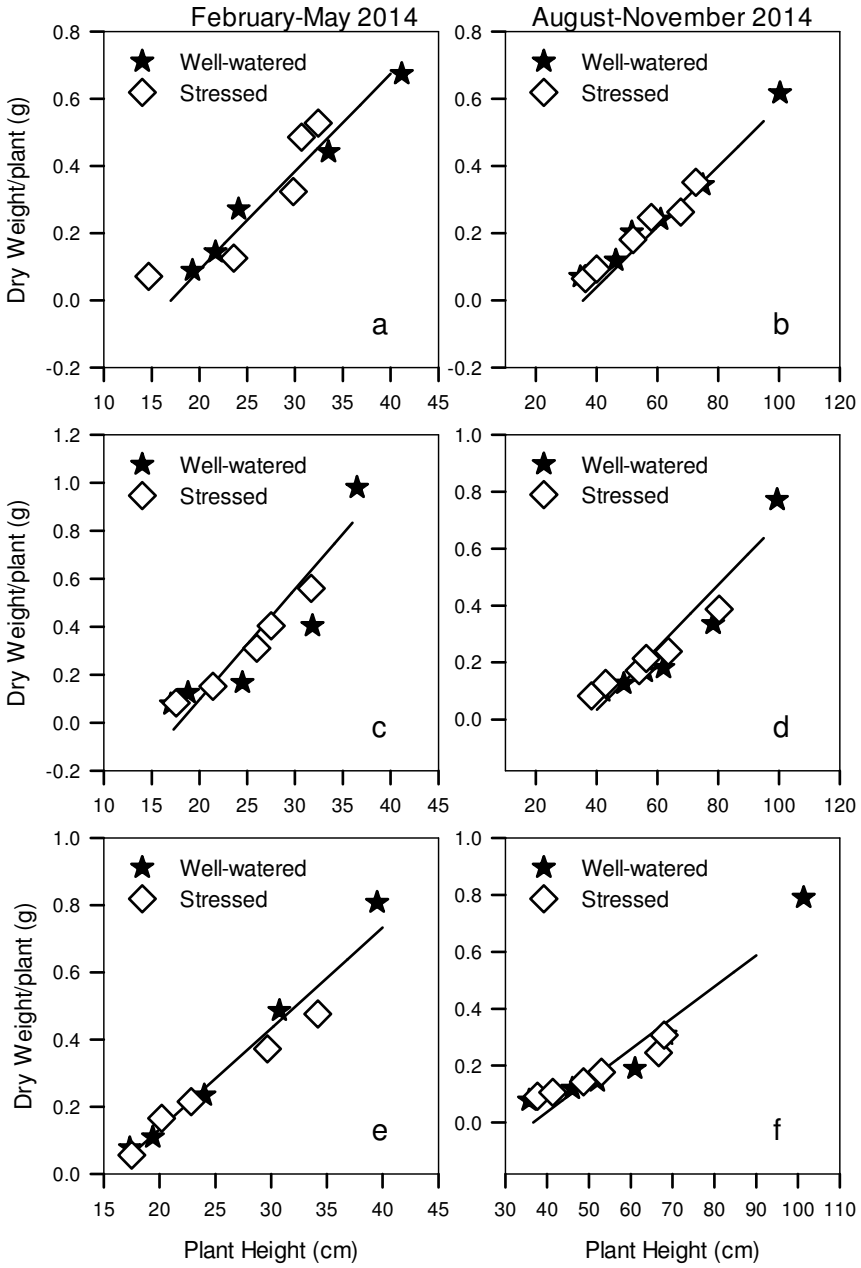


Figure 6.7: The relationship between plant height and dry weight for linseed cultivarsSummit (a,b), S19/12 (c,d) and Raja (e,f) grown during the February-May and August-November periods, 2014.

Decline in the rate at which plants accumulated biomass caused by water stress during the February-May 2014 season started at 0.43 FASW (Table 6.1). It was however reached at 0.62 FASW during the August-November 2014 season. In both seasons, severe water stress caused plants to cease accumulating biomass. This occurred when the ratio of biomass of the stressed to well watered plants was 0.5(Figure 6.8).

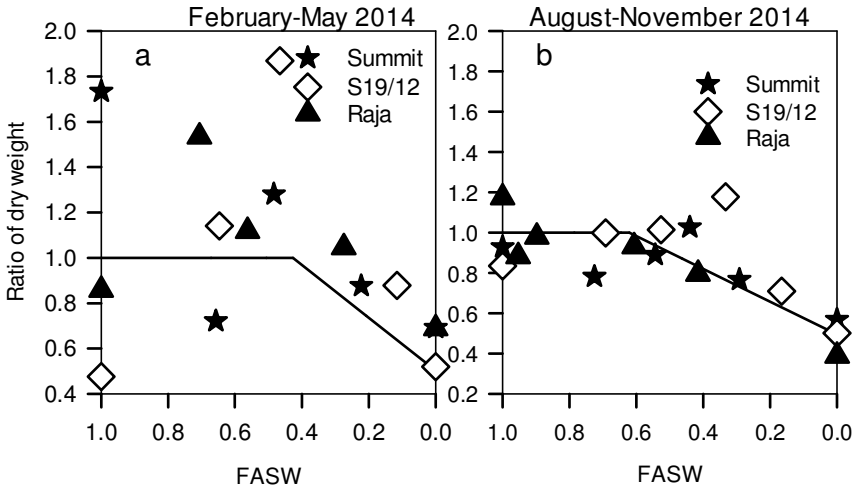


Figure 6.8: Ratio of plant dry weight of stressed plants to well watered plants in response to changing fraction of available soil water (FASW) for linseed cultivarsgrown during the February-May (a) and August-November periods (b), 2014.

There was a linear increase in plant dry weight with increase in tillers for all the cultivarsin both seasons (Figure 6.9). A unit increase in number of tillers resulted in

0.130-0.188 times increase in dry weight; for the three cultivarsin both seasons (Table 6.4).

Table 6.4: The slope and intercepts and their standard errors for the linear functions in Figure 6.9

Cultivar	**February-June**					**July-December**				
	Slope	Std err	Intercept	Std err	R^2	Slope	Std err	Intercept	Std err	R^2
S19/12	0.130	0.04	0.04	0.121	0.782	0.180	0.030	0.171	0.071	0.898
Raja	0.188	0.052	0.26	0.17	0.814	0.184	0.032	0.230	0.085	0.895
Summit	0.173	0.051	0.133	0.139	0.794	0.187	0.061	0.248	0.160	0.704

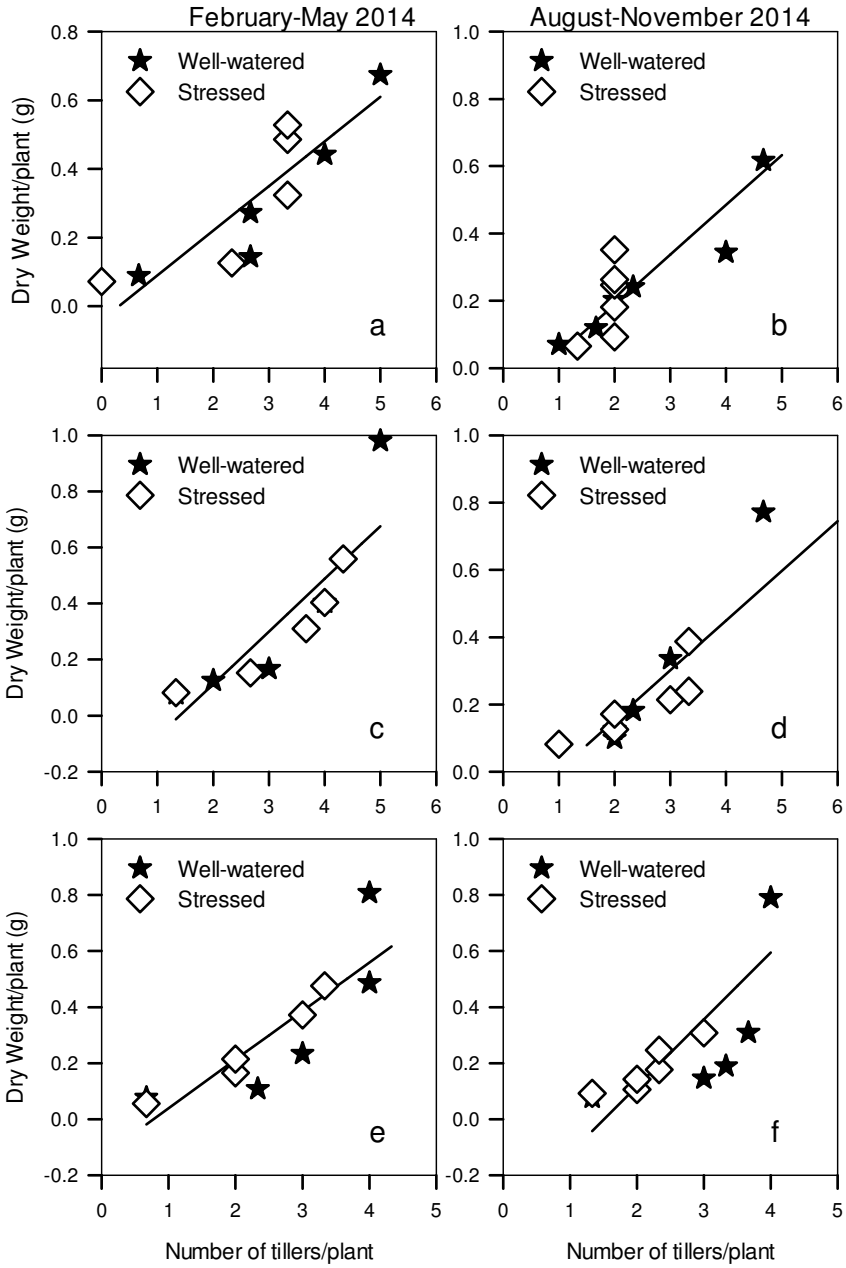


Figure 6.9:Relationship between tillering and accumulation of dry weight by linseed cultivarsSummit (a,b), S19/12 (c,d) and Raja (e,f) grown during the February-May and August-November periods, 2014.

6.3.6: Relative water content

Reduction in relative water content caused by water stress during the February-May 2014 season started at 0.33 FASW (Table 6.1). This decline begun at 0.75 FASW during the August-November 2014 season. As available water decreased, so did the plant's relative water content(Figure 6.10).

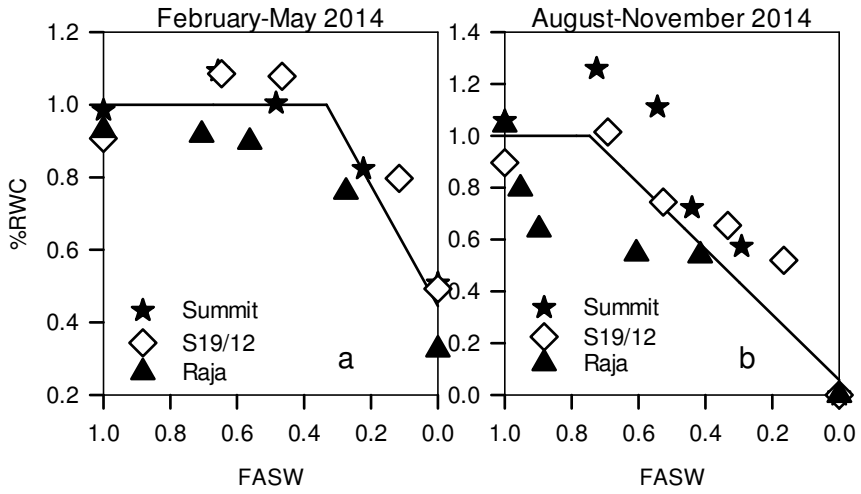


Figure 6.10: Relationship between relative water content of stressed plants and well watered plants,and changing fraction of available soil water (FASW) for linseed cultivarsgrown during the February-May (a) and August-November (b) periods, 2014.

6.4: Discussion

The three tested cultivarsdid not vary in their production of leaves. Results of a field research also found that these cultivarsdid not differ significantly in leaf production as discussed earlier in this thesis (Chapter 4). Exposure to conditions of water stressresulted in a significant reduction in the rate of production of leaves. This could be as a result of reduced rate of leaf initiation with setting in of water stress (Turay,McKenzieand Andrews, 1992). The decline in the rate of production of leaves was first indicated when 50% of available water had been used up in both seasons. Water stress can exert a strong influence on leaf area development by decreasing leaf appearance rate (Turay*et al.,* 1992), duration and leaf expansion rate (Turner, 1997) and increasing the rate of leaf senescence and abscission (Sinclair,Tanner& Bennett*,* 1984). Bazzaz & Harper (1977) found the total leaf area of linseed to be largely determined by the number of leaves and therefore concluded that a reduced leaf number due to water stress probably accounted for the low leaf area index in rain fed plants. For the three linseed cultivars, production of leaves was severely affected by water stress.

During both seasons, there was a relationship between production of leaves andaccumulation of dry matter in the threecultivars; anincrease in number of leaves was accompanied by a corresponding increase in dry weight.

A unit increase in number of leaves resulted in a linear increase in dry weight, a relationship which was similar for the three cultivars. Leaves arephotosynthetic sites

and therefore the more the leaves the more the photosynthates are produced and accumulated in the plant. Total dry matter accumulation has been shown to be a function of assimilating organs and the photosynthetic capacity of the leaf canopy (Bisco and Gallangher, 1977; Diepenbrock& Porksen, 1992).

Water stress caused a decline in height of the three tested linseed cultivars. Well watered plants reached heights of 39 cm. Stress produced shorter plants whose heights averaged 31cm; this was true in both seasons. Summit and Raja cultivarsreached the heights of 40cmduring the February-May season. These were taller than S19/12 which grew to 36cm high. As plant available water reduced so did the rate of plant growth in height. Many earlier studies on other crops such rice (Davatgar,Neishabouri & Soltani, 2009), maize (Muhammad,Hussain& Muhammad,2001;Cassel, 1986; Hernandez, 1980) and safflower (Mohammad,Majnounhusseini, Amiri, Moslehi& Omid2012) have reported reduction in plant height due to water stress which they attributed to inhibition of cell elongation or cell division. The same can be said of the three linseed cultivars. In this study, a reduction of 21% in height resulted due to water stress. There was a positive linear relationship between increase in plant height and increase in dry weight for the three linseed cultivars. This was similar to the results of fieldexperiments as reported in Chapter 4.Plant cell division causes plant elongation hence increase in plant height. As these cells mature, they have a direct contribution to increase in biomass.

Linseed has been reported to produce 4.93 (Sakandar, 2011) and 4.46 (Mohammad *et al*., 2012) tillers. In the current study, water stress caused a significant reduction in production of tillers during the two seasons, of up to 25%. Well watered plants produced 4-5 tillers which reduced to 3-2 tillers per plant for those plants where stress was imposed. There were no variations in number of tillers produced by the three cultivars, though S19/12 produced a high number of 4-5 tillers in comparison to Raja which produced lowly 2-3 tillers. Studies on wheat cultivars Inqlab-91 and Uqab-2000 byAkram (2011) found significant reduction in tillers due to water stress. Imposition of water stress at the stem elongation and anthesis stage caused a reduction from 698.8 to 663.0 number of tillers per m^2.A minimum reduction in tiller numbers per hill (from 19 to 18) on basmati ricecultivarsBasmati-Super, Shaheen-Basmati and Basmati-385 was reported by Akram,Ali, Sattar, Rehman& Bibi(2013). This was attributed to the fact that at the time of water stress, maximum tillers had been developed by the plants. In a greenhouse experiment, with two sugarcane genotypes (CP 80-1743 and CP 01-2390), Zhao,Barry and Comstock, (2012) reported significant reduction in number of tillers due to water stress. During the 2009 experiment, the tillers ranged from 1-4 and 2-6 tillers per plant for the stressed and well watered pots respectively. The numbers rose to 5-8 and 6-10 tillers per plant for the stressed and well watered pots respectively in 2010.Gabiana (2005) reported as many as twice branches/plant in irrigated (2.5) compared to the unirrigated (1.2) linseed plants. Increase in dry weight was linearly

related to increase in tillers. This is because each individual additional tiller had an accompanying biomass.

During both seasons S19/12 had higher dry weight (0.75-0.80) compared to Summit and Raja which averaged 0.60-0.66. This is perhaps from the higher number of tillers it produced during both seasons. The well-watered plants produced 0.73-0.82g/plant of dry weights in both seasons. This was higher than that of the stressed plants, which produced 0.35-0.52g/plant dry weights. This could have resulted from the individual direct contribution to dry weight by number of leaves and plant height, parameters which also varied significantly between the well-watered and stressed plants. As water stress affected individual parameters, the whole was translated into an effect on dry weight. The total biomass produced by a crop during its life cycle, in response to theexisting environmental conditions can be defined as dry matter (Hassan &Leitch, 2001). Environmental factors can indirectly influence crop dry matter productionthrough their effect on the rate of photosynthesis and respiration (Robertson, 1984).Chartzoulakis,Noitsakis and Therios(1993) reported 60-65% reduction in dry weight in Kiwifruit cv. Haywardgrowing under severe water stress, in a glasshouse. Halil,Tasand Higgs(2001) studied the effect of water stress on eggplant (*Solanum melongena* L. cv., Teorem F1). They observed a 27-43% reduction in dry weight under severe water stress conditions (60% pot capacity-40% pot capacity) which was attributed to metabolic regulation of adaption to water stress. Total dry matter production in unirrigated plots was significantly lower than in irrigated plotsthroughout

the life cycle of the linseed crop (Gabiana, 2005). Irrigation increased dry weight by 59% from 509g/m^2 to 763g/m^2. In this study, accumulation of dry weight reduced with reduction in available soil water. The tested linseed cultivarshad only accumulated 55% of potential dry weight by the time they completely dried.

Relative water content (RWC) reduced with decrease in available soil water for the three tested linseed cultivars(Figure6.10). During the February-May season, the %RWC started to decline at 0.33 FASW while the decline begun at 0.75 FASW during the August-November season (Table 6.1). The RWC parameter is considered as one of the easiestagricultural parameters that can be used toscreen for plants' drought tolerance (Boutraa,Abdella, Abdulkhaliq&Ali, 2010).Droughttolerantplant species maintained high RWCcompared with drought-sensitive species incultivars of sugarcane (Marcelo,Jifon, da Silva& Sharma, 2007). Stoyanov(2005) reported that water stress causes a decrease inRWC in beans species. Tambussi,Bartoli, Beltrano,Guiametand Araus,(2000) reported that cultivars of wheat under waterstress showed a decrease in the RWC. There are many reports aboutthe direct relationship between relative watercontent and drought resistance (Shimshi,Mayokal & Atsmon, 1982; Merah, 2001; Schonfeld,Johnson, Carver& Mornhinweg,1988) from which deductions on ability to adjust intracellular water relationsunder drought stress conditions have been made. The variation in the FASWs at which %RWC declined during the two seasons could have arisen from the differences in environmental relative humidity, which could have been higher during the August-November season than during the February-May season. This

could have lowered the rates of evapotranspiration thus enabling the soil to continue holding more water for longer (data not presented). The tested linseed cultivars can be considered as drought-sensitive since, during the February-May season they maintained a very low %RWC.

6.5: Conclusions and Recommendations

- From the study, it can be concluded that when the five linseed cultivars were subjected to water deficit, the rate of production of leaves, growth in height, dry weight accumulation and the relative water content began to decline at different soil water stress levels (0.2-0.7).
- The sensitivity of plant height varied within seasons where decline in rate of increase in height began at 0.56 FASW or 0.2 FASW in the February-May and August-November seasons, respectively.
- In both seasons, decline in rate of leaf production begun when half of the available soil water had been used up (0.5 FASW).
- Relative water content begun to decline at 0.33 FASW during the February-May season and at 0.75 during the August-November season.Due to the sensitivity of the RWC to water deficit during the August-November season, the three cultivars can be said to be susceptible to water stress.

- However, further studies need to be done to determine the minimum FASW levels required for normal growth and seed production by the various linseed cultivars.

CHAPTER SEVEN

GENERAL CONCLUSIONS AND RECOMMENDATIONS

7.1: Conclusions

Thirteen linseed cultivars evaluated for genetic diversity by SSR markers were found to be genetically different and fell into three clusters with the first cluster having one cultivar and the second and third clusters having 7 and 5 cultivars respectively, with no overlaps within the clusters.

Five linseed cultivars were found not to differ significantly in height and leaf production. However, Raja had significant higher number of tillers, dry weight, and seed yield

Application of nitrogen fertilizer at 0-150 kgN/ha levels did not significantly influence the growth parameters. While it resulted in significantly higher head production, this did not translate into higher seed yield.

Linseed in both Juja and Njoro contained acceptable quantities of oil (32.5-34.8%). This oil contained good amounts of essential fatty acids alpha-linolenic acid and oleic acids, with levels ranging between 44.2-48.5% and 19.0-24.2% respectively.

The five linseed cultivars tested were susceptible to water deficit.Optimal growth of the three linseed cultivars may not be achieved below 70% soil field capacity.

7.2: Recommendations

It is recommended that:

1. Linseed cultivation could be adopted in Juja.
2. Cultivar Raja could be usedfor cultivation in Juja and Njoro as well as other similar agro-ecological regions with minimal application of nitrogen.
3. Agronomic performance, oil content and oil quality evaluation should be evaluated on more cultivars, in more agro-ecological zones and using other mineral nutrients such as Potassium.
4. Crop water use for linseed should be determined inorder to optimize the timing, amount and response of linseed to water resources.

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APPENDICES

Appendix 1: Linseed showing colour differences in response to nitrogen fertilization (control vs 150 kgN/ha) in Juja.

Appendix 2: Average monthly rainfall and temperature for Juja and Njoro, 2012.

	JUJA		NJORO	
Month	Average rainfall (mm)	Average temp. (^oC)	Average rainfall (mm)	Average temp. (^oC)
Jan.	38	21.5	34	20.2
Feb	27	19.7	43	17.9
Mar.	33	20.2	16	18.3
Apr.	60	20.1	16	18.1
May	45	18.9	51	17.1
Jun.	11	17.3	45	15.9
Jul.	8	16.5	23	15.4
Aug.	7	16.7	28	15.5
Sep.	4	18.3	7	16.3
Oct.	30	19.5	8	17.1
Nov.	43	19.2	42	17.0
Dec.	27	19.0	38	17.1

Appendix 3: ANOVA tables

Number of leaves Juja February-June 2012

Dependent Variable: Lvs

Source	DF	Type I SS	Mean Square	F Value	Pr > F
rep	**2**	**70.18791213**	**5.09395601**	**0.42**	**0.2649**
N	**2**	**12.90019266**	**4.50096302**	**60.26**	**0.7733**
N*rep	**4**	**237.1309430**	**59.2827357**	**2.39**	**0.0832**
vareity	**4**	**848.3392680**	**212.0848170**	**8.56**	**0.0003**
vareity*N	**8**	**381.1023987**	**47.6377998**	**1.92**	**0.1100**

Number of leaves Njoro February-June 2012

Dependent Variable: Lvs

Source	DF	Type I SS	Mean Square	F Value	Pr > F
rep	**2**	**2600.044444**	**1300.022222**	**3.61**	**0.0427**
N	**2**	**1945.377778**	**972.688889**	**2.70**	**0.0877**
N*	**4**	**4082.222222**	**1020.555556**	**2.83**	**0.0469**
vareity	**2**	**1993.866667**	**498.466667**	**1.38**	**0.2696**
vareity*N	**4**	**4667.066667**	**583.383333**	**1.62**	**0.1718**

Number of leaves for Juja July-December 2012

Dependent Variable: Lvs

Source	DF	Type I SS	Mean Square	F Value	Pr > F
rep	**2**	**453.8231452**	**226.9115731**	**0.93**	**0.1687**
N	**2**	**380.4929421**	**90.2464711**	**0.62**	**0.2207**
N*rep	**4**	**825.9618202**	**06.4904551**	**0.76**	**0.1734**
vareity	**2**	**550.2634351**	**37.5658591**	**0.17**	**0.3506**
vareity*N	**4**	**2198.986565**	**274.8733212**	**0.34**	**0.0647**

Number of leaves for Njoro July-December 2012

Dependent Variable: Lvs

Source	DF	Type I SS	Mean Square	F Value	Pr > F
rep	**2**	**428.5777778**	**214.2888889**	**6.95**	**0.0042**
N	**2**	**30.04444441**	**5.022222**	**0.49**	**0.6202**
N*rep	**4**	**111.0222222**	**27.7555556**	**0.90**	**0.4792**
vareity	**2**	**870.3555562**	**17.5888889**	**7.06**	**0.0007**
vareity*N	**4**	**167.5111111**	**20.9388889**	**0.68**	**0.7050**

Number of tillers for Juja February-June 2012

Dependent Variable: TIL

Source	DF	Type I SS	Mean Square	F Value	Pr > F
rep	**2**	**1.244444**	**0.622222**	**2.37**	**0.0693**
N	**2**	**2.977777**	**1.488888**	**0.89**	**0.4236**
N*rep	**4**	**0.622222**	**0.155555**	**0.09**	**0.9837**
vareity	**2**	**15.46666**	**6.866666**	**2.31**	**0.0868**
vareity*N	**4**	**8.800000**	**1.100000**	**0.66**	**0.7224**

Number of tillers for Njoro February-June 2012

Dependent Variable: TIL

Source	DF	Type I SS	Mean Square	F Value	Pr > F
rep	**25.644444442.822222221.930.1668**				
N	**21.911111110.955555560.650.5290**				
N*rep	**40.622222220.155555560.110.9791**				
vareity	**437.555555569.388888896.430.0012**				
vareity*N	**88.977777781.122222220.770.6337**				

Number of tillers for Juja July-December 2012

Dependent Variable: TIL

Source	DF	Type I SS	Mean Square	F Value	Pr > F
rep	**23.124458871.562229441.990.1596**				
N	**24.308571432.154285712.740.0854**				
N*rep	**43.253333330.813333331.040.4101**				
vareity	**428.247222227.061805568.990.0002**				
vareity*N	**810.894444441.361805561.730.1437**				

Number of tillers for Njoro July-December 2012

Dependent Variable: TIL

Source	DF	Type I SS	Mean Square	F Value	Pr > F
rep	**21.911111110.955555560.930.4065**				
N	**20.311111110.155555560.150.8597**				
N*rep	**44.222222221.055555561.030.4108**				
vareity	**46.000000001.500000001.470.2432**				
vareity*N	**83.466666670.433333330.420.8951**				

Plant height for Juja February-June 2012

Dependent Variable: Hght

Source	DF	Type I SS	Mean Square	F Value	Pr > F
rep	**222.309544211.154772217.620.0031**				
N	**23.755629271.877814631.280.2971**				
N*rep	**414.699768173.674942042.510.0710**				
vareity	**46.049202291.512300571.030.4122**				
vareity*N	**816.096568542.012071071.380.2612**				

Plant height for Njoro February-June 2012

Dependent Variable: Hght

Source	DF	Type I SS	Mean Square	F Value	Pr > F
rep	**2**	**35.74514501**	**7.87257250**	**0.45**	**0.6408**
N	**2**	**40.66594292**	**0.33297140**	**0.52**	**0.6035**
N*rep	**4**	**332.9413667**	**83.23534172**	**0.11**	**0.1118**
vareity	**4**	**623.4720691**	**55.86800173**	**0.96**	**0.0138**
vareity*N	**8**	**373.5535764**	**46.69419701**	**0.19**	**0.3496**

Plant height for Juja July-December 2012

Dependent Variable: Hght

Source	DF	Type I SS	Mean Square	F Value	Pr > F
rep	**2**	**58.83956043**	**29.41978022**	**0.59**	**0.0977**
N	**2**	**1.207981706**	**0.039908005**	**0.05**	**0.9483**
N*rep	**4**	**55.84142311**	**3.960355812**	**0.30**	**0.3273**
vareity	**4**	**201.7209015**	**50.43022544**	**0.44**	**0.0088**
vareity*N	**8**	**56.85926517**	**0.107408106**	**0.30**	**0.7475**

Plant height for Njoro July-December 2012

Dependent Variable: Hght

Source	DF	Type I SS	Mean Square	F Value	Pr > F
rep	**2**	**3.667111111**	**1.833555562**	**0.36**	**0.1158**
N	**2**	**0.619111110**	**0.309555560**	**0.40**	**0.6755**
N*rep	**4**	**2.687555560**	**0.671888890**	**0.87**	**0.4988**
vareity	**4**	**86.83688889**	**21.70922227**	**7.96**	**<.0001**
vareity*N	**8**	**8.943111111**	**1.117888891**	**0.44**	**0.2310**

Dry weight for Juja February-June 2012

Dependent Variable: D1

Source	DF	Type I SS	Mean Square	F Value	Pr > F
rep	**2**	**0.061459140**	**0.030729571**	**0.88**	**0.2652**
N	**2**	**0.003506300**	**0.001753150**	**0.11**	**0.9006**
N*rep	**4**	**0.087283380**	**0.021820841**	**0.34**	**0.3924**
vareity	**4**	**0.022496360**	**0.007498790**	**0.46**	**0.7254**

vareity*N **60.164136670.027356111.680.3210**

Dry weight for Njoro February-June 2012

Dependent Variable: DW

Source	DF	Type I SS	Mean Square	F Value	Pr > F
rep	**2**	**0.00524321**	**0.00262161**	**0.09**	**0.9136**
N	**2**	**0.03400908**	**0.01700454**	**0.59**	**0.5639**
N*rep	**4**	**0.03686915**	**0.00921729**	**0.32**	**0.8619**
vareity	**4**	**0.33216367**	**0.08304092**	**2.88**	**0.0481**
vareity*N	**8**	**0.13626436**	**0.01703305**	**0.59**	**0.7753**

Dry weight for Juja July-December 2012

Dependent Variable: DW

Source	DF	Type I SS	Mean Square	F Value	Pr > F
rep	**2**	**0.02531776**	**0.01265888**	**2.57**	**0.0971**
N	**2**	**0.02063856**	**0.01031928**	**2.10**	**0.1446**
N*rep	**4**	**0.01363242**	**0.00340810**	**0.69**	**0.6041**
vareity	**4**	**0.06002587**	**0.01500647**	**3.05**	**0.0363**
vareity*N	**8**	**0.12621546**	**0.01577693**	**3.21**	**0.0727**

Dry weight for Njoro July-December 2012

Dependent Variable: DW

Source	DF	Type I SS	Mean Square	F Value	Pr > F
rep	**2**	**0.00038142**	**0.00019071**	**1.17**	**0.3265**
N	**2**	**0.00022283**	**0.00011142**	**0.69**	**0.5135**
N*rep	**4**	**0.00037548**	**0.00009387**	**0.58**	**0.6817**
vareity	**4**	**0.00508374**	**0.00127094**	**7.82**	**0.0003**
vareity*N	**8**	**0.00108511**	**0.00013564**	**0.83**	**0.5816**