THE EFFECT OF ETHYLMETHANESULFONATE (EMS) ON MORPHOLOGICAL CHARACTERISTICS AND SEED QUALITY DEVELOPMENT OF VERNONIA

(*Centrapalus pauciflorus* var.*ethiopica* Willd.**)**

By

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DECLARATION

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As supervisors we approve the submission of this dissertation for examination:

Signed ……....…….. Date …….

Dr Alfred Odindo (Supervisor)

Signed ……....…….. Date …….

Prof. Hussein Shimelis (Co-supervisor)

ABSTRACT

Vernonia (*Centrapalus pauciflorus* Willd.) belongs to the Asteraceae family (Compositae). The crop can produce epoxidized vernolic acid oil that can be used by industries to produce products such as paints.Crop production is significantly hampered by non-uniform seed maturity, lodging due to tall plant height, seed shattering and lack of appropriate technologies. There is need for research to address these challenges and improve productivity.

Firstly, this study compared two selected vernonia mutant lines (Vge-1 and Vge-4) and untreated controls with respect to morphological traits and seed quality development. A field experiment was conducted as a factorial design with 2 lines (Vge-1 and Vge-4) and 2 treatments (ethylmehanesulphonate treated and untreated seeds) at the University of KwaZulu- Natal Research farm at Ukulinga in Pietermaritzburg. The experiment was laid out in a Randomized Complete Block design (RCBD) with four replications, thus giving 16 experimental units (plots measuring 3mx6m). Data was collected on plants height, leaf number of secondary heads, mass of heads per plant and yield. Highly significant differences ($P<0.001$) were observed with respect to leaf number and seed mass.Vge-1 mutants plant produced more leaves (48 leaves per plant) compared with untreated controls (40 leaves), Vge-4 treated seeds had higher mass of heads per plant (2.46g) compared untreated controls Vge-4 with (0.75g). Vge-4 had seed yields of 3.5 ton/ha compared with untreated controls (3ton/ha). The effect of EMS application on growth parameters in vernonia lines resulted in an increase in leaf number, mass of seed per heads and seed yield of Vge-4; this line could be important than Vge-1 for potential use of vernonia as a new industrial crop.

Secondly, the study determined the pattern of seed quality development of Vge-1 and Vge-4 compared to untreated controls. Vernonia flowers were tagged at flowering stage and sampled at weekly intervals from seed development up to maturity. Twenty seeds from each line (both treated and untreated controls) at each developmental stage were used to determine solute leakage using conductivity meter (CM100). Percentage germination was determined using a growth chamber set at a constant temperature of 25^{o}C. Samples of twenty seeds were used for determination of seed moisture content using Kett's PM650 seed soil moisture meter (Kett instruments, USA). Viability tests were done using 2, 3, 4-triphenyl tetrazolium chloride (TZ) solution. Seed viability was evaluated by assessing the proportion of stained embryo according to the ISTA Rules and Methods for Seed Testing. Five seeds of each line (both treated and untreated controls) were scanned using an electron microscope to observe morphological changes during embryo development. The results showed that germination (%) was generally

low but differed significantly (P<0.01) between the lines; Vge-1 untreated seeds had the highest germination percentage (60%) compared with Vge-1 treated with (58%). The low germination percentage in vernonia could probably be attributed to seed dormancy.

Thirdly, the study investigated the effect of gibberellic acid (GA) and potassium nitrate on seed dormancy in vernonia lines. A total of 100 seeds per treatment (treated seeds of Vge-1 and Vge-4 and untreated controls) were subjected to three different temperatures regimes (25/25°C; 25/17°C or 30/17°C) and 0.7mM gibberellic acid or 1mM $KN0_3$. Seeds germination was assessed on daily basis by recording seeds with a radicle protrusion of at least 2mm. Highly significant differences and interactions (P<0.001) were observed between temperatures and dormancy breaking chemicals with respect to percentage germination, mean germination time (MGT) and germination index (GI).The GI increased with GA_3concentration application. The mean germination time (MGT) also improves for all treatments.

Fourthly, the effect of EMS on seedling growth was investigated. Harvested seeds of the two lines Vge-1 and Vge-4 were soaked in 0.372%, 0.744% and 1.1% EMS solutions for 2 hours and rinsed in water for 30 minute. The experiment was laid out as 2x4x4x3 treatment structure using a completely randomized design with the following factors: Vernonia lines with 2levels (Vge-1 and Vge-4); EMS concentration 4 levels (0.372%, 0.744%, 1.1 % and control); duration time 4level (0.5, 1, 1.5 and 2 hour) and temperature 3 levels (30^oC, 32.5^oC and 35^oC) replicated 3 times, giving a total of 96 treatments combinations and 288 experimental units. The treated seeds were sown in seedling trays filled with sterilized soil. The following data were collected; seedling emergence, seedling length, germination percentage and the presence of chlorophyll mutants. Highly significant differences (P<0.001) were observed between EMS treatments with respect to seedling vigor, germination percentage and seedling height. The seedling length decreased with increased EMS concentration. EMS concentration increased emergence percentage and germination index. Increasing EMS concentrations, temperature, exposure time and duration negatively affected on all the traits measured in the study. EMS had the effect of causing mutations as evidenced by the various chlorophyll mutants identified in the study. The major findings of this study suggest that EMS as mutagen was effective in inducing genetic variability in vernonia. This suggests that EMS can be used for creating new vernonia lines.

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DEDICATION

Most importantly, I would like to dedicate this work to our Heavenly Father, the Almighty who made everything possible, and carried me through all the challenges. I also dedicated this work to my supportive and loving wife Blandine Mbango for being a pillar at all the time when I wanted to give up and to our beloved daughters Cecile Bwandola-Bolondo-likola, Josee-Bwandola-Lolema, Kerene Bwandola Bekebeke, Andreas Bwandola-Boyanee and Boyanee-Lifafu-Mercy for everything I am because you are and you are because I am (Prophet TB Joshua). Finally, I dedicate this work to my late parents Bwandola-Getumbe-Stephane and Bolondo-Limbaya- Esther. May your souls rest in peace.

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LIST OF ABBREVIATIONS

AWS	Automatic weather station
EMS	Ethylmehanesulphonate
Vge-1 and Vge-4	Accession number of lines Vge-1 and Vge-4, respectively
DES	Diethylsulphate
EI	Ethylene mine
DMSO	Dimethyl sulfoxide
DMS	Dimethylsulphate
MNH	Methyl nitroso urea
ENU	Ethyl nitroso urethane
TMX	Maximum Temperature
MN	Minimum Temperature
PVC	polyvinylchloride
AOSA	Association of Official Seed Analysts
ISTA	International Seed Testing Association
SEM	Scanning electron microscopy
LM	Light Microscope

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1. CHAPTER 1: GENERAL INTRODUCTION

1.1 Background of the crop

Vernonia (*Centrapalus pauciflorus* Willd.) belongs to the family Asteraceae (Compositae) and is a potential new industrial oil crop. Vernonia is cultivated mainly for the production of triglyceride oil. Earlier, it was known as *vernonia galamensis* Cass Less. Until Robinson (1999) underlined that the African annual or perennial herbs of vernonia should be included to the genus Centrapalus Cass, as *Centrapalus pauciflorus* Willd. H. Rob. (Syn.*Centrapalus galamensis* Cass.). Vernonia (*Centrapalus pauciflorus)* is now recognized, according to Gilbert (1986), to include six subspecies, namely: *pauciflorus mutomoensis*, *nairobensis*, *afromomntana*, *gibbosa*, and *lushotoensis*. Subsp. p*auciflorus*is the most widely distributed; it is highly diverse and has four botanical varieties, namely var. *galamensis*, var. *petitiana*, var. *australis and* var. *ethiopica*) (Gilbert, 1986).

Vernonia is believed to have originated from Eastern Africa. It has the ability to grow under low rainfall and marginal conditions and is most suitable for dryland farming (Perdue Jr, 1988). Due to the high oil and vernolic acid content and its relatively low shattering nature, var. *ethiopica* has been the focus of research and its production in some parts of the world reaches semi commercial scales (Baye et al., 2001).Vernonia seed produces a naturally epoxidized oil with several industrial applications such as in additives in flexible polyvinyl chloride (PVC), in plasticizers, adhesives, epoxy resins, insecticides and crop oil concentrates (Thompson et al., 1994). Industries produce epoxy oils by modifying petrochemicals and through epoxidation of oils from seeds of soybean (*Glycine max* L.) and linseed (*Linum ustitatissimum L*.) (Ray, 1994). Synthetically epoxidized oil is expensive and contains volatile organic solvents with high emission to the environment during processing and end use. Therefore, the natural epoxy oil extracted from vernonia seed is possibly useful in substituting the volatile solvents. Vernonia is predominately self-fertilizing and a short day crop (Baye and Becker, 2004). The seed from Vernonia crop produce triglyceride oil which is environmental friendly, less expensive and less viscose compared to other artificial epoxy oil (Mohamed et al., 1999). Vernolic acid constitutes 72-80% of the acids present in the seed oil. Vernonia oil, moreover, contains linoleic acid (12-14%), oleic acid (4-6%), stearic acid (2-3%), Palmitic acid (2-3%) and trace amounts of Arachidic acid (Gilbert, 1986). Considerable variation in agronomic traits, oil content and fatty

acid compositions are reported in *C. Pauciflorus* collections from East Africa (Shimelis et al., 2008).

1.2 Problem statement

On the global oil production, 80% is used for human food, 6% used as animal feed, and the remaining is used in industrial applications (Derksen et al., 1995). In order to meet the necessary demand for oils, different plant species have been domesticated and cultivated for centuries, and more recently, have been bred specifically for edible and industrial end products. Mainly, sunflower, canola, cotton and sunflower are used as oilseed crops, are good sources of both common and uncommon fatty acids. There is a growing interest in finding new alternative crops for oil production. Vernonia oil is naturally epoxidized and is a potential substitute for some of the oils which are under pressure from environmental legislation. It has low viscosity which allows it to be used as a non-volatile ingredient in oil-based paints. Vernolic acid is an important resource in the manufacturing of paints and coatings and has great value in the oleo–chemical industry. Epoxy fatty acids are widely used in oleo–chemical industry as plasticizers and stabilizers of polyvinyl chloride (PVC), in reformulation of oil based paints, in cosmetics, and pharmaceutical applications (Bhardwaj et al., 2000). Currently, vernonia is under investigation to be planted commercially in South Africa. However, in order to ensure the wider adoption of vernonia, the general mode of use of the crop needs improving. But, the successful support of vernonia as an alternative and semi-arid crop hinges on the availability of information describing its agronomic practices. Production and succeeding commercialisation of vernonia is hampered by some agronomic problems such as seed shattering resulting in massive seed losses as well as lack of appropriate harvesting, seed threshing and cleaning and seed retention methods. In addition the uneven maturity could be associated with problems of seed dormancy (Nyamongo et al., 2009). Previous studies reported that ethylmehanesulphonate (EMS) is an effective chemical mutagen, extensively used to induce genetic variability in a number of crop plants (Kumar et al., 2005). There is little information on the effect of EMS on morphological characteristics and seed quality development in vernonia.

1.3 Significance of the study

This study will contribute to a deeper understanding of the seed quality and oil characteristics of vernonia as a potentially new industrial crop.

1.4 Aims and objectives of study

The overall aim of this study was to compare two selected vernonia mutant lines Vge-1and Vge-4 with respect to morphological characteristics and seed quality development. The specific objectives of the study:

1. To evaluate the morphological and seed characteristics of vernonia lines Vge-1 and Vge-4 treated with a and untreated (controls),
2. To examine the pattern of seed quality development in mutants of vernonia lines Vge-1 and vge-4 and untreated controls plants,
3. To determine the effect of gibberellic acid and potassium nitrate on seed dormancy in vernonia lines,
4. To investigate the effect of EMS concentration, exposure time and temperature on seedling vigor of vernonia lines Vge-1 and vge-4.

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2. CHAPTER II: LITERATURE REVIEW

2.1 Introduction

The family "Asteraceae" also known as "Compositae" is a largest angiosperm family consisting of about 1 300 genera and 25 000 species distributed all over the world and in almost all habitats. Most of the members of this family are annual or perennial; xerophytes, succulents or normal mesophytes; herbs, shrubs, climbers and occasionally trees. Asteraceae is one of the largest families of flowering plants (about 10%) with ca. 24 000-30 000 species in 1 600-2 000 genera, distributed in all continents but Antarctica and arid regions as well as tropical and subtropical mountains. Asteraceae are most abundant in dry places in temperate and subtropical regions as well as mountainous areas near the Equator. Many Asteraceae are cultivated as ornamentals and there are few species that are grown for edible parts, including the production of oil seed.

2.2 Origin of the crop

Centrapalus Pauciflorus (Willd.) originated in the eastern and south eastern parts of Ethiopia and is endemic primarily to East African countries (Baye et al., 2001). is an annual crop, growing naturally in marginal areas with as little as 200 mm seasonal rainfall and at an elevation ranging from 700 to 2400 m above sea level (Gilbert, 1986). The distribution of *Centrapalus pauciflorus* (Willd.) Subsp. *Centrapalus* Willd. V*ernonia filisquama* is recorded from several locations in northern Tanzania (Figure 2.1). *Vernonia pauciflorus* (Willd.) sub sp. *pauciflorus* includes 4 varieties distributed from West Africa, east to Sudan and Ethiopia, then south to Zimbabwe and Mozambique. This plant is widely distributed in eastern and western Hararghe in Ethiopia and is known to farmers by the local names "Ferenkundela", 'Dunfare', 'Kefathebogie' and" Noya", which have different connotations in different localities (Baye, 1996).Vernonia has been grown experimentally and semi-commercially in several places in the world, including Zimbabwe (Perdue Jr et al., 1989), Costa Rica, Dominican Republic, Italy (Perdue Jr et al., 1989), Ethiopia (Baye, 1996), and the United States (Thompson et al., 1994).

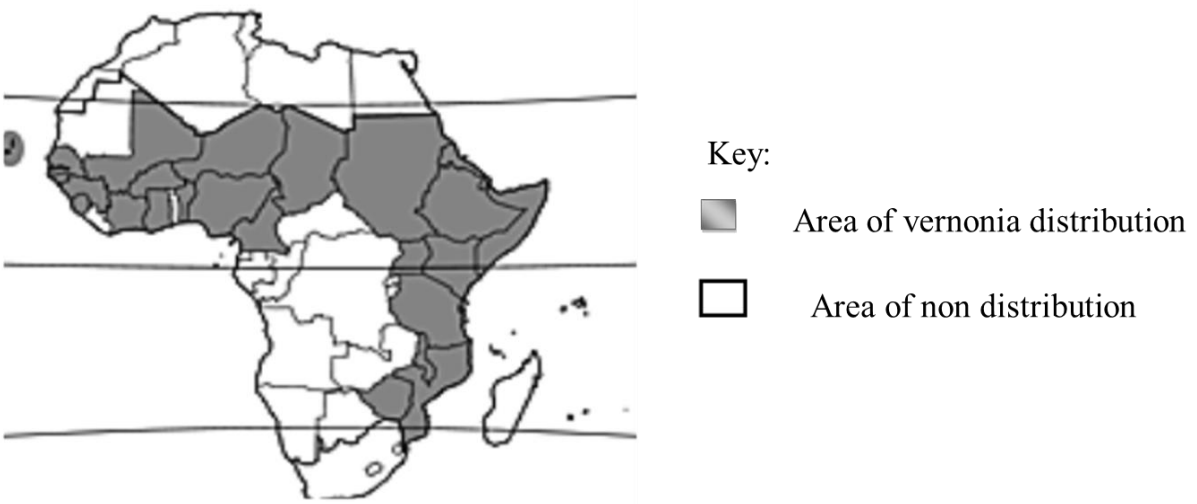

Figure 2.1: A map showing the distribution of vernonia in the Africa continent (Damania et al., 2008).

2.3 Plant genetic diversity and morphology

The greatest diversity of vernonia is found in east Africa while a single variety occurs in West Africa. The genus vernonia comprises of more than a thousand species which vary from annual herbs and shrubs to perennial trees (Baye et al., 2001). There are six major subspecies namely *afromomntana*, *galamensis*, *gibbosa*, *nairobensis*, *lushotoensis* and *mutomonesis*. Among these, galamensis shows the highest genetic diversity (Gilbert, 1986). It contains four botanical varieties namely *australis*, *ethiopica*, *galamensis* and *petitiana* (Gilbert, 1986).

2.3.1 *Centrapalus pauciflorus var ethiopica*

This species often called ironweed is the largest source of vernonia oil, which is rich in a useful epoxy fatty acid. Vernonia oil is used to make plastics, rubbery coatings, and drying agents. It is grown in many parts of Ethiopia, especially around the city of Harar, with an average seed yield of 2 to 2.5t/ha. It is reported that the Ethiopian strains of vernonia have the highest oil content; up to 41.9% with up to 80% vernolic acid (Perdue et al., 1986). Vernonia is similar to daisy which is quite variable in appearance between individual plants. It produces a flower which may be blue or purple, with long petals surrounding a centre made up of shorter petals called florets. The centre is filled with seeds. Depending on environmental conditions, the plant may be short and shrubby or extremely tall. It may send out few stems, each with one flower, or many stems with one flower or many stems with bunch of flowers.

When not found in cultivation, the plant may be found growing wild, often as a weed which invades disturbed areas and rangeland.

2.3.2 *Vernonia phenotypic description*

Florets are long and exerted at anthesis. The colour ranges from bright blue to pale mauve blue, purple, almost white and, sometimes are flushed pale yellow or green. They can grow up to 14 mm in length. Florets are tubular on the lower half and they expand slightly above. The stamens and stigma are similar in colour to the corolla, slightly exerted, and anthers obscurely articulate at base. Cypsela colours range from very dark brown to black uniformly covered with dense, slightly oppressed silky hairs. Outer pappy of barbellate setae are between 1 and 5 mm high. The inner part of copious is off-white to brown and the sordid barbellate setae is up to 8 mm long (Gilbert, 1986). Chromosome counts of accessions are 2n = 18. Sub-species readily hybridize among themselves (Gilbert, 1986). The shoot can grow up to 5 m in height, but are usually much shorter. The stem is ribbed, finely to coarsely hairy and sometimes branching near the top (Gilbert, 1986). Leaves are arranged in an alternate format and are sessile and membranous. The length and breadth can be up to 25 x 0.6-5 cm, with each leaf being broadest at about the middle. The length/breadth ratio ranges from 20:1 to 2.3:1 depending on taxon. The tip is usually foliaceous, often markedly reflexed. The innermost phyllaries are scarious, oblong to narrow-lanceolate with an acuminate tip, sometimes with prominent sub-apical teeth (Gilbert, 1986).

2.3.3 Palynology of vernonia

Palynology provides useful data for the intragenic classification of the large genera (Nyananyo, 1987). Seed coat morphology and other palynological features of *Talinumand Calandrinia* to produce a more acceptable classification of the species in these taxa (Nyananyo and Olowokudejo, 1986). Vernonia leaves are simple, alternate or opposite and they are not compound. The inflorescences are either head or capitulum with involucres of bracts and it is about 3 to 4 cm wide. Seeds are dark brown to black, 5-6 mm long x 1.5 rom wide with silky hairs, ca. 0.5 mm long. Each bristle in the tuftor Pappus at the large end of the seed is 7~8 mm long and iscovered with minute barbed setae. Flowers are of two kinds -the central ones called disc florets are tubular and the marginal ones called ray florets are ligulate (Dutta, 1974). Pollen or flower sperm is a fine to coarse powder consisting of micro gametophytes (pollen grains) which carry the male gametes of seed plants (Wodehouse, 1935).

2.4 Ecology

Vernonia is an annual crop, growing naturally in marginal areas with as little as 200 mm seasonal rainfall and at an elevation ranging from 700 to 2400 m above sea level (ASL) in the southern and south eastern parts of Ethiopia (Gilbert, 1986). Vernonia requires a rainy season that provides sufficient moisture to permit the main flower heads to develop. A longer rainy season that permits secondary flower heads to develop will result in poor uniformity of maturation and a risk of seed shattering. The plants tolerate substantial shading, which may make cultivation in agro forestry system possible as well as drained soil with pH varying between: 5.0-8.5. Poorly drained soils will cause the growth of the main stem to stop before flowering. Branches that develop from the base of the plant, may also wither and die (Favi et al., 2008).

2.5 Importance and uses of Vernonia

2.5.1 Agro-forestry

Vernonia is an important shade and other diverse habitats plant type, which makes it ideal for agro-forestry and increases its potential in agricultural systems that are important in preventing erosion and fighting desertification in arid and semi-arid lands (Perdue Jr, 1988).

2.5.2 Pet food additive

A number of species of this family grow in so many habitats and localities are good sources of animal feed. The press-cake after oil extraction is suitable for animal feed. It is a valuable source of crude protein (43.75%); it also consists of crude fiber (10.90%), ash (9.50%) and the carbohydrate fraction (6.57%) with sucrose (2.36%), fructose (1.90%) and glucose (0.77%). The major mineral elements, calcium (11.08 mg/g), potassium (14.18 mg/g), magnesium (6.90 %) and phosphorus (644 mg/g) not only meet the nutritional requirements but are also higher than in most other oilseeds (Ologunde et al., 1990).

2.5.3 Pesticides

Leaf trichome extracts from vernonia have shown potential for drug (Miserez, 1999) and these trichomes extracts have been shown to cause rapid mortality of both immature and adult whiteflies (Favi et al., 2008). Mass spectrometry analysis revealed that the peltatetrichome is a major source of prevernocistifolide-8-0-isobutyrate. This glaucolide-type sesquiterpene lactone was previously identified as a major constituent of the aerial parts of vernonia (Favi et al., 2008).

2.5.4 Production of plastics

Vernonia oil has potential as a plasticizer of polyvinylchloride (PVC) and as a structural component of polymers in the plastic manufacturing industry (Mebrahtu et al., 2009). There is a large industrial market for synthetically deoxidized vegetable oils (such as linseed and soy-bean), but the epoxidation process is expensive. Vernolic acid is already deoxidized, and can fill in market niches. Vernolic acid is much less viscous than the synthetically deoxidized oils. The latter are semisolids at 10 °C and not pourable at 0 °C and below, while vernolic acid can be poured even below freezing point. The low viscosity of vernonia oil should make it a good solvent in paint manufacture, and the highly reactive epoxy group will cause it to become chemically bound in the dried paint rather than evaporating in the atmosphere (Kaplan, 1989a).Vernonia oil could also be used as a natural source of plasticizers and stabilizers for producing PVC plastic, which is currently manufactured from petroleum. The leaves have been smoked as a substitute for tobacco in Ethiopia (Shenkute et al., 2012). In Tanzania the leaves are cooked in porridge, or drunk as a tea to treat chest pain. In Kenya the plant is used to treat stomach pain (Damania et al., 2008). Epoxy oils have an advantage over commercially epoxidized oils in that the location, number, and configuration of epoxy groups are rigorously known. Vernolic acid is characterized by its chemically active epoxy group.

2.5.5 Chemical constituents of the seed

The seeds of this plant contain 40% epoxy oil, which when hydrolysed, yields different fatty acids with variable composition as detected by gas chromatography (GC). The fatty acid profile of vernonia oil as reported by Ayorinde et al. 1990 is: vernolic acid (79% to 80%), linoleic acid (C18:2) 11% to 12%, oleic acid (C18:1) 4% to 6%, stearic acid (C18:0) 2% to 3%, palmitic acid (C16:0) 2% to4%). Thompson et al., (1994) also reported the fatty acid composition of vernonia oil as follow: vernolic acid (72% to 82%), linoleic acid (12% to 14%), oleic acid (4% to 6%), stearic acid (2% to 3%), Palmitic acid (2% to 3%), and a trace amount of arachidic acid.

2.6 Agronomy and production

Vernonia (*Centrapalus pauciflorus* Willd.), is commonly propagated by seed. The seed is small, and a firm, level seedbed is required (Damania et al., 2008). Vernonia seedling growth is slow and weeding is important. Pre-sowing herbicides have been applied successfully. Topping of young plants may reduce the risk of lodging and enhance uniform maturation (Damania et al., 2008). Different diseases attack vernonia and these include a leaf blight (*Alternaria alternate*), root rot (*Fusarium solani, Rhizoctonia solani* and *Sclerotium rolfsii*) and rust (*Pucinia spp.*). These are common in areas where Vernonia has been grown for several years (Damania et al.,

2008). Vernonia seeds matures unevenly and several harvesting of heads is done when the involucres surrounding the seeds are dry and spread out to release the fully mature seeds. At this stage 90% of the seeds have a black seed color and firm. The highest vernonia yields recorded in Ethiopia from local selections are 4 t/ha of seed, equivalent to 1.625 t/ha of oil (Damania et al., 2008).

2.7 Growth and development.

Vernonia seeds might exhibits dormancy for a few months after maturation. When seeds are not dormant germination takes about 10 days. Plants form a single unbranched stem ending with an inflorescence. Growth is indeterminate. Some plants may extent to a height of only 20 cm and form only a single flower head, whereas others become more vigorous and can grow to 2.5 m and form tall shrubs with many branches and flower heads. Flowering is induced by short day, but plants have been found in subsp. *pauciflorus var. Petitiana* (Gilbert, 1986) in southern and northern Ethiopia and Kenya that are only weakly qualitatively sensitive to day length.

2.8 Seed quality and components

Seed quality is a multifaceted concept with several aspects which include the following genetic quality, physical purity, germination, vigour, uniformity in size and freedom from seed-borne diseases (Basra, 2006). High viability, storability are also important aspects of seeds. Seed quality is described as the complete value/suitability of seed lot for its expected use; in this case it is defined in terms of physiological quality (viability, germination and vigor) (Copeland and McDonald, 2012). Viability as the property of the seed that allows it to germinate under a wide range of conditions (Copeland and McDonald, 2012). On the other hand, vigor refers to that aspect of the seed responsible for rapid, uniform germination, increased storability, good field emergence and an ability to perform well under field conditions (Perry, 1978). In practice, the relationship between seed viability and vigor is an intricate one. High seed quality (viability, germination and vigor) is essential for good crop establishment and how the crop will perform under field conditions. In general, poor quality seed may result in reduced germination and emergence rates, poor tolerance to sub- optimal conditions and low seedling growth rates (Oliveira et al., 1984). Germination (viability) and emergence (vigour) of vernonia is often irregular, variable and slow in the field; it has been reported to takes up to 10 days after sowing and seed may express some dormancy for few months after maturation. As a result, ripening of the heads of a plant may be uneven. Shattering of mature fruiting heads occurs. Vernonia is self-fertile, but rates of out-crossing ranging from 2.5 to 16% have been reported (Baye and Becker, 2004). Previous research indicated that the stage of maturity at harvest is one of the

most important factors that influences the quality of seeds (Demir et al., 2010). Harvesting too early may result in low yield and quality, because of the partial development of essential structures of seeds. Whereas, harvesting too late may increase the risk of shattering and decrease the quality of seeds due to ageing. Adverse environmental conditions such as raining may also result in sprouting of seeds on mother plants (Wang et al., 2008). Therefore, successful seed production depends on detection and implication of optimal time of harvesting. This time is a pre requisite for the production of maximum number of high quality seeds (Demir and Balkaya, 2005a). Maximum seed quality may be achieved at the end of seed filling period (Tekrony and Egli, 1997) or slightly after this phase (Ghassemi-Golezani et al., 2009). The end of seed filling phase coincides with the stage of as physiological maturity or mass maturity (Ellis and Pieta Filho, 1992). Low quality seeds can potentially decrease the rate and percentage of germination and seedling emergence, leading to poor stand establishment in the field and consequently yield loss in many crops such as corn (Moreno-Martinez et al., 1998), wheat (Ganguli and Sen-Mandi, 1990), cotton (Iqbal et al., 2002) and garden pea (Hampton and Scott, 1982). Therefore, it is necessary to examine and identify suitable techniques for production of high quality seeds from different crops. This study aimed to investigate the pattern on seed quality development of vernonia at different stages of development in order to determine the appropriate time for harvest and quality improvement of a vernonia crop.

2.8.1 Seed germination.

Seed germination is defined as the emergence and development of the seed embryo and the essential structures that indicate the seed's ability to produce a normal plant (ISTA, 2003). Germination is described as emergence of embryo from the seed by starting a variety of anaerobic activities, including respiration, protein synthesis and mobilization of food reserves after it has absorbed water (Copeland and McDonald, 2012). Laboratory germination tests can be used to determine germination potential of a seed lot, which can then in turn be used to compare the quality of different lots (ISTA, 2003). External conditions are controlled to give the most regular, rapid and complete germination for the majority of samples. Germination test is defined by radicle protrusion according to the seed physiologists but the International Seed Testing Association (ISTA, 2003) defines seed germination as the development of normal and vigorous seedling.

2.8.2 Seed viability

Viable seeds are those that are alive and have the potential to germinate when exposed to favorable germination conditions (Basra, 2006; Copeland and McDonald, 2012). Measurement

of viability is an essential requirement in assessing the value of seed for planting (Basra, 2006). A seed may be viable but not capable of germinating because the germination process has been blocked by physical and/or chemical inhibitors (Basra, 2006).

2.8.3 Seed vigour.

Seed vigour is defined as that quality of the seed which is responsible for rapid, uniform germination, increased storability, good field emergence and ability to perform over a wide range of field conditions (ISTA, 2003). A vigorous seed lot is one that is potentially able to perform well even under environmental conditions that are not optimum for the species (Basra, 2006). The germination rate (speed germination) was the recommended tool for evaluation of seedling emergence and Vigour (Maguire, 1962). Vigorous seeds are able to efficiently synthesize new materials and rapidly transfer these new products to the emerging embryonic axis resulting in increased dry mass accumulation (Knittle and Burris, 1976). Thus, vigor results are expressed as mg dry weight of germinable seedlings.

2.9 The use of mutagens in crop improvement

Crop improvement focuses on the need to continuously improve crop performance to meet the increasing demands for food, fibres, fuels; pharmaceutical and chemical raw materials. There is an urgent need for crop diversification to add new uses to existing crops and to introduce new crops to meet these new demands. This is driven by efforts to reach a more sustainable and environmentally friendly agriculture. These efforts also call for renewable resources, new and more specialized raw materials for the chemical industry, vegetable oils for fuel, disease and pest resistance sources which will reduce the use of pesticides, products with modified qualities and adaptation to stress conditions.

Plant breeding efforts combining genetic resources and induced mutations using classical, in vitro and innovative molecular approaches have been responsible for much of the intensified development of industrial crops in recent decades. In addition, these efforts have changed quality characteristics, which are more exacting in industrial crops (Ruane and Sonnino, 2011). Many of the critical steps in the relevant biosynthetic pathways are controlled by one or a few major genes, which can be modified by induced mutations. These lend themselves to modification by induced mutations and breeding manipulations.

The chemical mutagens most used for mutation induction belong to the class of alkylating agents [Ethylmehanesulphonate (EMS); Dimethylsulphate (DES); ethylene mine (EI); ethyl nitroso urethane (ENU), ethyl nitroso urea (ENH), methyl nitroso urea (MNH)] and azides.N - Methyl-N- nitro - guanidine (NE), and Dimethylsulphate (DMS) (Adamu and Aliyu, 2007).The

dose assessment for chemicals is determined by varying the concentration and duration of treatment, the solvent used [e.g. dimethyl sulfoxide (DMSO)], or the pH of the solution. These are the commonly used criteria for selecting optimal treatment doses in plants (Shah et al., 2008).

Ethyl methane sulfonate (EMS) is an alkylating agent used in chemical mutagenesis of both plants and animals. Alkylation of nucleotides induced by EMS in chromosomes can result in mis-pairing and changing of bases. This results in alkylation of guanine (G) which subsequently forms O6- thylguanine. O6-ethylguanine then forms a base pair thymine (T) and not cytosine (C). Original G/C pairs end up being replaced by A/T base pairs. At a low EMS frequency, G/C to C/G or G/C to T/A Trans versions are generated by 7-ethylguanine hydrolysis or A/T to G/C transition by 3-ethyladenine pairing errors. This can result in gain or loss of gene functions.

EMS is a highly recommended chemical mutagen for seeds, because it can be applied easily and the after effects can be monitored with ease. In plants, EMS usually causes point mutations, but loss of a chromosome segment or deletion can be also occur (Okagaki et al., 1991). Therefore, EMS has the potential of altering loci of particular interest without inducing a great number of closely linked deleterious alleles presents in exotic or wild germplasm or even from adapted inbred lines. EMS has potential to be effectively applied to develop new vernonia lines with high yield and/or enhanced agronomic traits. Primary to any mutagenesis of crop, the suitable chemical dose and optimal conditions for a particular line/cultivar should first be determined.

Higher doses can produce very drastic effects that may lead to death of the organism. A relatively lower dose may result in altered growth characteristics. Under optimal mutagenesis conditions, individuals of an EMS mutant population carry a high mutation load but remain vigorous and fertile. It is important, therefore, to determine the level of mutagen treatment necessary to achieve the maximal mutation load (Stephenson et al., 2010). With respect to EMS doses, the terms lower and higher are relative and may be different for each crop species. Seedling emergence growth and chromosomal aberration are the commonly used criteria for selecting optimal treatment doses in plants (Shah et al., 2008). Results of many studies suggest that use of chemically induced mutants such as EMS can also provide useful information for understanding the functions of essential genes by generating weak nonlethal alleles. Ethyl methane sulfonate mutagenesis in crop can similarly be used to create new lines (Kim et al., 2005).

2.10 Conclusions

Vernonia is an important crop that can be used for industrial purposes such as manufacturing of paints and PVC plastics. This is important in boosting the country's economy and promoting job creation. However, its production is hindered by problems associated with seed quality such as shuttering making it difficult to produce. As an underutilized crop, which is still to gain momentum in its agricultural production there is need for more information on its seed quality and its morphological characteristics for breeding programs. This study therefore, aimed at investigating morphological characteristics and seed quality development of two induced mutation vernonia lines.

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3. CHAPTER III: MORPHOLOGICAL AND SEED CHARACTERISTICS OF VERNONIA LINES TREATED WITH ETHYLMETHANESULFONATE

Abstract

This study compared two selected vernonia mutant lines (Vge-1 and Vge-4) and controls with respect to morphological traits and seed quality development. A field experiment was conducted at the University of KwaZulu- Natal Research farm (Ukulinga) in Pietermaritzburg. In a randomised complete block design (RCBD) with 2 factors. The treatments were 2 lines (Vge-1 and Vge-4) and mutation induction using ethylmehanesulphonate (EMS) (treated vs untreated). The EMS mutation induction showed a significant difference with respect to plant height and leaf number ($P<0.001$). The EMS mutation induction showed a significant difference with respect to plant height and leaf number ($P<0.001$). An interaction between the line and time was observed with respect to plant height ($P<0.05$).Treated Vge-1 plants developed more leaves than untreated Vge-4 plants. Treated Vge-4 head mass per plant increased compared to the untreated Vge-4. EMS increased mass of heads per plant, leaf number and yield for Vge-4 compare to Vge-1. Mutated Vge-4 has a potential for agricultural production due to its increased vigour and yield.

Key words: growth parameters, growth stages, vernonia,

3.1 Introduction

Vernonia (*Vernonia galamensis*) is a potential new industrial crop which is thought to have originated from eastern and south-eastern parts of Ethiopia (Baye et al., 2001). Vernonia seeds contain 35-45% triglyceride oil, vernolic acid and naturally epoxidised fatty acids. Epoxidised fatty acids are important in the formulation of oil based (alkyd-resin) paints (Nyamongo et al., 2009). These oil based paints have ingredients which react with sunlight and other air pollutants to form lower atmospheric ozone and smog (Perdue et al., 1986). Other potential markets for epoxy fatty acids include plasticizers, additives to polyvinyl chloride, polymer blends and coatings, cosmetic and pharmaceutical applications (Bhardwaj et al., 2000). Currently, no oilseed crop has been commercialized as a source of natural epoxidized oil. The oil and vernolic acid (18:1 epoxy) contents were firstly characterized 50 year ago (Earle et al., 1960). Substantial agronomic and utilization research studies conducted in the 1960's on this species were reviewed by Perdue et al., (1986).

The discovery of *Centrapalus pauciflorus* (Willd.) var. ethiopica led to renewed interest in the domestication of Vernonia (Perdue et al. 1986). During early 1970 it was first reported that

seeds of var. ethiopica contained about 42% oil of which 73% was vernolic acid, and was higher than the best selections developed from *Vernonia anthelmintic* (Iqbal et al., 2006).Extensive plant exploration efforts were undertaken in Africa to identify and collect potentially useful germplasm of various vernonia species.Several accessions of the *Centrapalus pauciflorus* (Willd.) complex were collected, and are now maintained in the United States Department of Agriculture (USDA)-Agricultural Research Service (ARS).

In 1989, USDA-ARS initiated a major research effort at the U.S. Water Conservation Laboratory (USWCL) to evaluate the potential for commercialization of vernonia as a new industrial oilseed crop. Research studies on germplasm evaluation (Kaplan, 1989b), photoperiodicity and identification of day- neutral germplasm in *Centrapalus pauciflorus* (Wild.) ssp and *Pauciflorus var. petitiana* have been reported (Thompson et al., 1994). Increasing levels of research activities are in progress to characterize the chemical properties and utilize *Centrapalus pauciflorus* (Wild.) seed oil and vernolic acid in industrial applications (Ologunde et al., 1990). A taxonomic description of the vernonia complex was made by Gilbert (1986). This information was utilized to define potential differences in the morphological, ecological and geographic adaptations of the germplasm pool (Perdue Jr, 1988).

The exploitation and management of the plant is extensively hampered by a number of factors such as: lack of seed maturing lines; seed shattering resulting in enormous seed losses. After cross pollination, fertilization occurs and the seeds develop to reach maturity and ripen ready to be dispersed. The seed is an achene (single fruit) and dehiscent and each single flower can produce a seed. In some species, the bracts surrounding the flower simply open and allow the seeds to be dispersed by the wind. In this case, the seed is often attached to its own 'parachute'. There are reports that in vernonia shattering (spontaneous dispersal of mature seeds from the plants following ripening) can be prevented or is significantly reduced by desiccation (dry out) the crop for day prior to the normal harvest time. If there are delays in the harvesting of the mature dry seed; seed losses may occur as a result of shattering. If harvesting is done early, shattering losses may be less but seed quality and the vernolic oil content may be affected. In a previous study, ethylmehanesulphonate (EMS) was applied as a mutagenic agent to two vernonia lines Vge-1 and Vge-4 to induce variability that could allow for the selection of less variable (more uniform) genotypes with respect to selected morphological and seed characteristics of vernonia lines namely leaf number, number of productive primary and secondary heads, yield and mass of heads per plant and investigate whether it is possible to select for genotypes with uniform seed development. The objective of this study was to

determine the morphological and seed characteristics of these vernonia lines Vge-1 and Vge-4 after treatment with EMS compared with untreated controls.

3.2 Material and Methods

3.2.1 Plant material

Vernonia seeds selected from (two accession lines Vge-1and Vge-4 were used in this study. Seeds of the two lines Vge-1and Vge-4 had been treated with ethyl methane sulfonate to introduce mutations. The seeds were planted and mutant plants selected on the basis of morphological characteristics. Seeds from these mutant plants were harvested and used in this study to compare the effect of EMS on morphological and seed characteristics of the mutants compared to untreated controls.

3.2.2 Experimental layout and design.

The study was conducted at the University of Kwa-Zulu Natal research farm, Ukulinga Pietermaritzburg, South Africa (30°24'S, 29 °24'E, and 775 m above sea level). The research site has a warm subtropical climate with an average annual rainfall of about 694 mm received mainly during the summer months (Everson et al., 2013). Long-term climatic data with respect to rainfall and temperature averages at Ukulinga from January to December are: 738mm respectively. The experiment was laid out in a randomized complete block design (RCBD) with a single factor and the following treatments; line (Vge-1 and Vge-4) and mutation treatment (treated vs untreated). The trial was replicated 4 times giving a total of 16 experimental units, each plot measuring 3 x 3m. Treatments were randomly allocated to the experimental units (plots) within each block and planting was done on 22 December 2011 at a spacing of 0.75m between row and 0.40m between plants. Three seeds were planted per hole and later thinned to one when the plants had reached a height of 1.5 cm.

Table 3.1: Maximum, Minimum temperatures (degrees Celsius) and rainfall (mm) during the experimentation (2011/2012).

	Dec	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**
Min**. Temp.** (°C)	16.1	17.9	18.3	16.4	12.4	12.5	9.1	9
Max **T**emp(°C)	26.1	28	29	27.3	24.5	24.5	21.6	21.5
Rainfall (mm)	105.8	80.2	47.9	138.6	23.6	10.4	9.4	10.4

3.2.3 Data collection

Plant height (Ph) was measured (in centimeters) from the base of the plant to the base to the apical meristem of the uppermost leaf of the plant. Leaf number (mutant/control) was determined by counting the number of leafs with at least 50% green area up till flowering. Each trifoliate leaf was considered as one leaf. The numbers of productive primary heads and numbers of productive secondary heads per were counted during plant development. Heads per plant, yield (t/ha) and mass of heads per plant. Measurements from two accessions linesVge-1and Vge-4 and control were taken randomly on 8 selected plants from the middle row and were used to compute the score for each treatment.

3.2.4 Data Analysis

Data was subjected to Analysis of Variance (ANOVA) using (Genstat software (Genstat®Version 12, VSN International, UK). Means were separated using Duncan's Multiple Range Test at the 5% level of significance.

3.3 Results

3.3.1 Vegetative growth

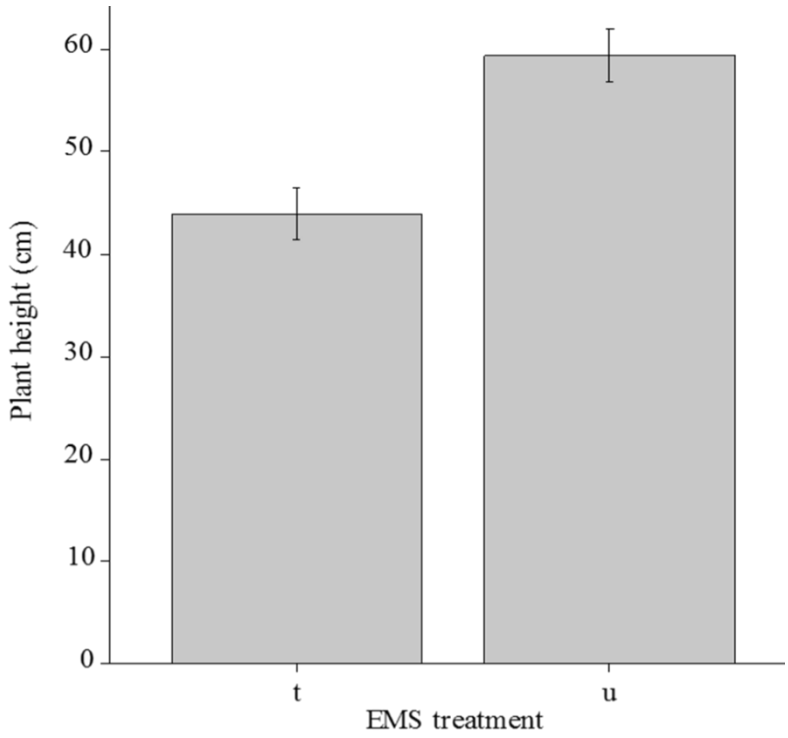
Table 3.2 shows the mean squares for the growth parameters (plant height and leaf number) changes with time during the vegetative stage of vernonia. Significant differences were observed between lines with respect to leaf number ($P<0.001$) and plant height ($P<0.05$). There was significant interaction ($P<0.05$) between lines/ cultivars and time with respect to plant height. Highly significant differences were observed between the EMS mutation treatments with respect to plant height and leaf number ($P<0.001$).

Table 3.2: Mean squares for the effects of cultivar and EMS treatment with time on vernonia plant height and leaf number.

Source of variation	**D.f.**	**Leaf number**	**Plant height**
Line	1	1386.1***	643.1*
Time	6	2206***	6678.6***
Mutation treatment	1	8861.6***	4042.4***
Line x Time	6	20.4	710.7*
Line x Mutation treatment	1	36.6	10
Time x Mutation treatment	6	90.9	27.8
Line x Mutation treatment x Time	6	7	87.7
Residual	81	111.9	153.2
Total	**111**		

N.B. Significant difference at 95% level*, 99% level **, 99.9% level ***

The differences in plant height between the EMS treatments (treated and untreated controls) are described in Table 3.1. Plant treated with EMS had a lower plant height (44 cm) compared to the untreated controls (57 cm). There was an increase in plant height over time regardless of the line. Effects of EMS treatment (Treated; t and untreated; u), variety (line v1; Vge-1 and line v4; Vge-4) and Time (weeks) on vernonia plant height. Vge-1 (V1) had a higher leaf number (48 leaves per plant) compared to Vge-4 (V4) which had 40 leaves per plant (Figure 3.1). The untreated plants had a higher number of leaves per plant (51) compared to EMS treated plants (35). There was a significant increase in number of leaves from week 1 (25 leaves per plant) until week 4 (53 leaves per plant), which later on remained constant (Figure 3.2).



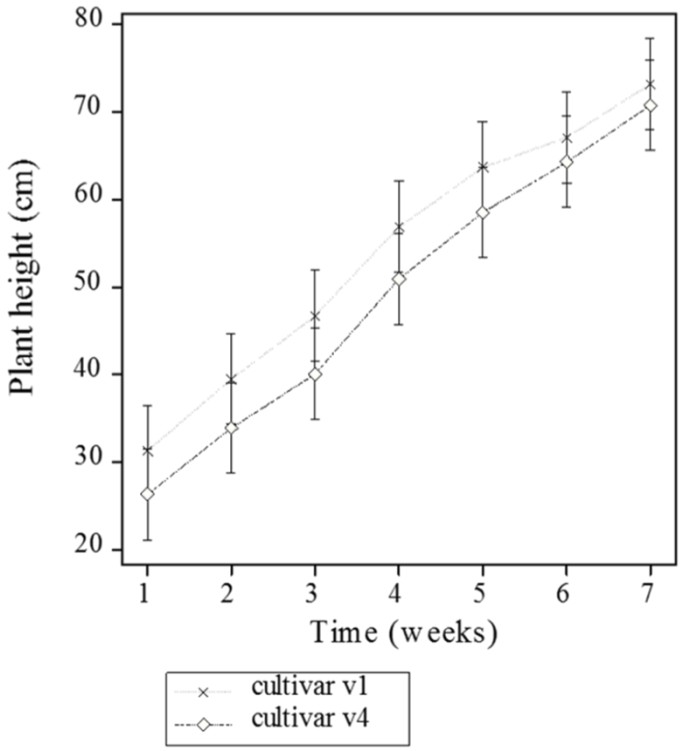


Figure 3.1: A comparison on the plant height and weekly development with the EMS treated (t) and the untreated (u) vernonia plants and, the changes in plant height over time between the two lines.

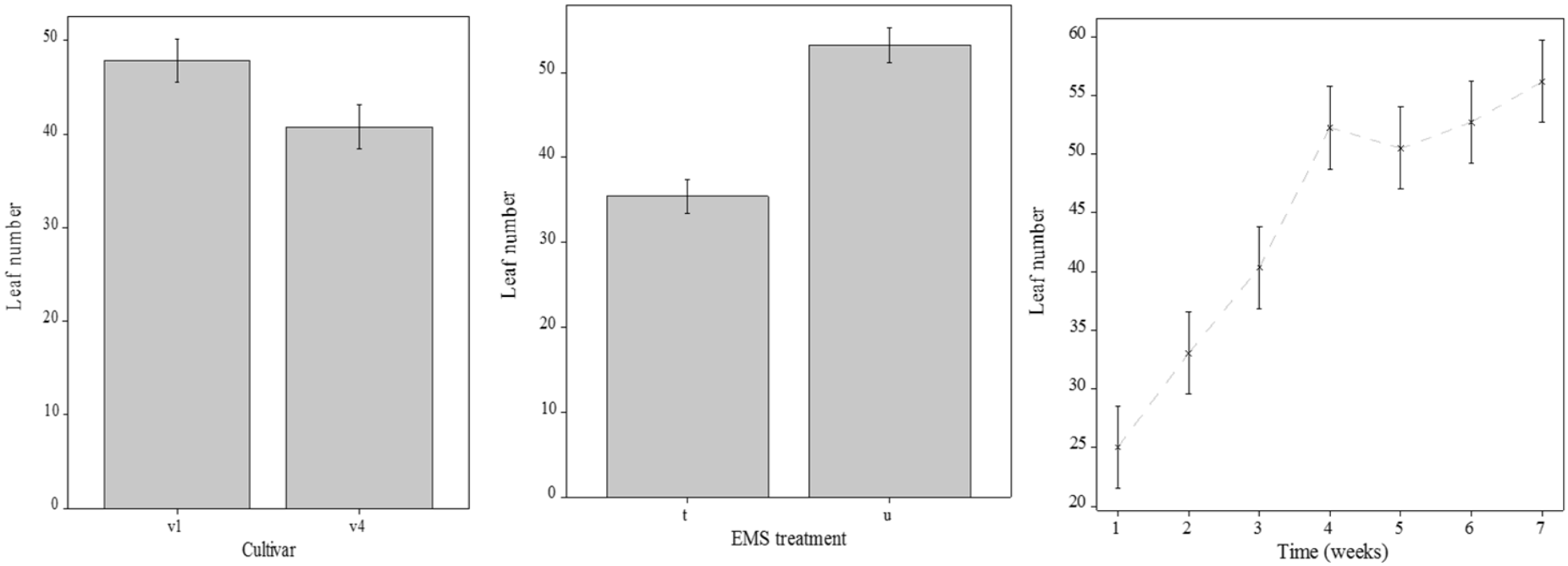


Figure 3.2: The effects of EMS treatment (Treated; t and untreated; u), variety (line v1; Vge-1 and line v4; Vge-4) and Time (weeks) on vernonia plant height.

3.3.2 Vernonia yield

Table 3.3 shows mean squares for the yield parameters (number of primary and secondary heads, mass of heads per plant and seed yield per hectare) for the different line (Vge-1 and Vge-4). There is a highly significant difference in yield between different cultivars and EMS treatment (P<0.001). A significant difference in primary heads (P<0.05) and yield (P<0.001) was observed with regards to EMS treatment. An interaction between the cultivar and EMS treatment was observed with respect to secondary heads and number of heads per plant (P<0.05).

Table 3.3: Mean squares for number of primary and secondary heads, heads per plant and crop yield between the 2 lines and mutation treatment.

Source of variation	**D.f.**	**Primary head**	**Secondary head**	**Heads per plant**	**Yield (t/ha)**
Line	1	0.51	16.869	0.007544	0.28784***
Treatment	1	33.474*	51.532***	0.016038	0.68303***
Line x Treatment	1	4.291	31.44*	0.019841*	0.00809
Residual	9	4.423	38.246	0.003496	0.01491
Total	15				

N.B. Significant difference at 95% level*, 99% level **, 99.9% level ***

Yield response of two different lines of vernonia (Vge-1 and Vge-4) treated with ethylmethylsulphonate.Vge-4 had a highest yield (3.38 tons/ha) compared to Vge-1 (3.1 tons/ha) (Figure 3.3). Figure 3.4 further on shows the effects of EMS treatment and cultivar on the number of primary heads per plant (g) and yield. The treated plants had highest number of primary heads per plant (8.5) compared to the control (5.5). The same observation applies to vernonia yield, treated vernonia plants having a yield of 3.4 tons per hectare compared to the control plants with 3 tons per hectare.

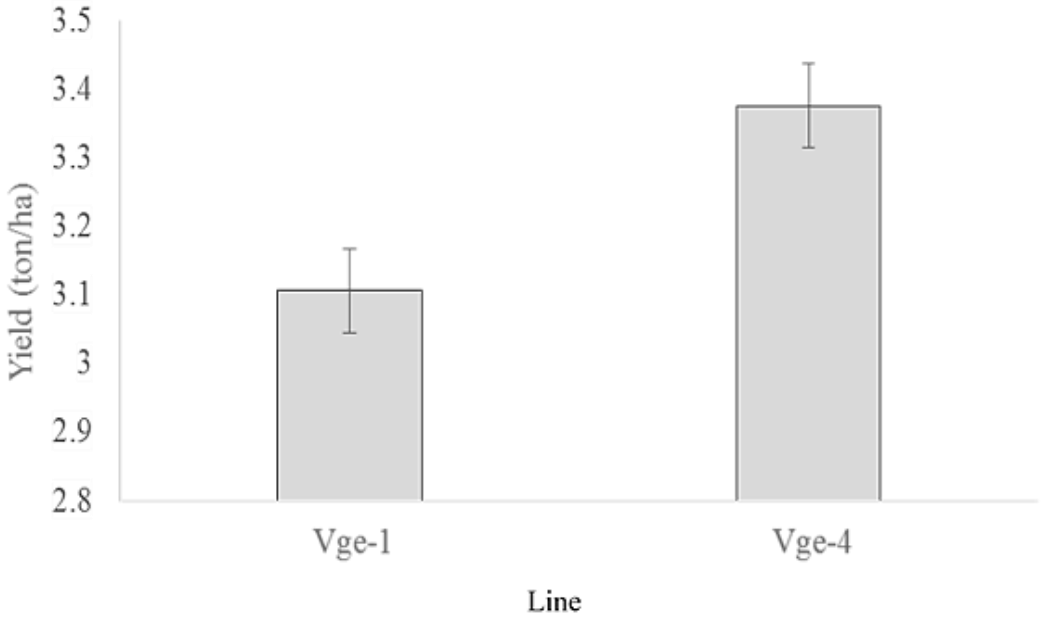


Figure 3.3: A difference in yield (t/ha) of two vernonia lines (Vge-1 and Vge-4) after harvesting.

The yield and number of secondary primary heads per plant were generally higher in EMS treated plants than untreated ones (Figure 3.4). The interaction of EMS treatment and line on the secondary heads and mass of heads per plant (g) shows that Vge-4 treated had the highest mass of heads per plant (0.88 g) compared to treated Vge-1 (0.78 g), untreated Vge-4 (0.75 g) and untreated Vge-1 (0.78 g) (Figure 3.5). Untreated Vge-1 had the highest number of secondary heads per plant (9.6) compared to untreated Vge-4 (5.2), treated Vge-1 (3.8) and treated Vge-4 (4.2).

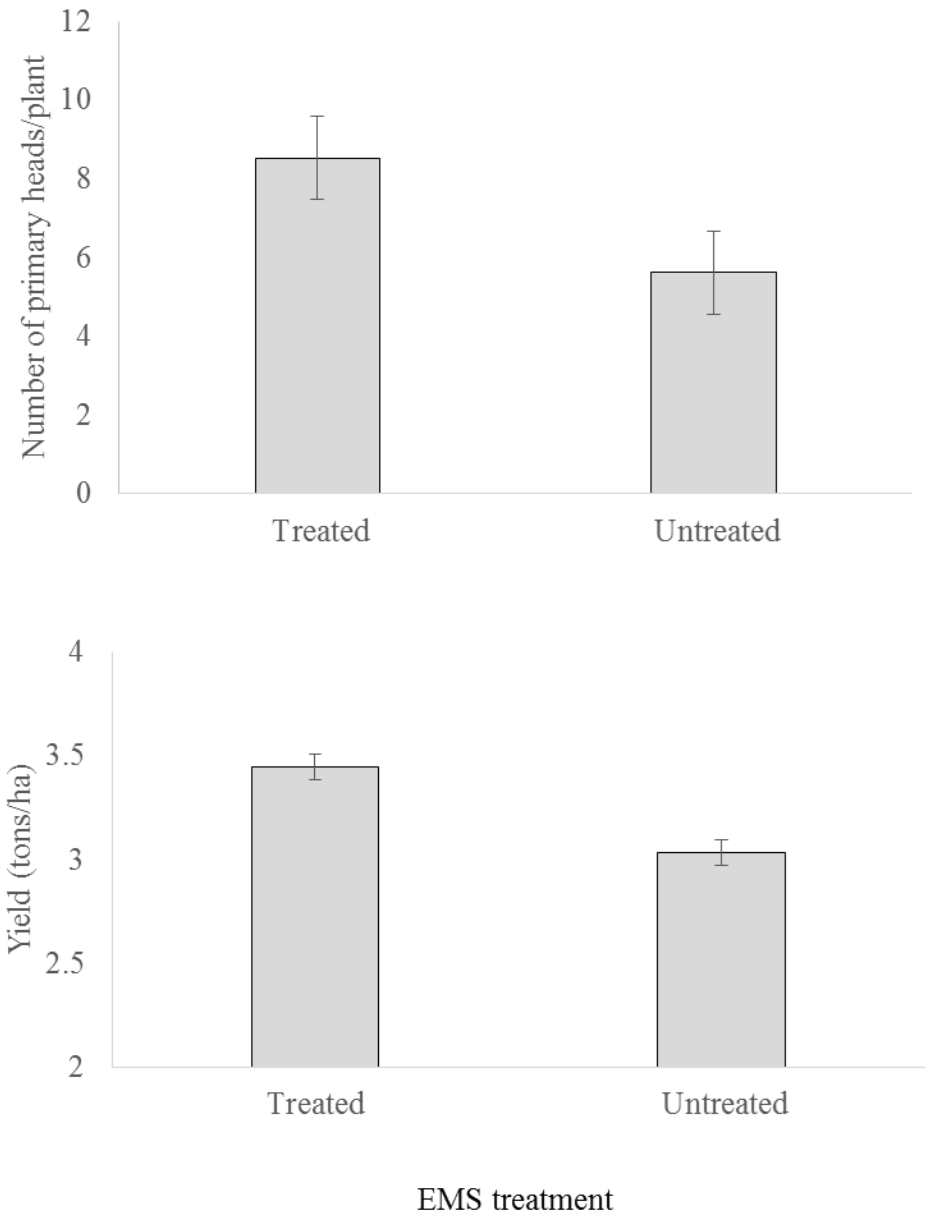


Figure 3.4: The effects of EMS treatment on yield and number of primary heads per plant after crop harvest.

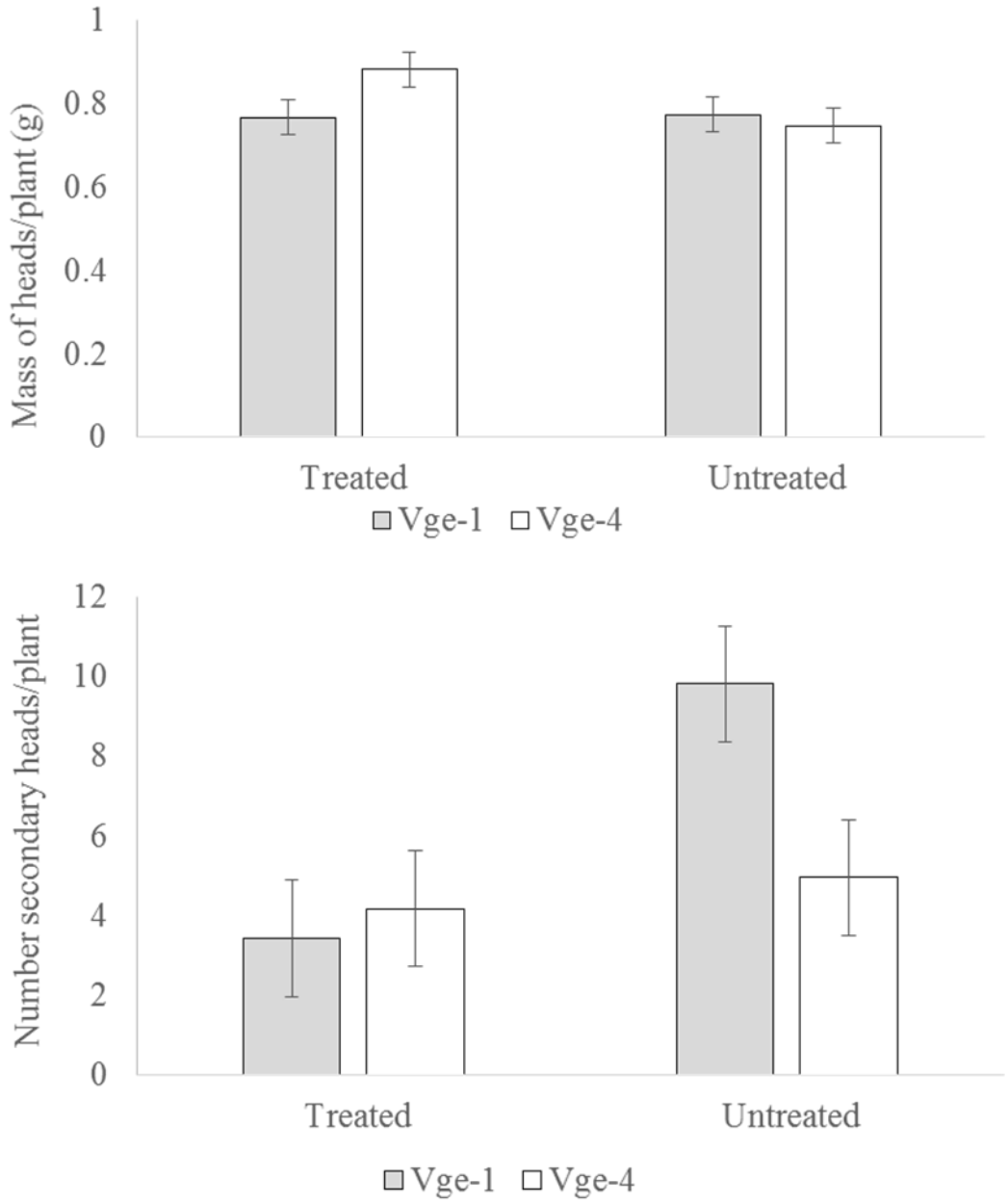


Figure 3.5: The interaction between EMS treatment and line on number of different Vernonia yield parameters (secondary heads per plant and mass of heads per plant).

3.4 Discussion

Treated plants had significantly shorter lower plant height compared the untreated plants (Figure 3.5). This observation shows that EMS treatment have an effect on Vernonia vegetative growth. According to Mih et al. (2008), genetic differences, which promotes early flowering in Vernonia leads to shorter plants? In this study the EMS treatment altered the genetic material of the lines, leading to shorter plants. As described by Shimelis and Hugo (2011), selection for primary secondary heads is associated with reduced plant height. In this study, EMS treatment gave rise to shorter plants which are of agronomic importance. Figure 3.1 and 3.2 showed an increase in plant height and leaf number with time respectively, which is a normal growth trend for all crops. However, the change in plant height was not significant between the 2 lines (Vge-1 and Vge-4) an implication that the lines were of the same. This is further evidence that the EMS induced shorter plants in Vernonia. A significantly higher crop yield was observed in Vge-1 compared to Vge-4 (Figure 3.3). This could be attributed to the fact that these two lines have different growth habits and morphological traits (Baye and Becker, 2004). Genotypic makeup influence number of seeds per pod and yield (Ahmad et al., 1996). Higher crop yield parameters were recorded in treated lines with respect to number of primary heads and seed yield (Figure 3.4). Although seed yield was comparably high in treated lines, it was still within the observed average (3 tons/ha) (Shimelis and Gwata, 2013). More secondary heads per plant were recorded in untreated Vge-1 lines while the mass of heads per plant were higher in treated Vge-4 lines (Figure 3.7). Vernonia genotypes which are determinate in growth, produced many productive primary and secondary heads, and large portions of flowers that were reproductive resulted in higher final yield. This is an indication that growers can successfully plant this genotype either early or late during the growing season (Shimelis et al., 2008). The results differ with those of Thompson and Lammers (1997) where seed number had proved to be the most consistent component of yield.

3.5 Conclusions

The application of EMS on growth parameters and yield on vernonia lines Vge-1 and Vge-4 increased secondary heads per plant, leafs number, mass of heads per plant and yield of Vge-4; this line could be important than Vge-1 for the potential use of vernonia as a new industrial crop.

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4. CHAPTER IV: THE PATTERN OF SEED QUALITY DEVELOPMENT IN MUTANTS LINES OF VERNONIA

Abstract

Seed quality of vernonia lines Vge-1 and Vge-4 treated with ethylmehanesulphonate was compared with untreated controls (untreated seeds). The experimental were laid out using a Randomized Complete Block Design (RCBD) and conducted in 2011-2012 at the University of KwaZulu-Natal, Ukulinga Research Farm in Pietermaritzburg. The treatments were 2 Vernonia lines (Vge-1 and Vge-4) and EMS treatments (treated and untreated). Seeds were sampled at four seed development stages and were used to determine laboratory germination. The tests were done using 20 seeds from each development stage and were replicated 5 times. Germination was recorded daily for 10 days. Germination percentage (%) was generally low but differed significantly ($P<0.01$) between the lines. Treated Vge-4 line had the highest water activity value of 0.25 compared to other treatments. However, mutant seeds of the two vernonia lines stained at 80 % against 90% for Vge-1 and Vge-4. However, electrical conductivity was different between the stages and lines.Vge-1 attained a highest germination percentage of 58 % compared with Vge-4 which had 35 %, Vge-1 had the highest mean germination time (MGT) of (59 days). Vge-4 line had low electrical conductivity of (40 µS/g) at stage four (maturity stage) than Vge-1 and controls (100 and 200 µS/g). Vernonia lines Vge-1 and Vge-4 showed a stained percentage of 90 to 80 %, were the highest seed water activity was observed at stage 1 and ranged between 0.4-0.45 for all treatments. However, the result on scanning electron microscopy (SEM) of embryo of the vernonia lines showed more or less the same embryo structure and seed coat colour during seed development. This study has showed that seed quality development of vernonia lines (Vge-1 and Vge-4) mutants were variable in all germination tests due to genetic differences resulting from induced mutations.

Keywords: water activity, seed quality, seed vigour, moisture content and electrolyte leakage.

4.1 Introduction

Vernonia is a weedy annual crop species, wild in much of tropical Africa resembling a thorn less thistle. It grows in areas close to the Equator and with large seed and best seed retention capacity only under short day conditions. It is grown for its seeds, which contain 40% oil of which 80% is a "naturally" epoxidized vernolic fatty acid (Baye and Becker, 2004). There is a large industrial market for epoxidized vegetable oils (such as linseed and soy-bean), but the epoxidation process is expensive.

Production of vernonia as an alternative industrial crop in marginal tropical and subtropical areas by small-scale low-input farming systems or commercial farmers could have various advantages. For example, it may help as a source of raw material for agro-processing industries and in the diversification of existing crop husbandry practice, thereby reducing a potential crop failure.

In South Africa, Vernonia production is characterized by semiarid climatic conditions receiving a low mean annual rainfall ranging between 300 to 600 mm with predominantly sandy-loam soil with reduced fertility (Thomas et al., 2003). Thus, the country's climatic and edaphic conditions may be highly suitable for the domestication of vernonia. However, detailed studies are required before large-scale production of the crop is possible. A major constraint in the production of vernonia is the indeterminate characteristics with lack of uniform maturity and the problem of shattering. Threshing is also one of the major problems encountered, which is difficult to separate the seed from the cover.

In a previous study (Chapter 3) EMS was used to induce mutations with the objective of creating less variables (more uniform lines). The objective of this study was to compare the pattern of seed quality development in seeds harvested from the plants grown from seeds treated with ethylmehanesulphonate and those from untreated control plants.

4.2 Materials and methods

4.2.1 *Plant material*

Vernonia seeds were initially collected from Ethiopia and the University of Western Cape, South Africa and used to develop new lines Vge-1 and Vge-4. The seeds of two different lines Vge-1and Vge-4 of vernonia were previously treated with ethylmethylsulphonate (EMS) to induce mutations. Treated seeds were planted and plants with clearly morphological deformations as a result of the mutagenic agent identified. Seeds from these plants were harvested and used in the study and compared with untreated control seeds. The seeds were planted in a trial at Ukulinga

Research station of the University of KwaZulu-Natal, South Africa. Seed development at various growth stages was investigated and compared to those from untreated controls. The following variables were measured at each stage of seed: germination percentage (%), mean germination time (days), electrical conductivity (EC), water activity, seed morphology during development using electron microscopy and seed viability using the tetrazolium test.

4.2.2 *Electrolyte leakage (EC)*

Electrolyte leakage was measured using a single seed conductivity meter (CM100, Reid and Associates, South Africa). Ten seeds from each vernonia lines were used and these were replicated 4 times. The seeds were placed inside the tray with individual cells, and each single cell was filled with 2ml of distilled water. Electrolyte leakage was measured at hourly intervals up to 24 hours.

4.2.3 *Water Activity Analysis (WA)*

Water activity (aw/T˚) of seeds was determined using reading Aqua Lab Series 4TE water activity instrument (also known as Equilibrated Relative Humidity, or ERH). Measurements were taken using imbibed 5 seeds of each vernonia lines. The seeds were replicated 4 times.

4.2.4 *Standard germination (SG)*

Germination capacity (%) was determined using the standard germination test. The tests were carried out in a growth chamber set at a constant temperature of 25 °C.A completely randomized design was used for the germination experiment (ISTA, 2003). The treatments were replicated 5 times and 20 seeds were uniformly placed in 9 cm Petri dishes on filter paper. Distilled water was added to keep the paper moist. Germination was recorded daily for 14 days after and which the germination (%) was calculated. The first count and final count were taken at 4 days and at the end of the germination period respectively. Only the normal seedlings were counted for the evaluation of the germination percentage (ISTA, 2003).

Mean germination time (MGT) was calculated according to equation 2.1 (Ellis and Roberts, 1981):

$$MGT = \frac{\sum D_n}{\sum n} \qquad \text{Equation } \mathbf{2.1}$$

Where;

n = number of seeds which were germinated on day D, and

D = number of days counted from the beginning of germination.

Mean germination time (MGT) of vernonia lines (treated) compared with control

4.2.5 *Viability tests*

Seed viability testing was done using the tetrazolium tests samples of 10 seeds of vernonia lines were replicated 4times and placed in a container filled with 100ml distilled water soaked seeds were cut longitudinally and were immersed in 1% of 2, 3, 4 triphyenyltetrazolium chloride (a salt) solution and placed in an incubator set at 25°C for three hours (Figure 4.1). The seed were evaluated viable (normal staining) and non-viable (none staining) according to the International Seed Testing Association Rules and Methods (ISTA, 2003).

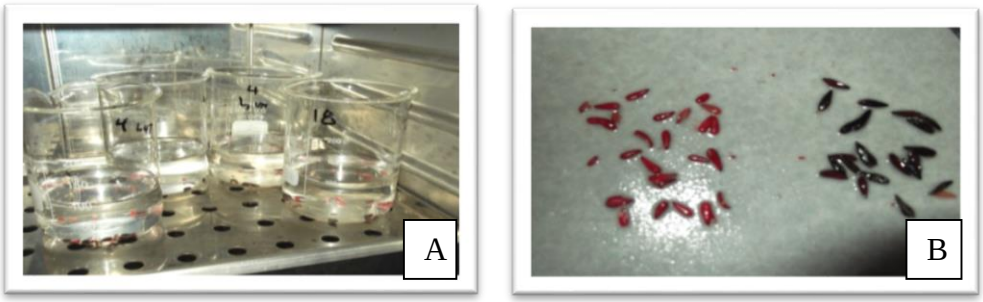

Figure 4.1: Stained seed of vernonia lines (Vge-1and Vge-4 treated seeds) soaked in 2, 3, 4-triphyenyltetrazolium chloride solution and A :(treated seeds of Vge-1and Vge-4) in an incubator.

4.2.6 *Electron Microscopy*

Morphological changes during seed development were observed using scanning electron microscopy (SEM). Seeds of the two vernonia lines (Vge-1 and Vge-4) were placed under light microscope (LM) and images were taken using a Nikon SMZ 1000 stereomicroscopic Zoom Microscope (Nikon Instruments, Tokyo, Japan). SEM images were also taken for two seeds of each treatment of vernonia lines using an AmRay3300 (Amray, Bedford, MA) to observed morphological changes of embryo development. The seeds were cut longitudinally into two pieces with a blade and observations made on the internal seeds structures.

4.2.7 *Data Analysis*

Data was subjected to Analysis of Variance (ANOVA) using Genstat statistical package (Genstat®Version 12, VSN International, UK). Means were separated using Genstat® at the 5% level of significance.

4.3 Results

Mean squares for the germination pecerntage and mean germination time for two vernonia lines Vge-1 and Vge-4 after EMS treatment during the 10 day period of germination test (Table 4.1). There was a significant interaction ($P<0.001$) between the time and lines with respect to seed germination and mean germination time.

Table 4.1: Mean squares for germination percentage and mean germination time between the two vernonia lines over a 10 day period.

Source of Variation	D.f	Germination (%)	Mean Germination Time (Days)
Time	9	7494.2***	5165.4***
Treatment	1	60.1	56
Line	1	10295.6***	10023.8***
Time x treatment	9	6	7.1
Time x Line	9	472***	489.6***
Treatment x Line	1	451	462.6
Time x Treatment x Line	9	45.3	50.5

The germination percentage and mean germination time for two vernonia lines and EMS treatment was described in figure 4.2. Vge-1 had the fastest germination rate and percentage compared to Vge-4. Vge-1 attained a highest germination percentage of 58 % compared to Vge-4 35 % over a 10 day period. Vge-1 had highest mean germination time of 58 days compared to Vge-4 with 35 days.

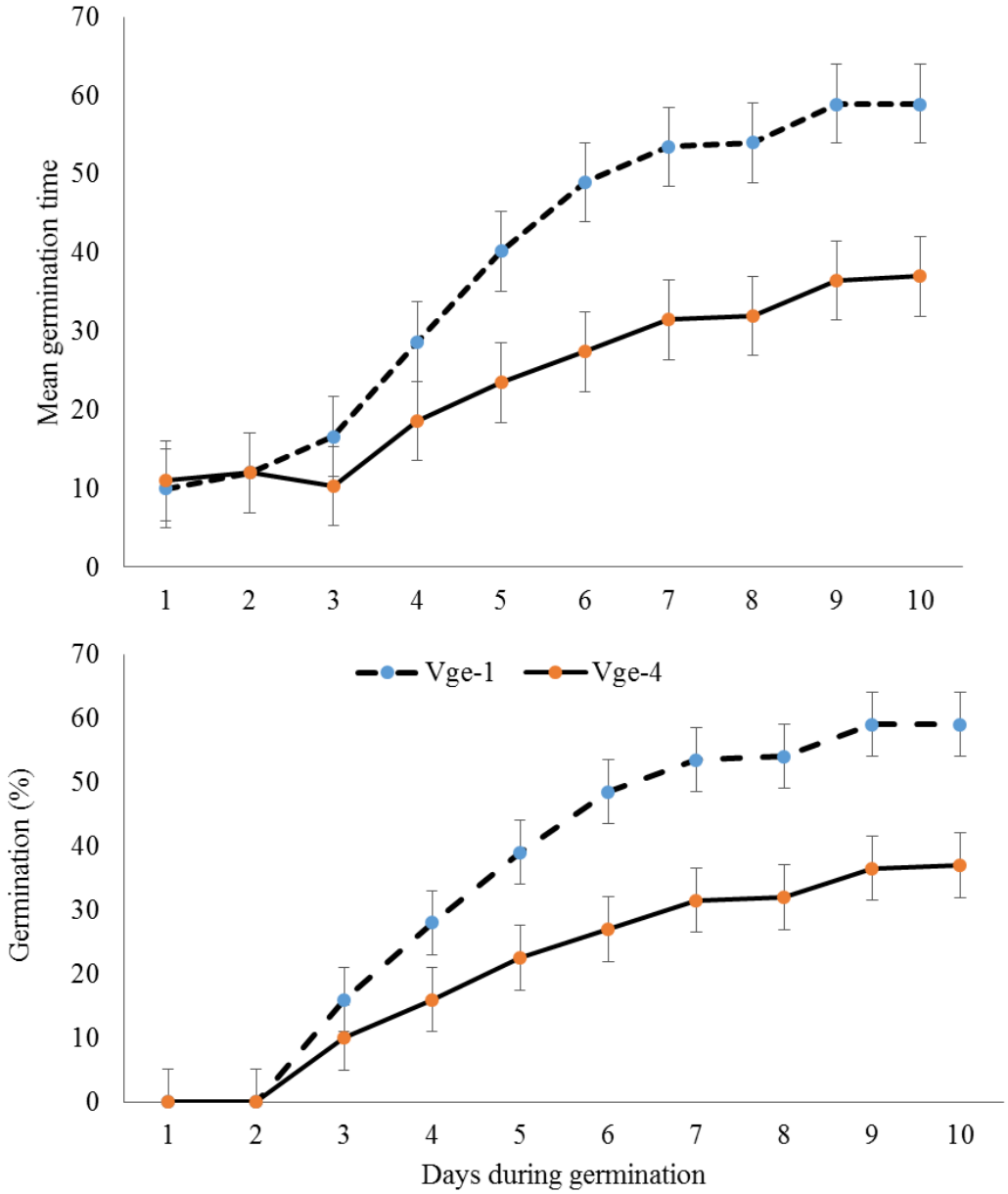

Figure 4.2: Germination percentage and mean germination time for two vernonia lines Vge-1 and Vge-4 during the 10 day germination period.

Mean squares for electrical conductivity and water activity for vernonia lines Vge-1 and Vge-4 after EMS treatment, significant differences in water activity at different stages of seed development were observed between the two treatments (EMS treatments) $P<0.001$ (Table 4.2).

Table 4.2: Mean squares for electrical conductivity and water activity for vernonia lines Vge-1 and Vge-4 after EMS treatment at different stages of seed development.

Source of variance	D.f	E.C	Water activity
Time	3	8509***	0.1694581***
Treatment	1	3405.7*	0.001232
Line	1	3379.6*	0.000353
Time x treatment	3	2137.6**	0.0035091***
Time x Line	3	2821.3*	0.001256*
Line x Treatment	1	3.3	0.007841
Time x Treatment x Line	3	2882**	0.000369

Water activity of seed from two vernonia lines (treated with EMS and untreated controls) sampled at four stages of seed development and after EMS treatment are described in Figure 4.3.

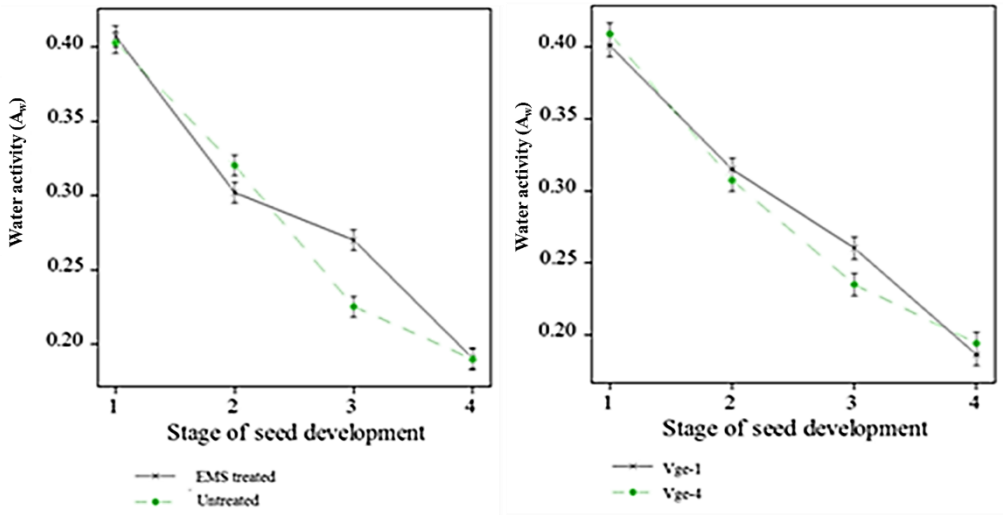

Figure 4.3: The effects of EMS treatment on water activity in different vernonia lines at different stages of seed development.

The seed staining results which were not significant different between treatments and lines. The Vge-1 seeds (treated and control) had the highest vigour with a all the sceeds stained (99.5 %) following a tetrazolium test. Lower values of tetrazolium test was observed in Vge-4 treated with EMS (75 %) and untreated Vge-4 lines (85 %).

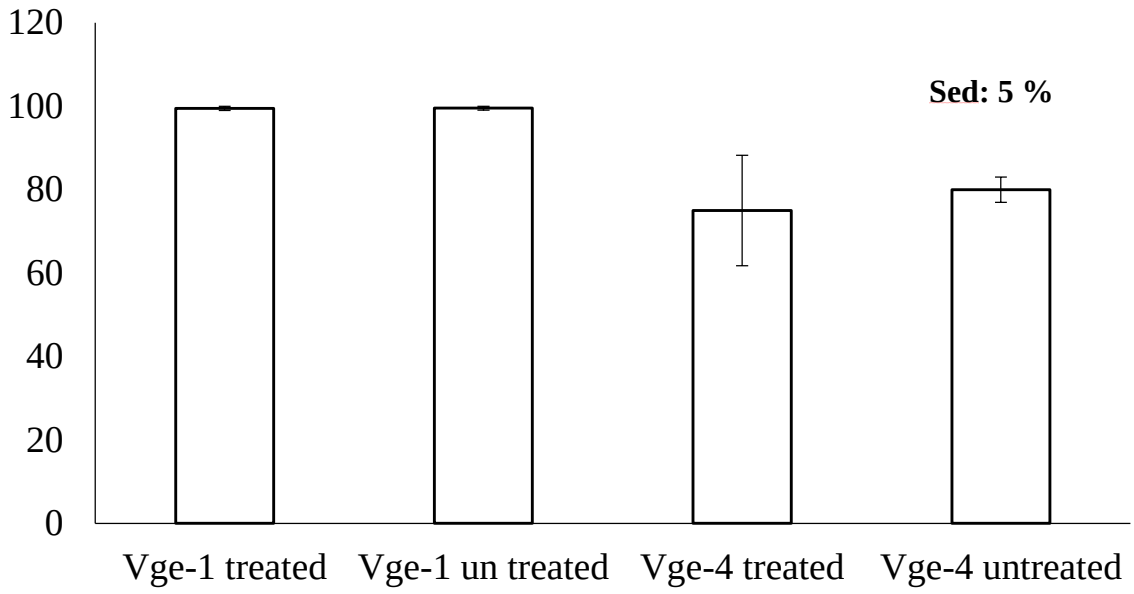

Figure 4.4: shows the seed staining results (%) following a tetrazolium (TZ) test of seed vigour between the two seed treatments and lines.

4.4 Discussion

Seed quality refers to the suitability of a seed lot for its future purpose; in this context it is defined in terms of physiological quality (viability and vigor) (Copeland and McDonald, 2012). Viability is described as the property of the seed that allows it to germinate under optimum conditions (Basra, 2006). Germination percentage refers to the amount of viable seeds in a sample while mean germination time refers to germination speed (seed vigour) (Copeland and McDonald, 2001).

During this study results showed that Vge-1 had the highest mean germination time and germination percentage compared to Vge-4. Vge-1 attained a highest germination percentage of 58 % compared to Vge-4 (35 %) over a 10 day period (Figure 4.2). In this study, Vge-1 had a MGT of 59 days compared to Vge-4 (38 days). This clearly shows that Vge-1 seeds were less vigorous hence germinated slowly, however there were more viable seeds in the sample. Seed vigour differences can be attributed to genetic differences between the two lines (Ghassemi-Golezani et al., 2009). Seed vigour refers to that aspect of the seed responsible for rapid, uniform germination, which have an ability to perform well during plant establishment (Copeland and McDonald, 2012) hence Vge-4 seeds are favourable despite problems with viability. During imbibition at the early stages of germination, the uptake of water is accompanied by a leaching of substances mainly potassium, phosphates, sugars and amino acids (Tekrony and Egli, 1997). Electrolyte leakage in germinating seeds results from the breakage of cell membranes, which will be repaired during imbibition hence higher leakage is associated with reduced seed vigour (Mc Donald et al., 1980). A direct relationship between electrical conductivity and seed viability has been reported by several researchers (Basra, 2006). In the study treated Vge-4 line had the lowest electrolytic leakage at stage four compared to other treatments. Higher electrolytic leakage in other treatments is associated with the loss of ability seed to re-organize cellular membranes completely and rapidly during early imbibition (Agarwal and Sinclair, 1996).

The effects of seed aging on electrolyte conductivity have been reviewed by several researchers. It has been found that as the aging period increases electrolyte conductivity increases in water melon (Demir and Balkaya, 2005b), tomato (Basra, 2006), pearl millet (ISTA, 2003) and maize (Ellis and Pieta Filho, 1992). Seeds with low viability show high electrical conductivity compared with seeds with high viability. Water activity was not different between all the treatments and decreased significantly during the study (Figure 4.3). The results on water activity of the seeds at different stages decrease gradually during seed development from the stage 1 to stage 4. The highest seed water activity was observed at stage 1 and ranged between

0.4-0.45 for all treatments compared with 0.15-0.2 for all treatments at stage 4. These changes can be explained by high moisture content of the seeds during development which declined with time until harvest maturity. Species with water impermeable seeds have physical dormancy and such seed generally have a water gap in seeds, which open in response to a suitable environmental signal (Baskin, 2003).

The seed quality development of vernonia lines was observed using Zeiss EVO Scanning Electron Microscopy (SME). Photograph scans of embryo of the vernonia lines showed more or less the same embryo structure and seed coat colour during seed development. Anatomical and functional evidence showed that the seed covers of vernonia seed is permeable to water.

The result also showed that accumulation of seed storage proteins in embryos of vernonia occurs at late developmental stages. It is important to note, however, that the hard funicular envelope acts as a partial barrier to water diffusion into the seed and to radicle protrusion. Changes occurring in the funicular cover during ageing and seed dehydration play an important role in water uptake dynamics and germination of the seed. The tetrazolium test is a biochemical test, whereby tetrazolium salt (2, 3, 5- triphenyl tetrazolium chloride) is reduced to a red coloured compound called formazan due to the effects of respiration enzymes. This is a test for testing seed viability and is expressed as a proportion of stained embryos (Tekrony and Egli, 1997). All the treated and untreated Vge-4 lines had lower staining percentage after a tetrazolium test compared to Vge-1 lines (Figure 4.4). This implies that most of the Vge-1 seeds were more viable compared to Vge-4 regardless of treatment with EMS treatment. This observation agrees with germination percentage results obtained during the study (Figure 4.2). Although Vge-4 seeds were more vigorous although most of them were not viable.

4.5 Conclusions

This study showed that seed quality development of vernonia lines (Vge-1 and Vge-4) change within different seed quality test in term of electrical conductivity, water activity, and viability using tetrazolium test, germination percentage, also mean germination time. These responses may not be necessary associated with the application of EMS. Germination percentage was generally low but differed significantly between the lines. This could be due to genetic differences.

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5. CHAPTER V: THE EFFECT OF GIBBERELIC ACID AND POTASSIUM NITRATE ON SEED DORMANCY IN VERNONIA LINES

Abstract

Vernonia (*Centrapalus pauciflorus* Willd.) is a new potential industrial oil crop, cultivated mainly for its seed to produce triglyceride oil. Vernonia seeds contained 35-45% of triglyceride oil rich in vernolic acid. The previous chapters showed that the germination percentage rate of two vernonia lines was generally low and this could be attributed to dormancy. Dormancy and germination requirements of vernonia seeds were investigated. Seeds of vernonia lines were subjected to different treatments of Gibberellic acid (GA_3)and potassium nitrate ($KN0_3$)concentration at 2 levels:(0.7mM of GA_3 and 1mM for $KN0_3$ and temperature 3 levels: 25/25˚C, 25/17˚C or 30/25˚C and the germination in growth chambers for 14 days. The germination percentage (%), mean germination time (MGT) and germination index (GI) significantly increased in all of treatments than the control. The highest germination were of the 65% with the range temperature of the 25/17˚C and germination index (GI) was obtained in growth germination chambers with a low temperature of 25/17˚C. The germination index improved to 70% with the application of GA_3**.** The mean germination time (MGT) also improved in all treatment than compared to untreated controls. The temperature regime (25/17°C) combined with concentration of gibberellic acid of 0.7mM increased seed germination percentage

***Keywords*:** Seed dormancy, vernonia accession lines, gibberellic acid, potassium nitrate

5.1 Introduction

Dormancy can be defined as the lack of germination of viable seed under favourable conditions (Bewley, 1997). Dormancy can also be defined as the inability of viable seeds to germinate under conditions considered adequate for radicle emergence (Benech-Arnold, 2004). This could be caused by a block to the process of germination as a result of some seed properties (Pritchard et al., 2000b). Dormancy is categorised into primary dormancy caused by maternal tissues and secondary dormancy caused by metabolic blocks when the germination environment is unfavorable (Basra, 2006). Studies showed that poor seedling emergence is attributed to dormancy in Cleome (*Gynandra indicum*) and (*Corchorus olitorus*) (Abukutsa-Onyango, 2007). Lettuce (*Lactuca sativa*) seeds failed to germinate at temperatures greater than 30 ºC hence exhibit secondary dormancy (Schwember, 2009). Temperature may break down the dormancy

either by enhancing permeability of the seed coat or through activating some seed biochemical processes that ultimately enhance the growth potential of the embryo (Daws et al., 2002). While some species require warm stratification to release dormancy, others may respond well to chilling (cold stratification), dry after-ripening, heat-shock or alternating temperatures (Keeley and Fotheringham, 1998). The level of seed dormancy has been shown to vary depending on the prevailing temperatures during seed development (Nyamongo et al., 2009). Therefore, the objective of this study was to determine the effect of different concentration of gibberellic acid (GA_3) and potassium nitrate (KNO_3) treatments on seed germination and devise an effective method for improving seed germination of vernonia lines (Vge-1 and Vge-4).

5.2 Material and methods

5.2.1 Handling of seed

Mature freshly harvested vernonia seeds selected from each of the two vernonia lines Vge-1and Vge-4. Untreated seeds of both lines were included as comparative controls with seed treated with ethylmehanesulphonate (EMS) could have also had an effect on dormancy if any on the two lines of vernonia.

5.2.2 Breaking dormancy of Vernonia seeds

The method used for germination and dormancy breaking as required for vernonia was adopted from the protocol according to Nyamongo et al. (2009) and applied in treating vernonia seeds. The Seed samples from each accession were incubated in 90 mm Petri dishes on two layers of filter paper moistened with 1 mM KNO_3 or 0.7 mM GA_3. The petri dishes were randomly arranged in growth chambers set at 3 temperature regimes (25/25 °C, 25/17 °C or 30/25 °C) and 12 h day length. The elevated part of the alternating temperature treatments coincided with the 12 h photoperiod. The concentrations of $KN0_3$ and GA_3 were chosen because they have been shown to be effective in stimulating germination in other studies (Daws et al., 2002).

5.2.3 GA_3 and $KN0_3$ preparation and handling of seeds

Fresh gibberellic acid and potassium nitrate solutions were prepared according to desired concentrations (0.7 mM of GA_3 or 1 mM for $KN0_3$). Predetermined quantities comprising of 0.0175 g or 0.05 g of GA_3 and $KN0_3$ was added in 500ml of distilled water and the solution shaken for a while to become homogenous. Seeds were pre-soaked for 20-30 minutes in the solutions and subsequently set up for germination. Germination was assessed as defined by protrusion of radicle (2 mm) up to one week.

5.2.4 Experimental design, layout and treatments

The experiment was laid out using a completely randomized design with the following factors: Vernonia lines (2 levels; Vge-1 and Vge-4 of control with 2 levels of Vge-1 and Vge-4 of mutant seeds), GA_3 and $KN0_3$ concentration (2 levels: (0.7mM of GA_3 and 1mM for $KN0_3$) and temperature (3 levels: 25/25, 25/17 or 30/25˚C) with two replications and 20 seed was put into each one of petri dishes according to the treatment combination. Each petri dish was watered with the concentration solution of 0.7mM GA_3 or 1mM $KN0_3$. Germination was monitored for up to14 days.

5.2.5 Germination tests

Germinated seeds were counted every day for 14 days. According to Sharma and Sharma (2010), seeds were considered germinated upon emergence of radicles (≥ 2 mm). The germination parameters recorded included germination percentage (**Eq. 1**) and mean germination time (**Eq. 2**) were recorded (Dezfuli et al., 2008).

GP = (number of germinated seeds/number of total seeds) × 100 **(1)**

MGT = ΣDn / Σn (**2)**

Where, n is the number of seeds which were germinated on day D, and D is the number of days counted from the beginning of germination.

Germination Index:

$$G.I = \frac{n}{d} \quad \textbf{(3)}$$

Where, n = number of seedlings emerging on day "g",

d= day after planting

5.2.6 Data Analysis

Data was subjected to analysis of variance (ANOVA) using GenStat® (2011, 14th edition, VSN International, UK). The least significant difference (LSD) test procedure was used to compare means at 5% level of significance.

5.3 Results

Significant differences on MGT treatments ($P<0.001$) and temperature exposure at 30ºC/25ºC regime ($P<0.05$). The results also showed a significant difference in germination percentage

with regards to temperature exposure ($P<0.05$), contrasts showing a significant difference ($P<0.01$) between $30^{o}C/25^{o}C$ and $25^{o}C/17^{o}C$ temperature exposures. There was also a significant difference in germination percentage with respect to different chemical treatments ($P<0.001$).

Table 5.1: Mean squares of mean germination time (MGT), germination percentage (%) and germination index (GI) for two vernonia lines (Vge-1 and Vge-4).

Source of Variation	D.f	MGT	Germination (%)	Germination index
Temperature	2	0.0786	528.1*	355
Contrast 1 (30/25 vs 25/17)	1	0.1525	1054.7**	703.16
Contrast 2 (25/25 vs 25/17)	1	0.0184	300	240.97
Contrast 3 (30/25 vs 25/25)	1	0.065	229.7	120.87
Variety	1	0.1689	78.1	5.18
Chemical	3	1.596***	3957.8***	981.23
Temperature x Variety	2	0.0033	209.4	70.64
Contrast 1 (30/25 vs 25/17) x line	1	0.0065	229.7	86.2
Contrast 2 (25/25 vs 25/17) x line	1	0.0008	18.8	3.19
Contrast 3 (30/25 vs 25/25) x line	1	0.0027	379.7	122.54
Temperature x Chemical	6	0.3123	99.4	10.76
Contrast 1 (30/25 vs 25/17) x Chemical	3	0.0911	67.2	10.63
Contrast 2 (25/25 vs 25/17) x Chemical	3	0.2478	100	19.3
Contrast 3 (30/25 vs 25/25) x Chemical	3	0.5978*	131.1	2.36
Variety x Chemical	3	0.4639	211.5	89.47
Temperature x Variety x Chemical	6	0.2342	42.7	33.54
Contrast 1 (30/25 vs 25/17) x line x Chemical	3	0.0948	45	43.55
Contrast 2 (25/25 vs 25/17) x line x Chemical	3	0.1965	74.3	33.35
Contrast 3 (30/25 vs 25/25) x line x Chemical	3	0.4114	8.9	23.72
Residual	48	0.1816	111.1	49.87
Total	71			

d.f. = degrees of freedom; m.s. = mean square

Germination percentages for vernonia plants under different temperatures (Figure 5.1.A) and chemical treatments (Figure 5.2.B). Generally there was an increase in germination percentage with decreasing temperature exposure; 25 ^{o}C/17 ^{o}C having the highest value compared to 30 ^{o}C/25 ^{o}C Germination percentage was higher for seeds treated with chemicals (GA_3, KNO_3 and GA + KNO_3) compared to seeds of the untreated control. Treatment with GA_3 had the highest germination percentage (68.1%) followed by a combination of KNO_3 and GA_3 (63.9%), KNO_3 (55 %) and the control had the least germination percentage of 36 %.

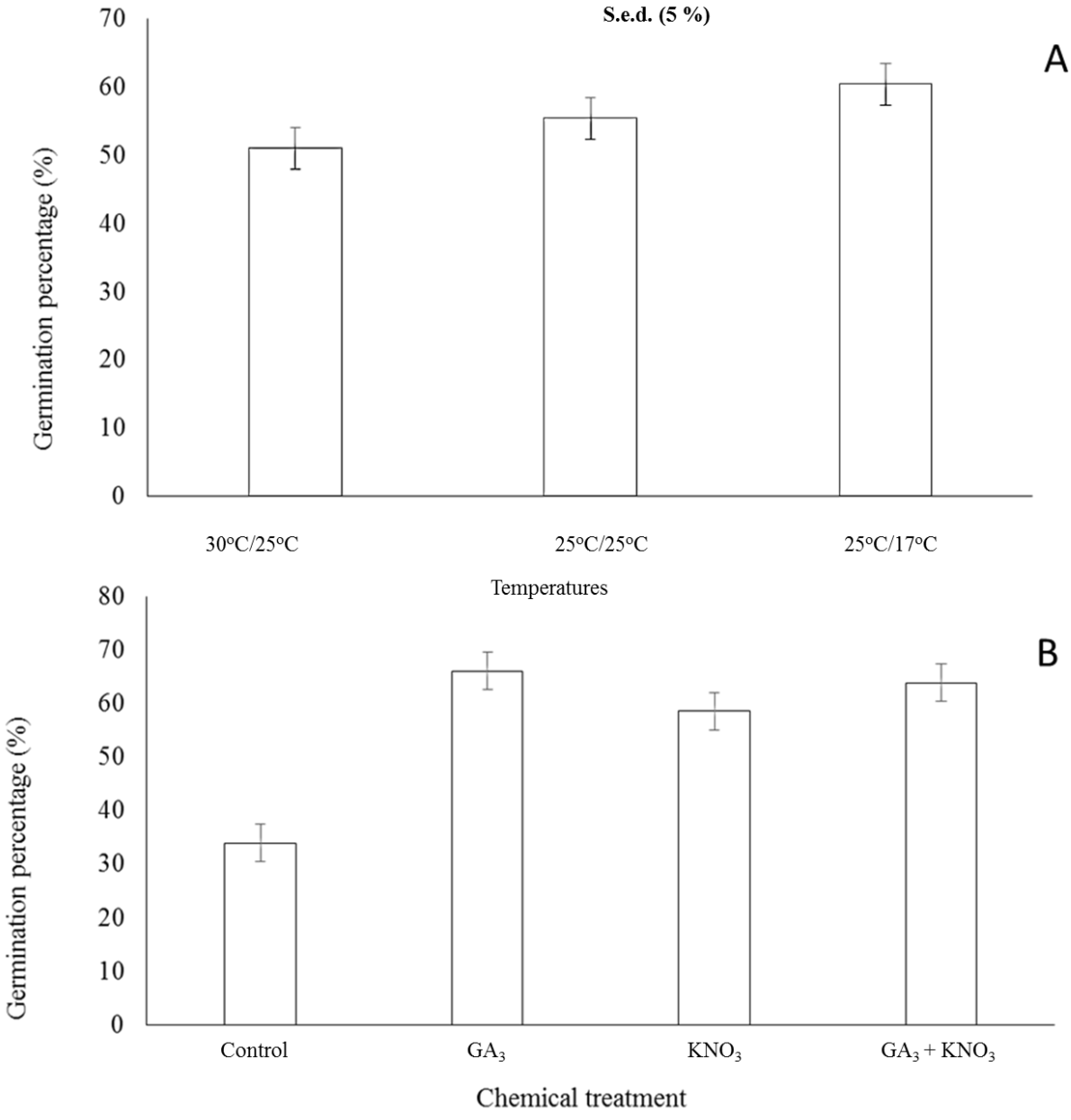

Figure 5.1: Germination percentages of vernonia seeds under different temperatures (A) and chemical treatments (B).

The effects of different chemical treatments and temperature exposures time on the mean germination time for two vernonia lines are described in figure 5.2. On contrary, the control had the highest MGT at 25 ºC/25 ºC (3.968 days) temperature which was significantly higher than 30 ºC/25 ºC (3 days).

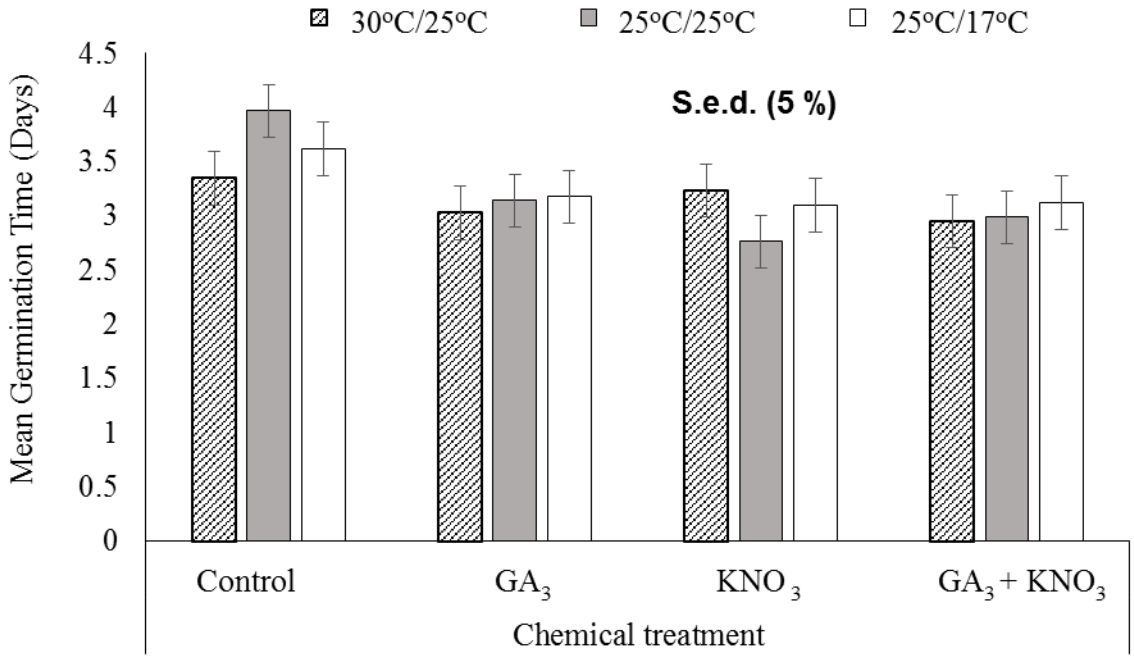

Figure 5.2: The effects of different chemical treatments and temperature exposures time on the mean germination time for two vernonia lines.

5.4 Discussion

Temperature plays an important role in controlling the growth and development of plants and the effect of temperature on seed germination is quite complex because it affects each stage of germination process in a different way and is not independent of other factors (Copeland and McDonald, 2012). The critical temperature level for seed germination is different for each species or cultivar (Reynolds and Thompson, 1973).

These results in the current study showed a significant difference in germination percentage with regards to temperature exposure ($P<0.05$), contrasts showing a significant difference ($P<0.01$) between 30 ^{o}C/25 ^{o}C and 2 ^{o}C/17 ^{o}C temperature exposures (Table 5.2). The result of this study revealed that the treatment with lower incubation temperature of 25/17 ^{o}C had increased the germination percentage (60%). However, GA_3 had the highest germination percentage (68.1%) for seeds treated with chemicals (Figure 5.1), whereas, the control had the highest MGT at 25 ^{o}C/25 ^{o}C (4 days) temperature (Figure 5.2).

These results are consistent with the results obtained by Ganaie et al. (2011) on A*rnebia a benthamii seed*, where the low values of MGT was confirmed for treated seeds in different concentration of KNO_3 and 25 ppm GA_3 in comparison with the control seeds. Similar results were also reported in *Descurainia Sophia* and *Plantago ovata* treated seeds where prechiling with 0.3% KNO_3 showed significant differences for MGT compared to the control seeds (Ali et al., 2013). However, result in the current study confirm that the GA_3 had highest value (68.1 %) of GI, while the treatment combination of KNO_3 and GA_3 (63.9%), KNO_3 (55 %) and the control had the least germination percentage of 36 %. Dewir et al. (2011) reported similar results for *S. palmetto* seeds treated with 1, 2 and 3% KNO_3 and 100, 250 and 500 ppm GA_3, where significant differences for germination index between the treated and control seeds were obtained. Zare et al. (2011) confirmed that the *Ferula assa foetida* seeds treated with higher concentration of GA_3 (2000 ppm) had higher germination rate values compared to the lower concentrations. Approximate results were ascertained as well in Lupinus (Dehgan et al., 2003) and *Anthriscus cerefolium* (Liopa-Tsakalidi and Barouchas, 2011).

The sensitive seeds buried deep in the soil, under leaf litter or below vegetation will not experience alternating temperatures and thus are unlikely to germinate until the soil is disturbed or the vegetation cleared (Daws et al., 2002). These ecological attributes perfectly fit *vernonia galamensis* which naturally occurs in disturbed areas (Gilbert, 1986) and is small seeded. Therefore the sensitivity of vernonia seeds to alternating temperature is perhaps not surprising. The results of the present study are in accordance to Alcorn & Kurtz (1959) who considered that

the germination of cactus seeds is stimulated by temperatures between 17 and 34 °C and 25 °C is frequently the optimum temperature for germination. Moreover, this effect agrees largely with the previous studies have shown that GA_3 enhances the germination of seeds exhibiting physiological, morphological or morph physiological dormancy (Ganaie et al., 2011).

The effectiveness of GA_3 treatment in breaking dormancy depends on the concentration and length of incubation. In the present study, increased GA_3 concentrations have positive effect on seed germination at low temperatures. However, as the incubation temperatures increased the germination decreased significantly with increase in GA_3 concentration both in light and continuous dark photoperiods. Legume species had a low germination percentage due to their hard seediness and mechanical scarification of seeds can break dormancy while it did not have a marked effect on germination percentage in Hedysarum spp. Because the effect of dormancy breaking method on germination percentage depends on the species, hard seediness is a common dormancy mechanism for the species of Leguminosae family; it leads to water and gases impermeability.

However, hard seediness depends on the genus, species and environmental factors by the time of seed development (Ellis and Roberts, 1981). The hard seediness and water-impermeability of many legume seeds is due to a palisade epidermal layer of thick-walled Malpighi a cells in the outer Testa (Exotesta). Osivand et al. (2005) found that the 95 percent of seed dormancy of *Astragalus siliquosus* caused by water impermeability of seed coat and 5 percent caused by physiological factors.

Furthermore, the most effective treatment for the water-permeability, with no side effect on embryo, was the sandpaper scarification. Applying the scarification and low temperature treatments simultaneously had a better response. The reason why germination percentage was lower in Astragalus squarosus and following disruption with sand paper of seeds may be due to inadequacy of 15 time disruption with sandpaper. Similar observation were made by Aydin and Uzun (2001) who found that germination percentage varied between 5 and 10 min disruption with sand paper period in clover (34 and 93% respectively). Therefore, 5 min disruption was not adequate. Many studies showed that fresh seeds were strongly dormant but after 80 days storage only a few seeds germinated at low and medium temperature regimes (Pritchard et al., 2000a).

5.5 Conclusions

In conclusion this study has shown that the interaction between temperature and gibberellic acid can have an effect on dormancy in vernonia. The temperature regime (25/17°C) combined with concentration of gibberellic acid of 0.7mM increased seed germination percentage. However, no genotypic differences were observed with regard to vernonia response to temperature and gibberellic acid concentration.

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6 CHAPTER VI: THE EFFECT OF ETHYLMETHANESULFONATE (EMS) CONCENTRATION, EXPOSURE TIME AND TEMPERATURE ON SEEDLING VIGOUR OF VERNONIA LINES

Abstract

Chemical mutagenesis using ethylmehanesulphonate is the most effective to select mutants with desirable plant attributes and agronomic values. This study investigated the effect of EMS concentration, treatment duration and temperature on seedling vigour of vernonia lines Vge-1 and Vge-4. Vernonia seeds samples of two lines Vge-1 and Vge-4 were treated using previously determined optimum mutagenesis conditions. The seeds were soaked with 0.372%, 0.744% and 1.1 % EMS solution for 2 hours, which was followed by rinsing in water for 30 minute and surface disinfection. Treated seeds were sown under controlled environment tunnels using a randomised complete block design.Significant differences ($P<0.001$) were observed between EMS concentrations with respect to seedling emergence, seedling length and germination percentage. The EMS treatments significantly delayed seedling emergence, seedling length and germination percentage during different incubation periods. Seedling length decreased with the increase in mutagens concentration. However, the extent of decrease varied among different seedling mutagens. Seedling length decreased with increased EMS concentration ($P<0.01$). EMS concentrations had an effect in all observed growth components (seedling vigour, emergence, seedling length, germination percentage).Increased EMS dose, temperature exposure time and duration were harmful to all the traits measured in the study. The effect of increased EMS concentration and exposure times also, resulted into the identification of various chlorophyll mutants within the vernonia lines.

Keywords: Chemical mutagenesis, ethyl methane sulfonate (EMS), Vernonia, mutation.

6.1 Introduction

Vernonia **(***Centrapalus pauciflorus* var. *ethiopica* Willd.**)** is an underexploited potential crop colonizing agricultural and arid lands which is endemic to tropical regions of East Africa including Kenya, Malawi and Ethiopia (Baye et al., 2001; Mebrahtu et al., 2009). Vernonia has a major potential as a manufacturing source of vernolic acid due to its high concentration of natural epoxidized oil (Shimelis et al., 2008). Crops like soybean (*Glycine max L.*) and linseed (*Linum ustitatissimum L.*) are currently used for epoxy oil production, but the oil still requires chemical epoxidation process. Vernolic acid is an important resource in the manufacturing of

paints and coatings and has great charge in the oleo chemical industry. Epoxy fatty acids are broadly used in oleo chemical (chemicals derived from plants or animal fats which are similar to petrochemicals) industry as plasticizers and stabilizers of polyvinyl chloride (PVC), in reformulation of oil based paints, in cosmetics, and pharmaceutical applications (Bhardwaj et al., 2000). With a great demand for natural epoxidized oil, there is need to study and develop the productivity of natural producers of epoxy acids like vernonia.

The chemical mutagens have been current in the development of novel germplasm in crop plants (Gunstone, 2006). Synthetic mutation can induce genetic variation of gene loci controlling economically important traits and/or eradication of unwanted genes from breeding lines (Malathi et al., 2004).

The mutants displaying appropriate traits can be developed openly as new cultivars. Chemical mutagenesis using ethylmehanesulphonate (EMS) can be used to induce useful genetic mutations in crop plants (Kim et al., 2005). Increasing the dose of EMS use alone or in combination with increasing duration of seed exposure to EMS in treatments has been shown to reduce germination and survival percentage in certain crops, and to induce variation in both qualitative and quantitative traits (Kumar et al., 2010; Shah et al., 2008). Because of its potency and ease with which it can be used, EMS is the most commonly used chemical mutagen in plants. EMS alkylates guanine bases and leads to mispairing-alkylated G pairs with T instead of C, resulting in primarily G/C- to-A/T transitions (Bhat et al., 2007).

The most important parameters for inducing mutation with EMS are concentration, duration of treatment, and solution temperature (Munyon, 1985). Thus, prior to the large scale generation of variants initial studies on induced mutations are usually conducted for finding optimum combination of these parameters together with the optimum dose to elicit the best response. Obviously any mutagenesis as gamma ray or EMS treatment makes plants vulnerable to negative effects on characters of M1 and the following generations, such germination, production of vital seeds, root and seedling lengths (Sasaki et al., 1998). Thus, it is important to optimize the best possible condition for generating large number of mutants having good seed germination for segregation without detrimental genetic damages.

Induced genetic variations have been used successfully in several crops to extract mutants with suitable agronomic traits such as herbicide resistance, early maturity and improved nutrition (Singh et al., 1980; van Harten, 1998). EMS is helpful in pre-breeding or genetic enhancement aimed to develop suitable germplasm (Minocha and Arnason, 1962; van Harten, 1998). It is known to induce a broad spectrum of mutations in plants (van Harten, 1998). Recently the

technique is being applied to generate mutants with altered agronomic traits for genetic studies and to predict the gene function through identification of an allelic series by *Targeting Induced Local Lesions IN Genomes* (TILLING) (McCallum et al., 2000).

The EMS usually renders point mutations (Okagaki et al., 1991)which is recommended for use on seed materials, since its application and monitoring of mutational events are relatively easy (Weil and Monde, 2007) . It is a highly recommended chemical mutagen for seeds, because it can be monitored with ease. In plants, EMS usually causes points mutations, but loss of a chromosome segment or deletion can also occur (Okagaki et al., 1991).

Therefore, EMS has the potential of altering loci of particular interest without inducing a great number of closely linked mutations. This allows the plant breeder to obtain useful alleles without the swarm of linked deleterious alleles present in exotic wild germplasm or even from adapted inbred lines. The most important parameters for inducing mutation with EMS are concentration, duration of treatments, and solution temperature. The chemical mutagen EMS has potential to be effectively applied to develop new vernonia lines with high yield and/or enhanced agronomic traits. Crucially to any mutagenesis of crops, the suitable chemical dose and optimal conditions for a particular line/cultivar must first be determined.

The greater doses can produce very drastic effects that may lead to the death of the organism. A moderately lower dose may result in altered growth characteristics. With respect to EMS doses the term lower and higher is relative and may be different for each crop species. EMS has also been reported to delay seedling emergence in crops (Greer and Rinehart, 2009). The effect of various EMS concentrations, duration times and temperature has not been exhaustively investigated.

The objective of this study was to investigate the effect EMS concentration, temperature, exposure time and treatments of vernonia seedling vigor of vernonia lines.

6.2 Material and methods

6.2.1 Presoaking

An ethylmehanesulphonate mutagenesis procedure suggested by Mba et al. (2010) was modified and applied in treating Vernonia seeds. The seeds (in the mesh bags) were soaked in 70% ethanol for 1 minute, and then washed in running water for 1 minute. Seeds were after that soaked in 30% sodium hypochlorite for 5 minutes, and then washed in running water for 1 minute. Seeds were dried by placing in a paper towel for 3 hours under Hume hood at 20-22 °C.

6.2.2 EMS Preparation and Pre-treatment of seeds

Naturally comparable and regular created disease free, dry and dormant vernonia seeds were selected from each of the two Vernonia lines Vge-1 and Vge-4. For two lines, the seeds were arranged into in different batches. Untreated seeds of two accessions lines Vge-1andVge-4 were included as untreated control seeds. Then, seed were positioned inside specially calculated and marked polyethylene mesh bags sealed and paper tags using to labels the bags; the opening of the mesh bags was closed by tying to strings around them. Vernonia seeds were treated with 0.372 %, 0.744 % and 1.1 % EMS solution for 2hours, which was followed by rinsing in water for 30 minute and surface disinfection. EMS doses required were calculated as: EMS = EMS dose [distilled water+2% dimethyl Sulfoxide (DMSO)]/100. I.e. desired concentration of 0.372% of EMS given the total volume under preparation in 1L (1000 m L) 2 % of absolute DMSO in 1000 m L solution was calculated and 0.93 ml, 1.88 ml and 2.25ml of EMS was added per 250ml water and DMSO solution to prepare three different concentrations above for the study. Volumes needed of distilled water were mixed with 2 %(v/v) dimethyl Sulfoxide (DMSO) into a bottle and autoclaved at 120°C for 15 min at 103.5kPa (15psi) and the concoction left to cool at room temperature. A sterile syringe was used to put in the required volume of EMS solution to the sterile water and DMSO fusion. The out coming solution was shaken energetically to give a homogenous suspension. At the end of the pre-soaking period, the bags were removed from distilled water and shaken to remove excess water. Excluding only the control bags, the seeds were soaked in the EMS solutions according to the desired combinations of the three concentrations of EMS (ethylmehanesulphonate) doses (3 levels: 0.93 ml, 1.88 ml, 2.25 ml) and untreated dose (0% EMS), temperatures(3 levels: 30 °C, 32.5 °C, 35 °C)and treatments duration (0.5, 1, 1.5 and 2 hrs) were applied. The temperature was maintained in a water bath during the duration of treatment. The seeds were washed (to remove excess EMS) under running cold tap water for 30minutes. The mesh bags were shaken off to take away excess water and the seeds sited on blotting paper for 2 hours to dry out.

6.2.3 Planting, Data collection and analysis

The seedling soil mix for this experiment was collected from controlled environmental facility (CEF) at the University of KwaZulu-Natal, Pietermaritzburg, South Africa, and sterilized in a steam chamber for 4 hours at 120 °C or 1 kpa = 121 °C and left to cool immediately for 24 hrs. The experiment was laid out using a completely randomized design with the following factors: vernonia lines (2 levels; Vge-1 and Vge-4); EMS concentration (4 levels: 0.93 ml, 1.88 ml, 2.25 ml and control); time (4 levels; 0.5, 1, 1.5 and 2 h) and temperature (3 levels; 30 ^{o}C, 32.5 ^{o}C and

35 ^{o}C) with 3 replications, resulting in 96 treatments combinations and 288 experimental units. Twenty treated seeds of the two lines (Vge-1and Vge-4) were sown at 1 cm depth in plastic trays measured 35 cm x 14 cm, 5 cm height filled with soil sterilized from the stream. One seed was planted per hole. The seeds were covered with extra soil and were sown according to the treatments combination and placed inside a tunnel at fluctuating day/night temperatures at CEF. The trays were watered at least once a day, before and after planting seeds to maintain optimal seedling growth. The number of mutant seedlings from each line and treatment was recorded during emergence and germination percentage were continuously assessed during developmental stage daily for each treatment combination from first emergence until no more seedling emergence was counted up to 30 days after planting. Seedling length was recorded (weekly) as an average shoot length of all seedlings per treatment.

6.2.4 Germination tests

Germinated seeds were counted every day for 14 days and seeds were considered germinated upon the protrusion of the radicles (≥ 2 mm) (ISTA, 2003). The following germination parameters were recorded:

1) Final Germination Percentage is: (FGP) = (number of germinated seeds/number of total seeds) × 100 **(1)**

2) Mean Germination Time (MGT) was calculated according to the following equation (Moradi et al., 2008).

$$MGT = \Sigma Dn / \Sigma n \quad \textbf{(2)}$$

Where, n is the number of seeds which were germinated on day D, and D is the number of days counted from the beginning of germination.

Germination Index:

Is computed by using the following formula:

$$G.I = \frac{n}{d} \quad \textbf{(3)}$$

Where, n = number of seedlings emerging on day “g”,

d= day after planting

6.2.5 Data Analysis

Data was subjected to general analysis of variance (without blocking) and Pearson correlation analysis using Genstat® (12th edition, VSN International, UK).The least significant difference (LSD) test procedure was used to compare means at 5% level of significance. Pearson's correlation procedure was conducted to estimate the degree of connection between agronomic traits.

6.3 Results

Table 6.1 shows mean squares for germination index, emergence and seedling length for vernonia seeds exposed to different EMS concentrations, temperatures and time of exposure. Significant differences ($P<0.001$) were observed between EMS concentrations and time of exposure with respect to seedling emergence. There was a significant interaction ($P<0.001$) between EMS concentrations and time of exposure with respect to seedling emergence. Significant differences ($P<0.001$) were also observed between EMS concentrations and vernonia lines with respect to seedling length. The interaction between the vernonia lines, EMS concentrations and time of exposure was also significant ($P<0.05$) with respect to seedling length.

Table 6.1: Mean squares for germination index, seedling length and emergence between different treatments, temperatures, lines and exposure period.

Source of variation	D.f.	Germination index	Emergence	Seedling length
Ems	3	47307.2	15769.1***	361.351***
Temperature	2	1943.8	971.9	11.048
Line	1	2396.9	2396.9**	36.087***
Time	3	9236.4	3078.8*	69.227***
Ems x Temperature	6	2571.3	428.6	4.051
Ems x Line	3	1553.6	517.9	4.195
Ems x Time	9	8124.4	902.7**	0.963
Temperature x Time	6	2286.9	381.2	8.556
Line x Time	3	413	137.7	27.103**
Ems x Temperature x Time	18	3577.8	198.8	3.294
Ems x Line x Time	9	2044.1	227.1	1.904*
Residual	224	67627.8	301.9	0.622
Total	287	149083.2		

D.f. = degrees of freedom; m.s. = mean square

The interaction between the vernonia lines Vge-1 and Vge-4, temperature of exposure (30 ^{o}C, 32.5 ^{o}C and 35 ^{o}C) and EMS concentrations (1.86 mL, 2.77 mL and 5.5 mL) on vernonia seedling length was shown (Figure 6.1). Vge-1 had comparably lower values of seedling length of 2.67mm (30 ^{o}C; 1.86 mL EMS), 1.68 mm (30 ^{o}C; 5.5 mL EMS), 4.88 (30 ^{o}C; control) and 6.41 (35 ^{o}C; control). However, Vge-4 recorded the following values: 4.42 mm (30 ^{o}C; 1.86 mL EMS), 3.41 mm (30 ^{o}C; 5.5 mL EMS), 8.88 (30 ^{o}C; control) and 9.5 (35 ^{o}C; control).

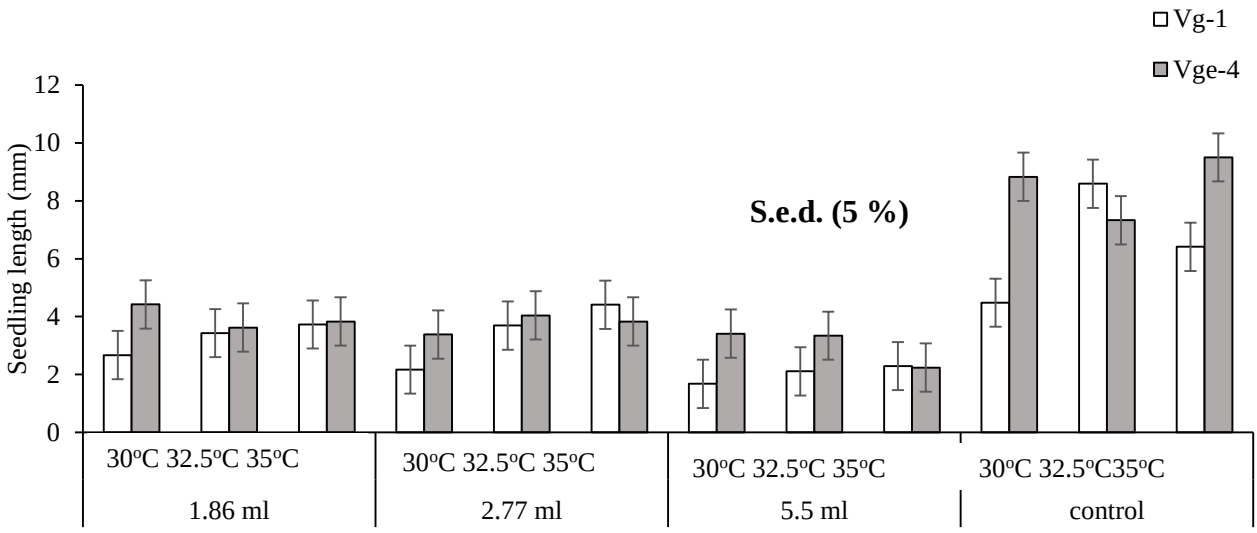

Figure 6.1: Effects of different temperature exposures and EMS concentrations on seedling length between two vernonia lines.

The effect of EMS concentrations and time of exposure on the emergence of two vernonia lines. The control had the highest emergence (63.9 %, 68.1 %, 68.3 % and 66.4 %) et all times of exposure (30 minutes, 60 minutes, 90 minutes and 120 minutes) respectively. Least emergence values were recorded within high concentrations of EMS treatment (5.5 ml) being (18.6 % and 18.5 %) at 30 minutes and 60 minutes exposures, respectively.

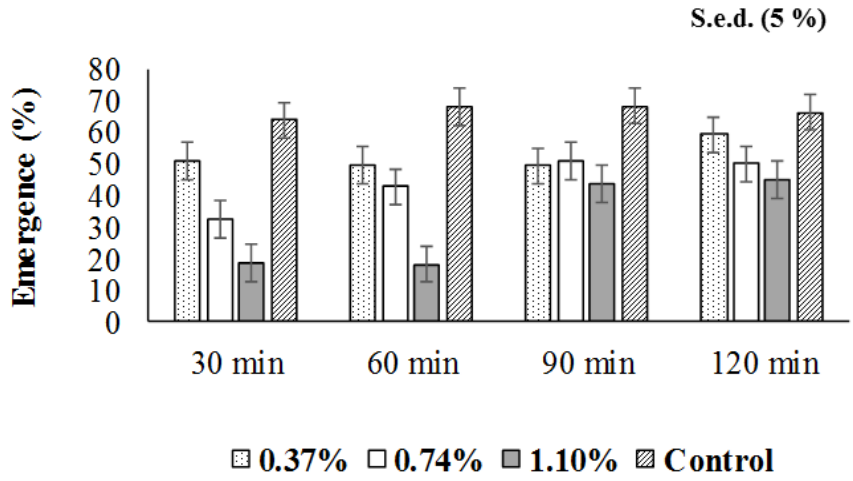

Figure 6.2: The effects of EMS concentrations and exposure time on seedling emergence of two vernonia lines.

The effects of exposure time on the seedling length of two vernonia lines are described in figure 6.5. The graph is characterised by increasing seedling length with increasing time of exposure being 3.64 mm, 3.94 mm, 4.47 mm and 5.26 mm at 30 minutes, 60 minutes, 90 minutes and 120 minutes, respectively.

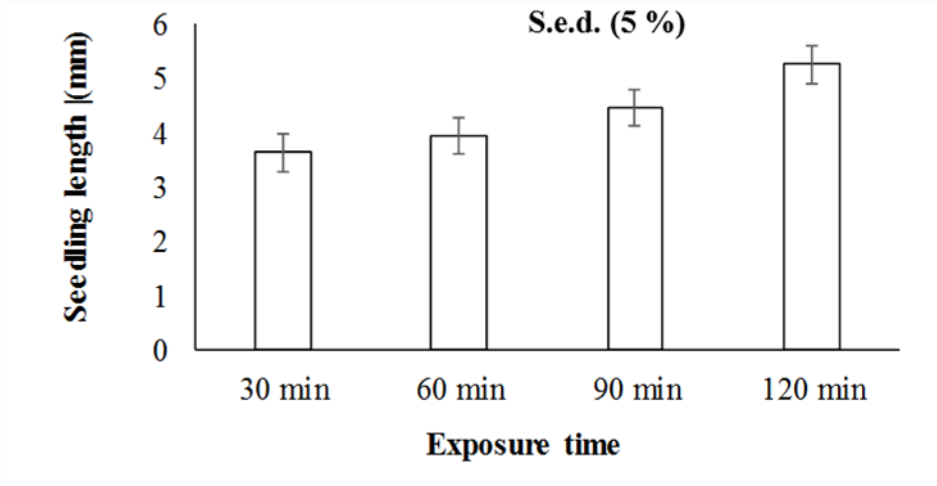

Figure 6.3: The effects of exposure time on seedling vigour (seedling length) of two vernonia lines

The emergence (%) of two vernonia lines Vge-1 and Vge-4 exposed to EMS chemical at different temperatures and exposure time are shown in Table 6.2. Vge-4 line showed a higher emergence value (52.7 %) compared to Vge-1 (44.5 %).

Table 6.2: Emergence (%) of two vernonia lines (Vge-1 and Vge-4) exposed to EMS chemical at different temperatures and exposure time.

Line	**Vge-1**	**Vge-4**
Emergence (%)	44.5[a]	52.7[b]
Sed	**2.9**	

6.4 Discussion

Mutagenic agents such as, EMS and certain chemicals can be used to induce mutations and generate variations from which desired mutants may be selected. Mutation induction has become a proven way of creating variation within a crop variety by way of inducing micro mutations in addition to the visible macro mutations. It offers the possibility of inducing desired attributes that either cannot be found naturally or have been lost.

The present study found significant interactions among lines, EMS doses, temperature regime and exposure time on emergence and seedling length in vernonia lines (Table 6.1).The control had higher seedling length compared to EMS treatment at different concentrations especially in Vge-4 at 30 and 35 ^{o}C (Figure 6.1). Studies by Pascual-Villalobos et al. (1994) have shown that EMS mutagenic treatment can produce normal or abnormal lines hence in this study; regardless of EMS concentrations resulting seeds generally had lower vigour probably due to chromosomal aberrations. Also, Khan et al. (2004) pointed out mutagenic treatments brought reduction in seed germination. Observed slight slow germination with the highest dose EMS treatment is indeed considered less than expected (Menda et al., 2004). Also, among the chemical mutagens and alkylating agents, EMS has especially been demonstrated to be the most effective.

Previous studies affirm that, chlorophyll mutants were frequently observed among EMS treatment group but were rare among those treated with physical mutagens (Chopra, 2005). The stimulating effect of physical mutation on germination may be attributed to the activation of RNA or protein synthesis. However, reduced seed germination due to mutagenic treatments may be the result of damage of cell constituents at molecular level or altered enzyme activity (Khan et al., 2004). Mehetre et al. (1994) in soybean have attributed reduction in germination percentage to the seed injury caused by higher exposures of gamma rays; while other studies correlated it with abnormalities in mitotic cycles and in metabolic pathways of the cells (De Micco et al., 2011).

Reduced seed germination is attributed to a delay and reduced seed germination to the effect of mutagen on meristematic tissues and chromosomal damages (Kumar and Yadav, 2010). Other studies indicated that increased EMS concentration could be accompanied by a corresponding greater amount of injury to seedlings with subsequent lethality (Kleinhofs et al., 1978; Mba et al., 2010). Singh et al. (1980) suggested that the effect of EMS in reducing germination could probably be attributed due to water potential difference, in which the higher EMS concentration may have lowered the water potential outside the seed and therefore the seeds could not imbibe enough water for proper germination. The badly damaged cells would produce only a few cell

progeny and growth will recur from those cells which are least damaged genetically. This implies that despite mutation induction, Vge-1 and Vge-4 were genetically different. In agreement with previous studies (Chapter 3), Vge-4 was generally more vigorous and high yielding compared to Vge-1. In agreement with table 6.2, emergence was higher in Vge-4 compared to Vge-1.The control had higher emergence compared to all EMS treatments. Moreover, there was a decline in seed emergence with increasing the concentrations of EMS (Figure 6.2). The graph further showed that increasing exposure time was associated with increasing emergence in at all EMS concentrations (Figure 6.2; 6.3). Other studies have shown that time and rate of seedling emergence and growth are influenced by the genetic constitution of the variety, seed dormancy, seed vigor, depth of planting, soil aeration, temperature and water supply (Forcella et al., 2000).

It is possible that chemical mutagens may prove to be a better alternative for inducing morphological mutations, as they induce mutations at a much higher rate and cause less chromosomal disturbances than radiations (Sharma, 2001). Various investigations suggest that the possible cause of these macro mutations may be chromosomal aberrations, small deficiencies or duplications and most probably gene mutations (Singh et al., 1980). Morphological mutations affecting different plant parts can be of enormous practical utility and many of them have been released directly as crop varieties (Shah et al., 2010). A wide range of morphological mutations induced by physical and chemical mutagens have been reported in different crop plants such as black gram (Kumar et al., 2005), chickpea (Shah et al., 2008). This implies that exposure time and concentrations play a role in inducing mutations. The optimum exposure time and concentrations for exposing the seed to EMS was 0.37 % after 120 minute.

6.5 Conclusions

The study concluded that ethylmehanesulphonate concentrations have an effect all observed growth components (seedling vigour, emergence and germination percentage). Increased EMS dose, temperature exposure time and duration were harmful to all the traits measured in the study. Also, various chlorophyll mutants were identified within the vernonia lines.

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7 CHAPTER VII: GENERAL DISCUSSION

It was clear from the literature that vernonia is a new potential industrial crop with very high content of vernolic acid in the seed oil. The species is known to naturally grow as a weed in fields or in woodlands under a wide range of agro ecological conditions in African countries. Its importance lies in the unique properties of the seed oil, which make it interesting both economically and ecologically and is most suitable for dry land farming (Baye et al., 2001; Gilbert, 1986; Perdue Jr, 1988). It remains one of the crops most neglected by science, yet empirical evidence and fragmentary research results suggest that it is a crop with great a naturally occurring epoxidized fatty acid, is primarily present in the oil as the triglyceride trivernolin with unique physical properties, in particular, low viscosity. Vernolic acid, beside other uses, is a useful raw material for manufacturing paints and coatings. As a major source of natural vernolic acid, there is no alternative to vernonia and, hence, a wide potential market is available.

The general aim of the study was to compare two selected lines of vernonia mutant plants and control with respect to morphological and seed quality characteristics.The specific objectives of the study were:

1. To evaluate the morphological and seed characteristics of vernonia lines Vge-1 and Vge-4 treated with ethylmehanesulphonate and untreated controls,
2. To examine the pattern of seed quality development in mutants lines of vernonia Vge-1 and vge-4 and untreated controls plants,
3. To determine the effect of gibberellic acid and potassium nitrate on seed dormancy in vernonia lines,
4. To investigate the effect of EMS concentration, exposure time and temperature on seedling vigor of vernonia lines Vge-1 and vge-4.

A new industrial oil seed crop for the production of natural epoxy oil (Mohamed et al., 1999; Thompson et al., 1994). Vernonia line germplasm production and management practices depend on local experiences and resources. Development of improved lines suitable for particular environments and production technologies are still in the early stages.

In the initial study, we evaluated the morphological and seed characteristics of vernonia lines Vge-1 and Vge-4 treated with EMS and untreated controls. Highly significant differences ($P<0.001$) were observed with respect to leaf number and seed mass. Vge-1 mutants plant

produced more leave (48 leaves per plant) compared with untreated controls (40 leaves), Vge-4 treated seeds had higher mass of heads per plant (2.46 g) compared untreated controls Vge-4 with (0.75 g). Vge-4 had seed yields of 3.5 ton/ha compared with untreated controls (3 ton/ha). The effect of EMS application on growth parameters in vernonia lines resulted in an increase in leaf number, mass of seed per heads and seed yield of Vge-4; this line could be important than Vge-1 for potential use of vernonia as a new industrial crop.

Secondlythe experiments to determine the pattern of seed quality development on mutants lines of vernonia, The results showed that germination percentage (%) was generally low but differed significantly ($P<0.01$) between the lines; Vge-1 untreated controls seeds had the highest germination percentage (60%) compared with Vge-1 untreated with (58%). Whereas, vernonia linesVge-1 and Vge-4 showed about 80-90% of seeds staining. The low germination percentage in vernonia could probably be attributed to seed dormancy.

The third experiment was conducted in controlled environmental facility (CEF) determine the effect of gibberellic acid and potassium nitrate on dormancy in vernonia lines. Highly significant differences and interactions ($P<0.001$) were observed between temperatures and dormancy breaking chemicals with respect to percentage germination, mean germination time (MGT) and germination index (GI). The germination index increased with GA_3concentration application. The mean germination time also improved for all treatments.

The fourth experiments were conducted in laboratory and the controlled environmental facility as a single factor experiment laid out in randomized complete block design to investigate the effect of EMS concentrations, duration time and temperature on seedling vigour of vernonia lines. Highly significant differences ($P<0.001$) were observed between EMS treatments with respect to seedling vigour, germination percentage and seedling height. The seedling length decreased with increased EMS doses/concentration. Ethyl methane sulfonate concentration had an effect in emergence percentage and germination index Increase EMS, temperature exposure time and duration negatively impacted on all the traits measured in the study. EMS had the effect of causing mutations as evidenced by the various chlorophyll mutants identified in the study. The major findings of this study suggest that ethylmehanesulphonate as a mutagen was effective in inducing genetic variability in vernonia. This suggests that EMS can be used for creating new vernonia lines.

7.1 Conclusions

Findings of this study show that ethylmehanesulphonate mutagen was effective for creating new vernonia lines Vge-1 and Vge-4. It is therefore recommended that studies be undertakes to promote its use and cultivation of Vge-1and Vge-4 as an alternative industrial oil crop.

7.2 Recommendations

- The fact that vernonia lines Vge-1 and Vge-4 treated and untreated showed lowest germination less than 70% and viability and vigour warrants further research,
- Further research is also needed to focus on low emergence imposed by the plant at establishment stages,
- Data obtained from the study will be valuable for modelling crop responses to different growth stages and to contribute to the establishment of a valid basis for advice on crop management,
- Vge-4 line showed to be more vigorous hence it can be selected for further improvement as it is more promising than Vge-1.
- Mutation induction can be done successfully with lower doses of EMS (0.34 %) and for longer period (about 120 minutes).

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