IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 13, Issue 5 Ver. II (Sep – Oct 2018), PP 70-80
www.iosrjournals.org

Physiological Determination (Manavaca Solution) of Alluminium Toxicity Tolerance In Selected Wheat Lines (Triticum Aestivum

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Abstract: *This study was conducted to determine the aluminium tolerance of fifteen strains of wheat. Aluminium (Al) toxicity is one of the major limiting factors for wheat production on acid soils. It inhibits root cell division and elongation, thus reducing water and nutrient uptake, consequently resulting in poor plant growth and yield. The studies involved field work where wheat was grown in three locations (Chepkolel, Moiben and Kaptagat) arranged in RCBD. Tiller numbers, spikelets number, main tiller height, main head length, seed yield and 1000 kernel weight measured. Physiological test was then carried out using Magnavaca (nutrient) solution in the laboratory to determine length of root growth. Where sufficient number of wheat seeds was set to germinate on trays between germination papers moistened with 20 ml of 18 mΩ* H_20 *and placed in a growth chamber set at* 26^oC *in darkness for four days. Seedling seminal roots were then inserted through the mesh bottoms and allowed to grow in nutrient solutions for four days then RNRG determined. DNA extraction was done by using Dellaporta protocol and the DNA analysis was done using simple matching (UPGMA) software, followed by amplification using SSR primers. The PCR products were then electrophoresed on 3% agarose gel containing 7 µLethidium bromide (EtBr), at 100 V for 1 hour, and then observed under a UV transilluminator and photographed using gel documentation unit. Bands then scored, where (**1**) indicate presence, (**0**) indicate absence. The data was analyzed using SAS and means separation using LSD. Field screening showed that two varieties; Robin and Njoro(BW)11 were found to have aluminium tolerance. Significant variation in yield and yield related components were either due to environmental influence and interaction between environment and genotypes. There is positive significant correlation* $(P \leq 0.05)$ *between yields per hectare and tiller number, implying that tiller number can be used to determine yielding potential on acid soils. Also main tiller head length and tiller number were positively significantly correlated meaning that they have more impact on grain yield. There is very close relationship between Relative Net Root Growth (RNRG) at 148µM and 222 µM .*

Key Words: *Growth,Mollecular,phytotoxicity,Tillering,Screening*

Date of Submission: 14-09-2018 Date of acceptance: 29-09-2018

I. Introduction

Wheat (*Triticumaestivum L*) is an annual crop and is the second most important cereal grain in Kenya after maize, according to National Development Plan (NDP) (2009). The crop is grown largely for commercial purpose on a large scale but this pattern is changing with small scale farmers taking up wheat farming on smaller plots. Wheat is the leading source of protein in human food having a higher protein than other cereals. It takes between 110 and 130 days to be harvested depending on species, climate and soil conditions. It is estimated that an average yield ranging from just one ton per hectare to 2.3 tons per hectare is realized and the acreage under wheat in Kenya is about (100,000-120,000) hectares translating into only 350,000 tons per year against consumption of approximately 720,000 tons (NDP 1997-2001).The demand for wheat flour in Kenya at present cannot be sustained by local production, so the country relies on imports to meet almost half of its consumption (World Food Programme annual report, 2008).Wheat provides almost 20% of all human food energy. It is made into various products including Chapaties, Roti, Mandazi, Cakes, Doughnuts, Biscuits, Bread (leavened, flat and steamed), baby foods and food thickeners. It is also used as a brewing ingredient in certain beverages. In addition, wheat farm by-product (hay) is also widely used as animal feed (Weegels*et a.,l* 1996).

Wheat does not grow well under very warm conditions with high relative humidity, unless nutrient availability is very favorable. In addition, wheat diseases are generally encouraged by such climatic conditions. Good soils for production are well aerated, well drained and deep, with 0.5% or more organic matter and optimum soil pH ranges between 5.5 -7.5 (Bhagat*et al.,* 2009).

In wheat, Aluminium(Al) is one of the major factor that limit its productivity in acidic soils (Kochian*et al.,* 1995). Acid soils $(pH \leq 5)$ increase the phytotoxic levels of trivalent Al (Al^{3+}) whereas at higher pH, other non-toxic forms such as $Al(OH)_2$ and $Al(OH)^{2+}$ are more prevalent (Delhaize and Ryan, 1995). Alluminium toxicity primarily affects the division and elongation of root apex. When Al^{3+} penetrates into the root it binds to the negative charges of phospholipids in the plasma membrane leading to rigidification and disruption of membrane function and enhancement of oxidative stress (Jones *et al.*, 2006). These physiological changes in the root cell results in the poor uptake of nutrients and water that ultimately affect wheat crop yield. Alluminium toxicity is mostly severe in soils with low base saturation, poor Ca and Mg.

Acid soils (pH<5.5) are widely distributed constituting over 40% of arable lands in the world (Hang, 1984). In Kenya, acid soils cover 13% (7.5 million hectare) of arable lands. They are widely distributed in the highlands areas and they cover significant parts of central, Rift valley, western and Nyanza provinces (Kanyanjua*et al*., 2002), which are the major maize and wheat growing areas. Acid soils are generally low in fertility and in such soils; the growth of wheat is limited by Al toxicity and Phosphorous deficiency (Magnavaca*et al.*, 1982)

Preliminary observations have shown that, the level of Aluminium ions found in acid soils of Kenya could be toxic to wheat. In addition, since most of wheat is produced in Rift-valley and most of the soils in the wheat growing areas are acidic, Al stresses at such low pH affect wheat production due to its toxicity.

Statement of the problem

Low yields of wheat are often associated with biotic and abiotic stress and it is of great concern to both farmers and breeders. The most speculated abiotic factor is the Aluminium toxicity. This situation is least known by farmers and therefore it is necessary to look for ways of dealing with this problem in order to promote the production of this high valued crop. Breeding crops with increased Al tolerance has been a successful and active area of research. However, the underlying molecular, genetic and physiological bases are still not well understood. Recent progress by a number of researchers has set the stage for the identification and characterization both of the genes and associated physiological mechanisms that contribute to Al tolerance in important wheat species grown on acid soils. The use of agricultural lime as a soil amendment material has proved beneficial (Nekesa, 2006). Though lime corrects soil acidity, it increases cost of production and burdens the farmer more. Breeding Aluminium tolerant wheat varieties will be of great importance to farmers

Objective

The main objective of this study was to physiologically determine the aluminum toxicity tolerance using nutrient solution (magnavaca solution) of selected wheat lines in the field.

II. Literature review

The origin and evolution of wheat

The term wheat is normally used to refer to cultivated species of genus *Triticum.* The genus *Triticum* is complex and includes diploids, tetraploids, and hexaploids with chromosome numbers of 14, 28 and 42 (Sakamura, 1918). The basic chromosome number in the genome is 7. Durum wheat carries two genomes, the A in common with einkorn wheat and the other the B genome. A cross between durum and bread wheats often has 14+7 chromosome at meiosis. The bread wheat on the other hand carries genome A and B plus a third genome D. The genus *Triticum* evolved through amphiploidy. (Figure 1) below

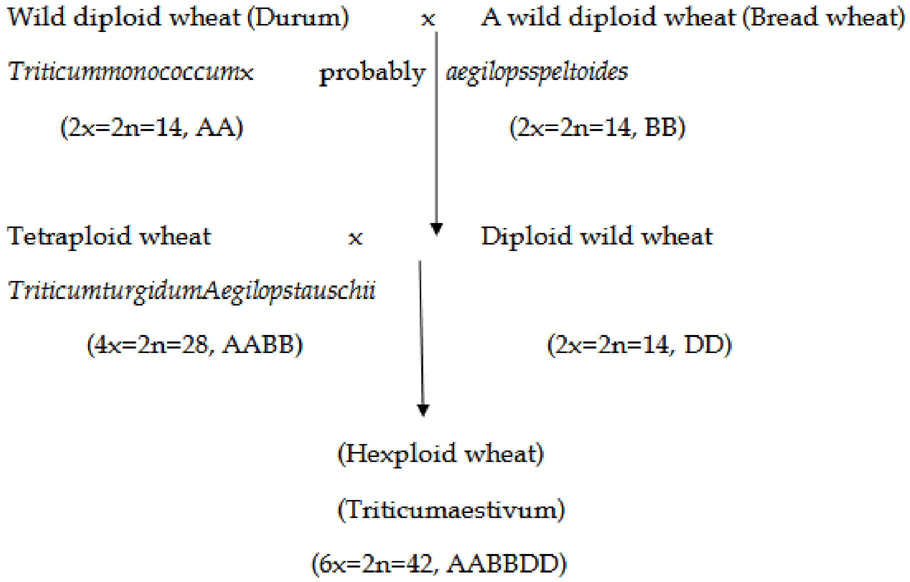


Figure 1: Evolution of durum and bread wheats (Knott, 1989)

BOTANICAL CHARACTERISTICS OF WHEAT

Wheat (*Triticumaestivum L*) is a cereal of Gramineae family that also includes cereals such as rice, maize, oats, sorghum and others. Wheat is an herbaceous annual plant up to 1.2 metresheight,stems are erect and presents a structure of a cane, that is they are hollow inside except at the nodes where it is not apical but produced by stretching of tissues above nodes (meristems).The leaves grow from the nodes .like the rest of the grasses, they have two parts: the petiole sheath that surrounds and protects the meristems and growth zone and the limbo that is elongated in shape and as parallel veins (Caligari and Brandham 2001).

Wheat flowers are gathered in spikes. Each spike consists of the main axis or rachis on which the spikelets are distributed laterally. These consist of a main axis from which some filaments and terminated by the glumes that enclose the flower. Beside the glumes, flowers are protected by two bracts; the inner is called palea and the outer is called lemma. The latter is topped with a beard that gives the ear of the wheat feathery appearance.

Wheat flowers do not have petals and sepals. Each female flower consists of an ovary from which two styles emerge, attached with two feathery sticky stigmas. The male flowers have three stamens that can be gold, green or violet. Fertilization and maturation of wheat produces the grain, a fruit of caryopsis type (Shewry and Hey 2015).

Wheat grain has a protective coating or husk (bran). This husk consists mainly of fibre which is completely removed when wheat is milled. The external casing is called pericarp, the central layer or integument is the mesocarp and inner layer epicarp. They consist mainly of minerals, protein and vitamins. The intermediate layer between the outer casing and the endosperm or albumen consists mainly of oils and dyes.Endosperm is (80-90) % of the gross weight and consists of carbohydrates such as starch. This is the reserve substances for growth of new plant.

Germ occupies bottom of the endosperm, consist of proteins, oils, enzymes and vitamins of group B. It consist of radical (embryonic root) and plumule (embryonic shoot).From this part of the grain the new plant is formed (Day *et al*., 2006)

LOCATION OF GENE RESPONSIBLE FOR ALUMINIUM RESISTANCE

The resistance to aluminum in wheat is located in the long arm of the chromosome 4D-(MAS wheat). The gene, Ta*ALMT1* on chromosome 4D controls the aluminium-activated efflux of malate from roots common in Aluminium resistant genotypes, also there is the gene Xcec on chromosome 4BL, which activate citrate efflux. Moderately resistant genotypes are encoded on loci 5AS, 6AL, 7AS, 2DL, 3DL, and 4DL. (Reynolds *et al*., 2009)

DIAGNOSIS OF ALUMINIUM TOXICITY IN PLANT

The diagnosis of Aluminium toxicity from visual signs in plants is unreliable (Matsumoto *et al*., 2001) and critical plant concentration of aluminium are ill defined. The aluminium concentration in leaves of Lucerne is of little value in determining toxicity (Pineros*et al*., 2002). A value above 150mgAl/kgDM in subclover leaves may indicate toxicity (Ma, 2002).Soil exchangeable aluminium concentration is used as a guide to the likelihood of aluminium toxicity. Aluminium levels greater than 15mg/kg may be a problem and above 50mg/kg is toxic, in which case the economics of liming should be considered to overcome this problems (Wenzl*et al*., 2002).Testing for aluminium tolerance in the field has a number of disadvantages some of which include: presence of other toxic elements and variability in the aluminium content throughout the field. This constraint can be alleviated by exposing seedlings to known levels of aluminium in nutrient solutions.

ALUMINIUM (Al^{3+}) TOXICITY SYMPTOMS IN PLANTS

The main symptom of aluminium toxicity is a rapid inhibition of root growth, which may translate to a reduction in vigour and crop yields (Kochian*et al.*, 2005). Roots injured by aluminium becomes stubby and thick, dark coloured, brittle, poorly branched and rubberized with reduced root length and volume (Nguyen *et al*., 2003). The rapid inhibition of radical growth can be seen in the primary and lateral root apexes, which become thick and turn brownish-grey (Rout *et al.*, 2001). These symptoms become evident after a few minutes or hours of the plants being exposed to micromolar concentration of aluminium in hydroponic solution

(Rengel and Zhang 2003). Radical inhibition coincides with a decline in cell division (Frantzios*et al.*,2001) and elongation of the root cells, which then induces significant lignifications of the cell wall by crossing with pectin (Rout *et al*., 2002; Jones *et al*., 2006) This alteration prevents water absorption which is essential for the transport of nutrients through the cell wall, eventually causing a decrease in yield and grain quality (Zheng and Yang, 2005; Roman *et al*., 2002). The shoot is also inhibited due to limiting supply of water and nutrients.

Furthermore, aluminium toxicity also triggers membrane lipid peridoxidation and apoptosis or programmed cell death (PCD) (Pan et al 2001). It has been reported that prolonged exposure to this element can induce and produce responses of a rapid change in other biochemical and physiological processes (Rengel and Zhang, 2003). This is why the symptom of aluminium level resembles phosphorous deficiency, preventing plant growth and turning mature leaves dark green, stems purple and killing leaf apexes (Wang *et al*., 2006), in other cases, aluminium toxicity reduces calcium transport. This makes young leaves curl, preventing the development and growth of the petiole (Rout *et al.*, 2001). According to some scholars excesses of aluminium also induces symptoms of iron deficiency, which was observed in *Triticumaestivum* (Rout *et al*., 2001). Aluminium affects the normal operations of the cell membranes, causing enzymatic disorders and affecting the DNA nuclear (Pan *et al*., 2001). It acts on the phosphate groups, altering their topology and recognition by polymerase DNA, modifying their functioning of the explicate machinery due to increased rigidity of double helix (Route *et al*., 2001; Zhang *et al*., 2002). In addition, aluminium is closely linked to other DNA associated molecules, such as phosporylated proteins (histones) (Kochian 2005). Aluminium interferes in the normal operations of Golgi apparatus and in the peripheral cells of the apex of intact roots, in their quiescent centre, meiotic activity and DNA synthesis (Rout *et al*., 2001). Aluminium may also affect the mechanism that controls the organization of cytoskeleton microtubules as well as the polymerization of tubulin by delaying disassembly during mitosis (Franzios*et al*., 2003). This would affect the direction of the microtubule, which is closely related to cell expansion (Zheng and Yang, 2005).

ALUMINIUM TOLERANCE MECHANISM

Species vary in their ability to grow in acid soils with severe aluminiumphytotoxicity (Jones and Ryan 2003). A study done by Rangel *et al*., (2007) on bean seedlings showed a reduction in root growth when sown in high aluminium concentration. In a similar study done by Musa and Munyinda (1986) on wheat, aluminium treatment was observed to reduce the root length but increased lateral roots as the aluminium concentration increased in solution. However, there was no differential effect with shoot length across aluminium concentration; Reid *et al*., (2001) also found that aluminium injury to barley was characterized by a decrease in root length exposed to different aluminium concentration.

Study on sweet sorghum by Munyinda*et al*., (2008) showed a reduction in root length as well as the number of roots as aluminium concentration was increased. Aluminium tolerance mechanism as been classified into two; a) those that exclude aluminium from root cells and b)those that allow aluminium to be tolerated once it has entered the plant cells (Barcelo and Poschenrieder., 2002). Species in tropical areas are very resistant to aluminium stress and some of these species can accumulate high concentration of aluminium in the leaves, greater than 1% of their dry weight (Jones and Ryan, 2003). However certain plants referred to as aluminium accumulators may contain over ten times more aluminium without any injury for example Tea plants. The aluminium content in tea can reach as high as 30 mg of dry mass in older leaves (Matsumoto *et al*., 2001). By contrast, cereals like; *Zea mays, Hordeumvulgare* and *Triticumaestivum* and others do not accumulate as high aluminium internally but rather use the aluminium exclusion mechanism through exudation (Caniato*et al*., 2007). This may be one of the most widely use mechanism by the most species studied. Nevertheless, important differences are manifested in some of the features of these mechanism in each species, including the nature of inducibility by the organic acids released and whether aluminium induced gene action plays a role in tolerance (Kochian, 2001)

EXTERNAL TOLERANCE MECHANISM (EXCLUSION)

Some species detoxify aluminium in the rhizophere by excluding organic exudates from their roots (Liet*et al*., 2002). This exudation is located in the radical apexes of some species as this region is very sensitive to aluminium toxicity due to constant cell division and elongation (Mossor-Pietraszewska., 2001). The organic acids commonly secreted are malate, citrate and oxalate. Malate and citrate are present in all cells given they are involved in mitochondrial respiratory cycle (Jones and Ryan. 2003). Organic acids level vary between species, cultivar and even between tissues of the same plant under identical growth conditions. In addition, organic acid biosynthesis and accumulation increased drastically in response to environmental stress (Lopes and Bucio, 2000). It has been observed that tolerant genotypes exclude a greater amount of organic acids than sensitive genotypes, which would support the notion that organic exudation is an aluminium tolerance mechanism (Delhaize *et al*., 2004). However, it has been reported that aluminium sensitive species of wheat showed a greater accumulation in cortical tissue (5 to 10 times more) than the tolerant genotypes exposed for the same period of time (Kashif*et al*., 2004). Some organic acids such as citrate, malate and oxalate are able to form stable complexes with aluminium (Guo*et al*., 2007), where aluminium-citrate complex bond is strongest, followed by Al-oxalate and Al-malate complexes which are insoluble and not available for plants (Jones and Ryan., 2003).This is because Al is a metal that tends to form strong complexes with the oxygen donor. The aluminium-citrate complexes is important in terms of plasma membrane transport because citrate is been used to desorbs Al from root cells and its release has been suggested by some scholars to be an important Al resistance mechanism in snap bean and corn. A study conducted by (Ryan *et al*., 2001) demonstrated that aluminium induced released of roots organic acids from Al –resistant genotypes. Different wheat cultivars were screened for Al stimulated malate release and it correlated with Al-resistance.

Effect of acid soil in crop production

Acid soils, pH≤5.5, are one of the most important limitations to agricultural production worldwide. Earlier studies by Von Uexkull and Mutert (1995) indicated that approximately 30% of the world's total land area consists of acid soils, and that as much as 50% of the world's potentially arable lands are also acidic. The production of staple food crops, and in particular grain crops, is negatively impacted by acid soils. For example, 20% of maize and 13% of rice production worldwide is on acid soils (Von Uexkull and Mutert, 1995). Furthermore, the tropics and subtropics account for 60% of the acid soils in the world. Thus, acid soils limit crop yields in many developing countries where food production is critical.

The impact of acid soils in the agronomic context is substantial. The value of all production on acid soils is estimated at US$ 706 billion with the greatest proportion being accounted for by forest and grasslands (Sumner, 2001). The value of arable and permanent crops is only US$ 129 billion. However cultivation of genotypes with tolerance to acidity could increase productivity by a further US$43 billion and if some grasslands and forests were reassigned to arable production, the figure could be substantially higher (Sumner, 2001). Research on acid soil tolerant wheat varieties will substantially increase its productivity.

III. Materials And Methods

Materials

Thirteen newly develop selected lines and two varieties (checks) developed by KALRO Njoro were used in this study. The two varieties were selected basing on tolerance against aluminium toxicity, while the develop lines had been selected from the crosses made on several varieties as shown in appendix (1) with the aim of getting genotypes which could resist effects of acid soils. These lines and two varieties are as follows:

ELD/AS/14/L1,ELD/AS/14/L2,ELD/AS/14/L3,ELD/AS/14/L4,ELD/AS/14/L5,ELD/AS/14/L6,ELD/AS/14/L7,ELD/AS/14/L8,ELD/AS/14/L9,ELD/AS/14/L10, ELD/AS/14/L11,

ELD/AS/14/L12,ELD/AS/14/L13,ROBIN,(Resistant to aluminium toxicity),NJORO BW (11), (Resistant to aluminium toxicity)

Study sites

The study was conducted both in the fields and laboratory. The field sites included University of Eldoret which is located at 0^0 57'N; 35^0 31'E, 13Km North East of Eldoret town at an altitude of 2,143m a.s.l and mean annual temperatures of 16^0C with soil pH of 4.5. Moiben located at 0º58'N; 35º'31'E, 43Km from Eldoret town at an altitude of 2454m a.s.l and mean annual temperatures of 18ºC with soil pH of 4.8. Kaptagat located at 0º46'N; 35º 48'E, 40Km from Eldoret town at an altitude of 2800m a.s.l and mean annual temperatures of 14 ºC with soil pH of 4.0.The laboratories used to carry out the study are botany laboratory of the University of Eldoret and molecular laboratory of KALRO Njoro.

Field preparations

The three fields used were chosen and soil sampling done. The samples were taken for soil analysis where soil pH and aluminium levels were established. Hand primary cultivations were done for all the sites and three weeks later secondary cultivations were carried out. The fields were then divided into experimental plots measuring 4 metres by 3 metres. Seeds which were pre-treated against fungal attack then planted in each plot organized in Randomized Complete Block Design (RCBD), at a rate of 125Kg/ha, an inter row spacing of about 5cm. A total of three replicates each with fifteen experimental plots and each had four rows (seed lines) were planted using NPK 23:23:0 fertilizer drilled together with the seed at planting at the recommended rate of 150Kg/ha.Wheat Aphids (RWA) and other insects were controlled using Bulldock star EC (Beta-Cyfluthrin 12.5g/l+ Chlorpyrifos 250g/l) on a 14 day interval starting at the end of tillering (GS 29) up to early dough stage (GS 83) at a rate of 20ml/ha.

Common wheat agronomic practices were carried out as described by Kinyua and Ochieng (2005).Weeds were controlled at the end of tillering using Buctril MC (BromxynilOctanoate 225g/l and MCPA Ethyl Hexyl Ester 225g/l) at a rate of 1.25L/ha mixed with Puma Komplete (Fenoxaprop-P-ethyl 64g/l+ iodosulfuron-methyl-sodium 8g/l+Mefenpyr-diethyl 24g/l).

Screening wheat for Tolerance to Aluminium Toxicity (Magnavaca test)

Aluminium induced inhibition of root growth in nutrient solution is used to quantify Al tolerance, using basal nutrient solution as described by Magnavaca*et al.,* (1982). Sufficient numbers of seeds for solution culture study were set to germinate on trays between germination papers moistened with 20 ml of 18 mΩ H_20 and placed in a growth chamber set at 26 ^{o}C in darkness for four days.

Seedling seminal roots were then inserted through the mesh bottoms of polyethylene cups (polypots) and introduced into the nutrient solution so that only the roots were immersed in the nutrient solution. The seedling polypots then filled with black beads that prevented harmful light rays from decomposing iron chelate component of the nutrient solution; black lids and tubs wrap with black polythene were also used to protect the iron chelate. Each tray was filled with nutrient solution under continuous aeration and carried 60 seedlings. The experiments then carried out in a growth chamber set at 26^{o}C day and 23 ^{o}C night temperatures, a light intensity of 330 µmolphotons m^{-2} sec^{-1} in a 14/10-hr day/night photoperiod was used. Aluminium then added as Al K $(SO_4)_2.12\ H_2O$ at a concentration of 0 (control), 148 µM and 222µM (treatments). These concentrations correspond to free Al^{+3} µM activities of (0) and (27) as estimated with the speciation software program GEOCHEM-PC (Parker et al 1995). The seedlings were then given 24-hr acclimatization period in low pH (4.0) nutrient culture solution lacking Al after which the initial seminal root length (*ISRL*) measured (day 0). Then, the basal solution replaced with similar solutions where Al is applied in treatment sets. Final seminal root lengths (*FSRL*) measured after 3 days.

Net seminal root length (NSRL) and relative net root growth (RNRG) computed as follows;

(i) NSRL=FSRL-ISRL

(ii) $$\text{RNRG}=\frac{\text{NSRL(Al)}}{\text{NSRL (control)}}$$

Magnavaca*et al.*, (1987)

Where;

NSRL = Net seminal root length; is the difference in root length between day 0 and day 3 of Al exposure.

ISRL = Initial seminal root length; is the seminal root length at day 0.

RNRG=Relative net root growth.

RNRG is the ratio of seedlings grown under Al stress to seedlings growth under control (no Al).

IV. Results

Means of yields and yields related components in Chepkoilel

Tiller Numbers

In this site, means for tillers were different depending on lines and varieties.ELD/AS/14/L11 had the highest mean of 5.33 and significantly different, while Njoro BW11 had the second highest mean of 5.00 compared to the other lines. The two lines which had the least number of tillers were ELD/AS/14/L5 and ELD/AS/14/L12. Both had mean of 3.33.

Spikelets Number

In this site spikelets number varied depending on the variety and line. The line with high spikelets was ELD/AS/14/L13 with a mean of 72, it was significantly different from others followed by ELD/AS/14/L6 with a mean of 68.67.The other lines which had the same spikelets were; ELD/AS/14/L1, ELD/AS/14/L4 and ELD/AS/14/L10, all had a mean of 66.00. Some lines produced less number of spikelets. These lines are ELD/AS/14/L2 with 60.33 and ELD/AS/14/L9 with 56.00.

Tiller height

Tiller height was measured and showed different results on various lines and varieties. The tallest variety was ELD/AS/14/L14 with a mean of 94.00 centimeters, it was significantly different (Table 2), followed by line ELD/AS/14/L7 with mean height of 91.33 centimeters. The shortest line was ELD/AS/14/L13 with 77.67 centimeters and the shortest variety was Njoro (BW)11 with 74.33 centimeters.

Head length

In Chepkoilel head lengths was measured in centimeters and the longest head was line ELD/AS/14/L13 with 10.67 centimeters.It was significantly different (Table 2), followed by line ELD/AS/14/L6 with 9.67 centimeters. Some of the lines with shortest heads were ELD/AS/14/L5 and ELD/AS/14/L14, both had mean of 8.00 centimeters while line ELD/AS/14/L9 had a mean of 7.67 centimeters.

Yield per hectare

Yields were measured in this site and the line with the highest yields was ELD/AS/14/L16 with a mean of 4.12 tons per hectare, it was significantly different (Table 2). It was followed by ELD/AS/14/L13 with a mean of 3.94 tons per hectare. The increase in yields for the two lines could be attributed to high number of spikelets and longer heads depicted. The lines which had lower yields were ELD/AS/14/L4, ELD/AS/14/L5 and ELD/AS/14/L11. They had 2.77, 2.54 and 2.60 tons per hectare respectively. The lowest yields were obtained from ELD/AS/14/L12, Robin and Njoro (BW)11 which had 2.45,2.22 and 1.65 tons per hectare respectively. The last line and the two varieties also had lower number of spikelets and shorter head lengths

1000 Kernel weight

1000 Kernel weight in Chepkoilel site was measured and the results tabulated, their means showed that ELD/AS/14/L13 had the highest mean of 46.00 grams and it was significantly different (Table 2), followed by ELD/AS/14/L6, which had 44.67grams. The lowest weights were recorded on ELD/AS/14/L2, Njoro(BW)11 and Robin which had 29.00grams, 29.00grams and 26.00grams respectively

Table 1: Chepkoilel means of yield and related components

Variety	Tiller number	Spikelets number	Tiller Height(cm)	Head Length(cm)	Yield/Hectare	1000 Kernel WT(gm)
V1	4.67^{ab}	66.00^{abc}	84.67^{cde}	8.67^{cde}	2.84^{cdefg}	36.33^{abcde}
V2	4.67^{ab}	60.33^{cd}	85.00^{cde}	9.00^{bcd}	3.74^{ab}	29.00^{de}
V3	4.67^{ab}	65.00^{abc}	89.00^{abcd}	8.33^{def}	2.88^{bcdefg}	32.33^{cde}
V4	4.33^{ab}	66.00^{abc}	83.00^{def}	8.67^{cde}	2.77^{cdefg}	34.33^{bcde}
V5	3.33^{b}	67.33^{abc}	81.67^{ef}	8.00^{ef}	2.54^{efg}	38.00^{abcd}
V6	4.33^{ab}	68.67^{ab}	88.33^{abcd}	9.67^{b}	4.12^{a}	44.67^{ab}
V7	4.00^{ab}	65.33^{abc}	91.33^{ab}	9.33^{bc}	3.35^{abcde}	38.67^{abcd}
V8	4.33^{ab}	61.33^{bcd}	89.67^{abc}	8.33^{def}	3.25^{abcdef}	44.33^{ab}
V9	4.33^{ab}	56.00^{d}	88.67^{abcd}	7.67^{f}	3.57^{abc}	41.00^{abc}
V10	4.67^{ab}	66.00^{abc}	86.33^{bcde}	8.67^{cde}	3.44^{abcd}	35.33^{abcde}
V11	5.33^{a}	64.00^{bc}	86.00^{bcde}	9.33^{bc}	2.60^{defg}	41.67^{abc}
V12	3.33^{b}	62.67^{bcd}	84.67^{cde}	8.33^{def}	2.45^{fgh}	42.33^{abc}
V13	4.33^{ab}	72.00^{a}	77.67^{fg}	10.67^{a}	3.94^{a}	46.00^{a}
V14	4.33^{ab}	64.00^{bc}	94.00^{a}	8.00	2.22^{gh}	26.00^{e}
V15	5.00^{ab}	62.00^{bcd}	74.33^{g}	8.33^{def}	1.65^{h}	29.00^{de}
LSD	1.83	7.52	6.05	0.86	0.89	11.16
CV(%)	25	6.98	4.22	5.91	17.66	17.91

Means of yields and yields related components in Moiben

Tiller Numbers

In this site, ELD/AS/14/L6 and ELD/AS/14/L8 had the highest number of tillers of a mean of 9.00 and significantly different (Table 3), while ELD/AS/14/L 4, ELD/AS/14/L5, ELD/AS/14/L13 and ELD/AS/14/L15 had the second highest value of tiller mean of 8.67. ELD/AS/14/L3 and ELD/AS/14/L 11 had equal means of 8.00 while ELD/AS/14/L9 had the lowest mean value of 6.33.

Spikelets Number

In Moiben site, spikelets number varied depending on the line and variety with the highest line ELD/AS/14/L6 with 75.33.It was significantly different (Table 3). ELD/AS/14/L13 had fairly high spikelets number of 74.67, while line ELD/AS/14/L9, ELD/AS/14/L8 and ELD/AS/14/L11 had 58.00, 66.33 and 43.33 means respectively. These were the lines with the lowest spikelet numbers.

Tiller Height

The tallest variety in this site was Robin with a mean height of 89.33 centimeters. It was significantly different (Table 3). Line ELD/AS/14/L3 had means of 88.00 centimeters. It was the second tallest line. Some lines grew moderate tiller heights for example ELD/AS/14/L7, ELD/AS/14/L6, and Njoro(BW)11. They had means of 85.33 centimeters, 84.33 centimeters and80.33 centimeters respectively. The shortest lines in this site were ELD/AS/14/L9 and ELD/AS/14/L13 with 79.00 centimeters and 75.67 centimeters respectively.

Head Length

In Moiben site the longest heads were observed in ELD/AS/14/L13 with a mean of 10.67 centimeters. It was significantly different (Table 3) while line ELD/AS/14/L1 and ELD/AS/14/L6 had both mean of 9.67centimeters and they were the second in head length growth. Some lines which had short head lengths were ELD/AS/14/L8 and ELD/AS/14/L9, both had 8.00 centimeters and the variety which had the shortest head length was Robin with7.33 centimeters.

Yield per Hectare

The means for yield was weighted in this site and recorded. The highest productivity was observed in line ELD/AS/14/L6 which had a mean of 4.14 tons per hectare. It was significantly different (Table 3). Closely, line ELD/AS/14/L13 weighed 3.76 tons per hectare. The increase in yields in the first two lines could be due to their increase in their spikelets numbers and long heads. The varieties which produce lower yields in this experiment were Robin and Njoro (BW)11. They had 2.25 tons per hectare and 1.66 tons per hectare respectively. The poor performance can be attributed to their reduced head lengths.

1000 Kernel Weight

In this site, the means for 1000 Kernel weight was recorded and the highest was line ELD/AS/14/L13 with 56.00 grams. It was significantly different (Table 3). The other line which had higher weight was line ELD/AS/14/L6 which recorded 53.00 grams. The lines which had moderate 1000 Kernel weight were ELD/AS/14/L1, ELD/AS/14/L7 and ELD/AS/14/L10 they had 46.33, 45.33 and 45.00g respectively. The lines which gave lower results are Njoro (BW)11 and Robin with 38.67g and 36.00g respectively.

Table 2: Moiben means of yield and related components

Variety	**Tiller number**	**Spikelets number**	**Tiller Height(cm**	**Head Length(cm**	**Yield/Hectare**	**1000 Kernel WT(gm)**
V1	7.33^{ab}	66.67^{ab}	83.67^{abc}	9.67^{b}	2.86^{bcd}	46.33^{cde}
V2	8.33^{ab}	64.00^{ab}	80.67^{abc}	8.67^{cde}	3.57^{ab}	51.00^{ab}
V3	8.00^{ab}	70.00^{ab}	88.00^{ab}	8.67^{cde}	2.89^{bcd}	42.33^{ef}
V4	8.67^{ab}	67.33^{ab}	82.67^{abc}	8.33^{de}	2.89^{bcd}	44.33^{ef}
V5	8.67^{ab}	66.00^{ab}	85.00^{abc}	8.67^{cde}	2.56^{cde}	48.00^{bcde}
V6	9.00^{a}	75.33^{a}	84.33^{abc}	9.67^{b}	4.14^{a}	53.00^{ab}
V7	7.00^{ab}	62.00^{ab}	85.33^{abc}	8.67^{cde}	3.37^{abc}	45.33^{de}
V8	9.00^{a}	56.33^{bc}	87.33^{ab}	8.00^{ef}	3.27^{abc}	47.67^{bcde}
V9	6.33^{b}	58.00^{bc}	79.00^{bc}	8.00^{ef}	3.59^{ab}	51.00^{abcd}
V10	7.67^{ab}	65.33^{ab}	81.33^{abc}	9.00^{cbd}	3.25^{abc}	45.00^{e}
V11	8.00^{ab}	43.33^{c}	86.67^{ab}	9.33^{bc}	2.82^{bcd}	44.67^{e}
V12	7.33^{ab}	62.67^{ab}	86.33^{ab}	8.67^{cde}	2.46^{cde}	52.33^{ab}
V13	8.67^{ab}	74.67^{a}	75.67^{c}	10.67^{a}	3.76^{ab}	56.00^{a}
V14	7.00^{ab}	62.00^{ab}	89.33^{a}	7.33^{f}	2.24^{de}	36.00^{g}
V15	8.67^{ab}	68.00^{ab}	80.33^{abc}	9.33^{bc}	1.66^{e}	38.67^{g}
LSD	2.47	14.74	9.69	0.82	0.95	5.81
CV(%)	18.59	13.75	6.92	5.55	18.81	7.42

Means of yields and yields related components in Kaptagat

Tiller Numbers

In this site, the line which had the highest mean for tiller numbers is ELD/AS/14/L13 which had 4.67. It was significantly different (Table 4). Lines ELD/AS/14/L1, ELD/AS/14/L 6 and ELD/AS/14/L 11 had mean value of 4.00. The lines and a variety with lowest tiller numbers in this site were ELD/AS/14/L8, ELD/AS/14/L9 and Njoro (BW)11 had all mean of 2.67. Generally Moiben had the highest tiller mean, followed by Chepkoilel and the lowest being Kaptagat.

Spikelets Number

In this site, spikelets means varied. Line ELD/AS/14/L 6 had a mean of 66.00 and significantly different (Table 4) while line ELD/AS/14/L13 had a mean of 65.33. It was the second highest. The other lines which had moderate spikelets are; ELD/AS/14/L1 and ELD/AS/14/L10 which had 64.00 spikelets and 62.00 spikelets respectively. Line ELD/AS/14/L2 had a mean of 55.33 while the line which produced the lowest number of spikelets is ELD/AS/14/L3 which had a mean of 48.67. Comparatively, the line which produced high spikelets numbers across the three sites is ELD/AS/14/L 13.

Tiller Height

In this site variety ELD/AS/14/L14 was the tallest with a mean of 88.33 centimeters and significantly different (Table 4) while the second tallest line was ELD/AS/14/L10 which had 88.00 centimeters. The other line which had taller tillers is ELD/AS/14/L11 with 86.67 centimeters. Lines with the shortest tillers in this site are; ELD/AS/14/L 4, ELD/AS/14/L7, ELD/AS/14/L13 and ELD/AS/14/L 5 which had means of 77.33 centimeters, 77.00 centimeters, 76.00 centimeters and 74.67 centimeters respectively. In general the tallest lines and varieties were found in Moiben and shortest lines and varieties were found in Kaptagat

Head Length

In this site, head lengths was measured and recorded. The line which had longest head was ELD/AS/14/L 13 with 9.00 centimeters. It was significantly different (Table 4). The second longest head was observed in line ELD/AS/14/L6 which had 8.67 centimeters. ELD/AS/14/L9 and ELD/AS/14/L11 had the same head lengths, while line ELD/AS/14/L5 and ELD/AS/14/L12 had the same means of 7.33 centimeters each. The lines with shortest head lengths in Kaptagat were ELD/AS/14/L3 and ELD/AS/14/L8. They had 7.00centimeter and 6.33 centimeters respectively. Generally the variety with longest head across the three sites is ELD/AS/14/L13.

Yield per Hectare

In this site, the yield was measured in tons per hectare. Line ELD/AS/14/L6 had the highest productivity of 4.06 and it was significantly different (Table 4). The second highest line in terms of productivity was ELD/AS/14/L13 which had 3.88 tons per hectare. The line and varieties with lowest production were ELD/AS/14/L3, Robin and Njoro (BW)11. They had the weight of 2.81, 2.16 and 1.58 tons per hectare respectively.

Kernel Weight (1000)

The line with the highest Kernel weight in this site was ELD/AS/14/L6 which had 36.00 grams. It was significantly different (Table 4). The other line which had high kernel weight was ELD/AS/14/L13 with 34.33 grams. While the lowest varieties in this component were Robin and Njoro (BW)11, they had 15.33 grams and 19.00 grams respectively. All lines performed better than checks (Robin and Njoro (BW)11) in this component across the three sites.

Table 3: Kaptagat means of yield and related components

Variety	**Tiller number**	**Spikelets number**	**Tiller Height(cm**	**Head Length(cm**	**Yield/Hectare**	**1000 Kernel WT(gm**
V1	4.00^{ab}	64.00^{ab}	81.33^{abcd}	8.33^{abc}	2.92^{bcdef}	26.33^{ef}
V2	3.00^{ab}	55.33^{bc}	82.00^{abcd}	8.00^{abc}	3.67^{abc}	33.00^{abc}
V3	3.33^{ab}	48.67^{c}	83.67^{ab}	7.00^{cd}	2.81^{cdef}	22.33^{gh}
V4	3.00^{ab}	58.00^{abc}	77.33^{bcd}	8.00^{abc}	2.70^{cdef}	24.33^{fg}
V5	3.67^{ab}	62.67^{ab}	74.67^{d}	7.33^{bcd}	2.48^{defg}	28.00^{de}
V6	4.00^{ab}	66.00^{a}	82.33^{abc}	8.67^{ab}	4.06^{a}	36.00^{a}
V7	3.67^{ab}	57.00^{abc}	77.00^{bcd}	8.00^{abc}	3.28^{abcde}	28.67^{de}
V8	2.67^{b}	57.33^{abc}	81.33^{abcd}	6.33^{abcde}	3.20^{abcde}	34.33^{ab}
V9	2.67^{b}	64.00^{ab}	81.00^{abcd}	7.67^{abcd}	3.51^{abcd}	31.00^{bcd}
V10	3.00^{ab}	62.00^{ab}	88.00^{a}	8.33^{abc}	3.37^{abcde}	25.33^{efg}
V11	4.00^{ab}	59.33^{ab}	86.67^{a}	7.67^{abcd}	3.20^{abcde}	30.67^{cd}
V12	3.67^{ab}	62.00^{ab}	82.00^{abcd}	7.33^{bcd}	2.37^{efg}	32.33^{bc}
V13	4.67^{a}	65.00^{ab}	76.00^{cd}	9.00^{a}	3.88^{ab}	34.33^{ab}
V14	3.00^{ab}	63.33^{ab}	88.33^{a}	9.00^{a}	2.16^{fg}	15.33^{i}
V15	2.67^{b}	64.00^{ab}	86.00^{a}	7.00^{cd}	1.58^{g}	19.00^{h}
LSD	1.68	9.77	7.56	1.36	1.03	3.48
CV(%)	29.62	9.64	5.52	10.45	20.53	7.53

Identification of yield (tons per hectare) and yield related components

Tiller numbers recorded in the three sites showed that moiben had the highest mean of 7.97. It was significantly different .Chepkoilel had 4.37 while Kaptagat posted a mean of 3.44. The other component measured was spikelets number. Moiben posted significantly different higher mean of 64.44. The other two sites Chepkoilel and Kaptagat had 64.11 and 60.59 means respectively.

The main tiller height was measured in centimeters on sampled wheat plants and the results showed that Moiben had the highest mean of 85.70 centimeters. It was significantly different. It was followed by Chepkoilel with a mean of 84.62 centimeters, while Kaptagat had a mean of 81.80 centimeters.

Head lengths were also measured and recorded in the three sites. Moiben had the greatest mean of 8.84 centimeters long and significantly different (Table 5). The other two sites Chepkoilel and Moiben posted a mean of 8.73 centimeters and 7.77 centimeters respectively.

Yield was also measured in the three sites and Moiben had the highest productivity of 3.12 tons per hectare. It was significantly different. Chepkoilel was second with 3.02 tons per hectare and kaptagat had 3.00 tons per hectare.

1000 kernel weight measured revealed that Moiben was the highest with 46.82 grams. It was significantly different. The other two sites Chepkoilel and Kaptagat posted means of 37.26 grams and 27.64 grams respectively.

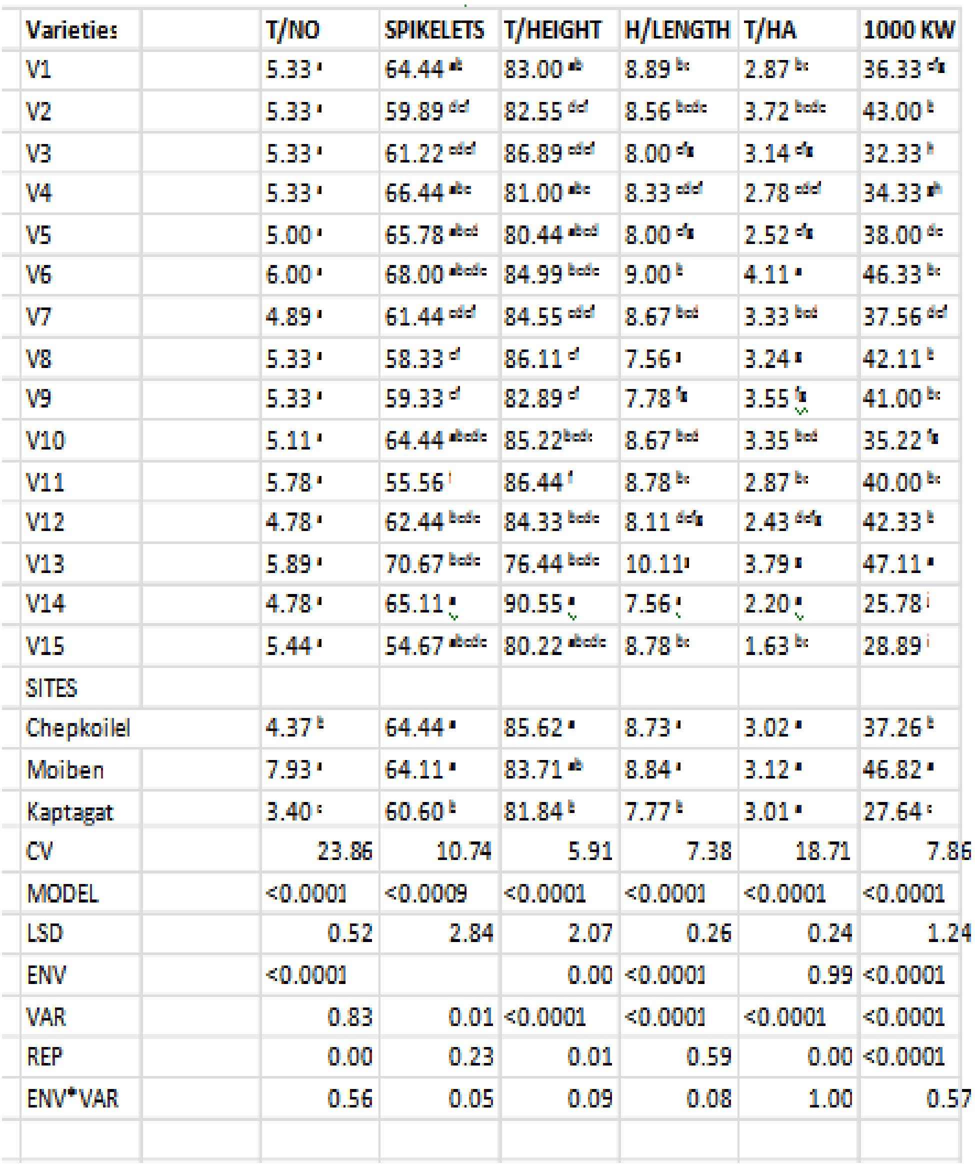
Table 4: Means of the Yields (tons per hectare) and yield related components

	Tiller Number	Spikelets Number	Tiller Height(cm)	Head Length (cm)	Yield per Hectare	1000 kernel Weight
Chepkoilel	4.37	64.11	84.62	8.73	3.02	37.26
Moiben	7.97	64.44	85.70	8.84	3.12	46.82
Kaptagat	3.44	60.59	81.80	7.77	3.00	27.64

Yield per hectare per line in the three sites

The highest performance was realized on line ELD/AS/14/L 6 with 4.4 tons per hectare and it was significantly different from the other lines . Line ELD/AS/14/L13 was the second in performance and it recorded 4.0 tons per hectare. This was also significantly different from the other lines. Line ELD/AS/14/L2 was the third in production with 3.8 tons per hectare; this line differed from the other lines significantly. Line ELD/AS/14/L 9 registered 3.6 tons per hectare. This also posted significantly different tonnage per hectare as compared to the other lines under the study and it was the fourth in productivity. Line ELD/AS/14/L10 and ELD/AS/14/L7 posted 3.5 tons on average and both also were significantly different from the other lines as they were the fifth in productivity. Line ELD/AS/14/L8 recorded 3.4 tons per hectare. Its results were significantly different from the other lines. It was the sixth in productivity. Line ELD/AS/14/L1 and ELD/AS/14/L3 productivity was 2.8 tons per hectare and they posted significantly different mean from the other lines as they were seventh in productivity. Line ELD/AS/14/L4 and ELD/AS/14/L11 had 2.7 tons per hectare. These tons were significantly different from the other lines under the study and eighth in productivity. Line ELD/AS/14/L5 had 2.6 tons per hectare**.** This average is significantly different from the other lines and second last in productivity of the eleventh position (Table 6). Line ELD/AS/14/L15 had 2.0 tons per hectare and the last in productivity. It was significantly different from the other lines under the study.

Table 5: Means of yields (tons per hectare) and related components across the three sit



Varieties		T/NO	SPIKELETS	T/HEIGHT	H/LENGTH	T/HA	1000 KW
V1		5.33^{a}	64.44^{ab}	83.00^{ab}	8.89^{bc}	2.87^{bc}	36.33^{efg}
V2		5.33^{a}	59.89^{def}	82.55^{def}	8.56^{bcde}	3.72^{bcde}	43.00^{b}
V3		5.33^{a}	61.22^{cdef}	86.89^{cdef}	8.00^{efg}	3.14^{efg}	32.33^{h}
V4		5.33^{a}	66.44^{abc}	81.00^{abc}	8.33^{cdef}	2.78^{cdef}	34.33^{gh}
V5		5.00^{a}	65.78^{abcd}	80.44^{abcd}	8.00^{efg}	2.52^{efg}	38.00^{de}
V6		6.00^{a}	68.00^{abcde}	84.99^{bcde}	9.00^{b}	4.11^{a}	46.33^{bc}
V7		4.89^{a}	61.44^{cdef}	84.55^{cdef}	8.67^{bcd}	3.33^{bcd}	37.56^{def}
V8		5.33^{a}	58.33^{ef}	86.11^{ef}	7.56^{g}	3.24^{g}	42.11^{b}
V9		5.33^{a}	59.33^{ef}	82.89^{ef}	7.78^{fg}	3.55^{fg}	41.00^{bc}
V10		5.11^{a}	64.44^{abcde}	85.22^{bcde}	8.67^{bcd}	3.35^{bcd}	35.22^{fg}
V11		5.78^{a}	55.56^{f}	86.44^{f}	8.78^{bc}	2.87^{bc}	40.00^{bc}
V12		4.78^{a}	62.44^{bcde}	84.33^{bcde}	8.11^{defg}	2.43^{defg}	42.33^{b}
V13		5.89^{a}	70.67^{bcde}	76.44^{bcde}	10.11^{a}	3.79^{a}	47.11^{a}
V14		4.78^{a}	65.11^{a}	90.55^{a}	7.56^{a}	2.20^{a}	25.78^{i}
V15		5.44^{a}	54.67^{abcde}	80.22^{abcde}	8.78^{bc}	1.63^{bc}	28.89^{i}
SITES							
Chepkoilel		4.37^{b}	64.44^{a}	85.62^{a}	8.73^{a}	3.02^{a}	37.26^{b}
Moiben		7.93^{a}	64.11^{a}	83.71^{ab}	8.84^{a}	3.12^{a}	46.82^{a}
Kaptagat		3.40^{c}	60.60^{b}	81.84^{b}	7.77^{b}	3.01^{a}	27.64^{c}
CV		23.86	10.74	5.91	7.38	18.71	7.86
MODEL		<0.0001	<0.0009	<0.0001	<0.0001	<0.0001	<0.0001
LSD		0.52	2.84	2.07	0.26	0.24	1.24
ENV		<0.0001		0.00	<0.0001	0.99	<0.0001
VAR		0.83	0.01	<0.0001	<0.0001	<0.0001	<0.0001
REP		0.00	0.23	0.01	0.59	0.00	<0.0001
ENV*VAR		0.56	0.05	0.09	0.08	1.00	0.57

Treatment means within columns followed by the same letter are not significantly different at P≤0.05

V. Discussion

Comparison between yield and yield related components to phytotoxic aluminium.

Aluminium phytotoxic levels are of great concern to breeders and researchers. The results of the test lines and the checks gave varied results. It is widely accepted that aluminium tolerance in wheat is species and genotype dependent as reported by Jones and Ryan (2003). Aluminium toxicity seems to have high impact on the lines tested because the yield realized in Moiben was higher compared to the other two sites, since acidity was less in Moiben than the other sites. A study conducted by Barcelo and Poschenrieder (2002) revealed that some species in tropical areas are very resistant to aluminium stress.

Tiller number per plant was significantly higher in Moiben compared to Chepkoilel and Kaptagat. This shows that the phytotoxic aluminium really affect the tiller number. The sizes of the head of the main tiller also appeared affected by the aluminium problems. On the other hand, 1000 kernel weight was higher in Moiben followed by Chepkoilel then Kaptagat. This significantly shows that a high aluminium effect reduces the yield substantially.

The spikelets per head were also highest in Moiben and lowest in Kaptagat. This can be attributed to higher aluminium levels in Kaptagat and lower aluminium levels in Moiben.

The main tiller height recorded in the three sites also showed that Moiben had the highest and Kaptagat the lowest. To some extent this can also be attributed to higher acidity recorded in Kaptagat compared to other sites.

VI. Conclusion

Great genotypic variability of the lines studied and the environmental influence as the capability of masking the effects of the aluminiumphytotoxicity as depicted by field screening. The uneven distribution of aluminium in the fields also can also influence the effect of aluminiumphytotoxicity.There is positive significant correlation ($P \leq 0.05$) between yields per hectare and tiller number, implying that tiller number can be used to determine yielding potential on acid soils. Also main tiller head length and tiller number were positively significantly correlated meaning that they have more impact on grain yield.

There is very close relationship between RNRG at 148µM and 222 µM indicating that both levels of aluminium concentration can be used to ascertain aluminiumphytotoxicity in these lines.Lines ELD/AS/14/L1, ELD/AS/14/L4, ELD/AS/14/L6, ELD/AS/14/L8, ELD/AS/14/L13, Robin and Njoro BW11 were resistant to aluminiumphytotoxicity and they should be considered in breeding for tolerance to Al-toxicity. A prerequisite to any genetic improvement programme for wheat, it requires knowledge of the extent of genetic variation present within *Triticum* species. Various tools such as morphological, molecular and biochemical markers can be used to assess genetic diversity in crop plants

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