Sky Journal of Agricultural Research Vol. 5(6), pp. 129 - 138, August, 2016
Available online http://www.skyjournals.org/SJAR
ISSN 2315-8751 ©2016 Sky Journals

*Full Length Research Paper*

# Effect of waterlogging on selected physico-chemical parameters in maize

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Accepted 29 July, 2016

**A study was conducted to determine selected physico-chemical aspects of waterlogging tolerance in acidic soils for maize crop improvement. This experiment was conducted in low lying flood prone regions of western Kenya where waterlogging is a severe problem affecting maize production. Nine cultivars selected from a previous screening study were subjected to waterlogging to select traits for tolerance to waterlogging. Seventeen day old seedlings planted in acidic ferralsol soil (pH 5.5) were exposed to field capacity flooding for 10 days, drained and growth monitored. The effect of flooding on number of leaves, leaf area, root collar diameter, seedling height and grain yield was determined. Waterlogging reduced leaf number, area and chlorophyll concentration, plant height, nutrient uptake and grain yield but increased leaf wilting and death and root porosity. The number of wilted or dead leaves, leaf chlorophyll concentration, root porosity and yield formed good screening criteria for tolerance to waterlogging in maize. The tolerant germplasm could be incorporated in maize breeding programmes to develop genotypes that are high yielding and tolerant to waterlogging.**

**Key words:** Water stress, phosphorus, chlorophyll and nitrogen.

## INTRODUCTION

Maize is ranked first as a staple food crop in most developing countries (Donswell et al., 1996). The area under maize cultivation in Africa is approximately 80.8 million hectares. Maize is grown on about 50% of the arable land in Kenya with annual turnover of over 6 billion Kenya shillings (Gudu, 2003). Ninety percent (90%) of the Kenyan population depends on maize as a staple with per capita consumption from 28 to 125 kg (Gudu, 2003). Maize has been estimated to contribute more than 20% of the total value of agricultural production in Kenya (Ayaga, 2003). It contributes 25% of all the agricultural employment and occupies between 1.4 and 1.6 million ha of cultivated land, producing between 2.5 to 3.0 million tons of grain maize (Gudu, 2003). Maize contributes 75% of total cereal consumption, 44% of total energy supply and 32% of total protein supply.

The extent to which waterlogging affects maize yield is determined by several factors including timing of waterlogging in relation to stage of development, frequency and duration of waterlogging and air-soil temperatures during waterlogging (Lauer, 2001). Another symptom of waterlogging is leaf chlorosis that starts with older leaves as nitrogen is mobilized to the developing leaves. Waterlogging increases chlorophyll breakdown, which leads to reduced photosynthesis, hence reduced yield (Bryan and McKersie, 1996). Waterlogging also reduces nitrogen uptake and thus leads to nitrogen shortage in the plant aggravating chlorosis. Nitrogen deficiency is also compounded by the fact that waterlogging stimulates denitrification and leaching. Generally waterlogging leads to a decrease in uptake and transport of mineral ions through the roots causing nutrient deficiencies (Collaku and Harrison, 2001).

Various techniques have been used as morphological markers to screen crops for tolerance to waterlogging. In barley the percentage chlorosis of leaves has successfully been used as selection criterion for tolerance to waterlogging (Zhou et al., 2004). In lucerne (*Medicago sativa* L.), chlorophyll fluorescence has been

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**Table 1.** The source and coding of maize accessions used to test response to waterlogging.

| S.No. | Code | Accession/cultivar | Source |
|---|---|---|---|
| 1 | K24 | H625 | KARI – Kitale / Kenya Seed |
| 2 | E2 | 93 | Ecuador |
| 3 | BR2 | Brazil Synthetic | Brazil |
| 4 | KAN2 | Accession from Rae (Kano Plains) | Kano Plains |
| 5 | C8 | CIMCali96ASA 3 | CIMMYT |
| 6 | KAT1 | DLC1 | Katumani |
| 7 | K3 | KTLN70168 | KARI / Kitale |
| 8 | K27 | F | Kitale |
| 9 | K8 | KTLN70143 | KARI / Kitale |

**Note**: The codes stand for the first letters of the places from which the seeds were obtained.

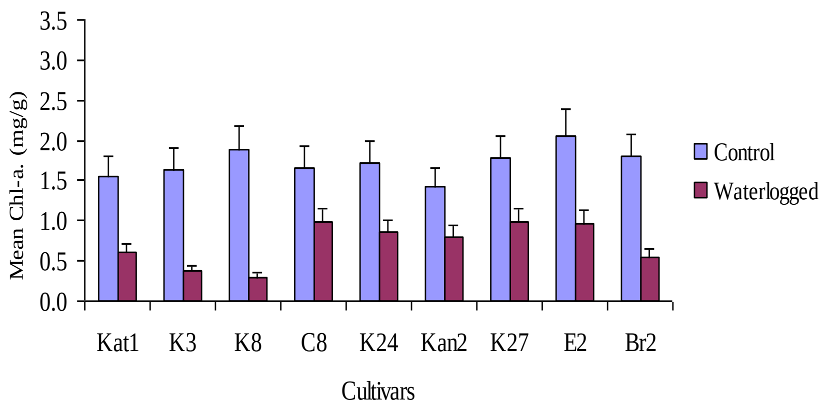


**Figure 1.** Effect of waterlogging on the chlorophyll-a concentration (mg/g) in leaves of 27-day old maize seedlings following 10 days of waterlogging stress.

used as a selection criterion (Christiane and Shabala, 2003). Due to destruction of chlorophyll by waterlogging, photosynthesis is affected; therefore the leaves of waterlogged lucerne fluoresce more. In maize early and increased adventitious rooting, enhanced root porosity and grain yield can be used as markers for waterlogging tolerance (Zaidi et al., 2005). The scope for genetic gains is tremendous for excessive moisture tolerance. This can be exploited in a systematic manner (Zaidi et al., 2005). Plant breeding programme involves obtaining cultivars with tolerance to abiotic stresses and is characterized by several steps including introduction, genotype characterization, crosses and field selection (Zimmer et al., 2004). The genetic mapping of such traits involves the choice of suitable parents with contrasting phenotypes and enough polymorphism to allow the use of molecular markers. In breeding maize for tolerance to waterlogging, tolerant and susceptible lines are compared to discriminate the various genotypes regarding tolerance to waterlogging. This helps to identify the most contrasting genotypes to be used as parents for mapping populations. This study was conducted to determine selected physical and chemical aspects of waterlogging tolerance in acidic soils for maize crop improvement in particularly low lying flood prone regions of Western Kenya where waterlogging is a severe problem affecting maize production

## MATERIALS AND METHODS

### Experimental site

The study was conducted at the Chepkoilel Campus farm located at an altitude of 2180 m above sea level, $35^0$ 18′ E, $0^0$30′ N and is a maize and wheat growing area (Jaetzold and Schmidt, 1983). The experiment was carried out in the Botany green house at temperatures of 18 ± 2°C and 32 ± 3°C night/day.

### Materials

A total of 71 maize cultivars were initially used in this study. Fifty one commercial accessions were obtained from the Kenya Agricultural Research Institute (KARI), these included accessions from International Maize and Wheat Improvement Centre (CIMMYT), Brazil, Ecuador, USA, and South Africa (Table 1). Twenty land races were collected from individual farmers from Migori, Homa Bay, Kano Plains and Bumala (Figure 1)

**Table 2.** Ratio of chlorophyll-a to chlorophyll-b in leaves of 27-day-old maize seedlings after 10 days of waterlogging compared to control plants.

| | Ratio (Chlorophyll-a / Chlorophyll-b) | |
|---|---|---|
| **Cultivar** | **Control** | **Waterlogged** |
| KAT1 | 3:1 | 2:1 |
| K3 | 2:1 | 2:1 |
| K8 | 3:1 | 1:1 |
| C8 | 2:1 | 2:1 |
| K24 | 3:1 | 2:1 |
| KAN2 | 2:1 | 2:1 |
| K27 | 2:1 | 2:1 |
| E2 | 3:1 | 2:1 |
| BR2 | 3:1 | 1:1 |

**Sowing and experimental design**

The experiment was carried out in potted soils filled with 2 kg of acidic ferralsol soil of pH 5.5 collected from Chepkoilel farm. A total of 213 pots (diameters: 15 cm at the top, 12 cm at the bottom and depth of 17 cm) were sown with three seeds of a particular cultivar/accession with three replications using randomized complete block design (Table 2). DAP (diammonium phosphate) fertilizer was applied at the time of sowing at the rate of 247 kg/ha equivalent. The fertilizer was thoroughly mixed with the soil before sowing. The seedlings were allowed to grow for fourteen days after emergence before being subjected to blanket flooding. Tolerance of the 71 accessions was accessed by considering the score of number of dead leaves, number of wilted leaves, number of young leaves with dead tips and the general plant health.

Nine selected accessions (seven tolerant and two susceptible) previously selected after a preliminary screening process were used in the final morphological analysis. Three seeds of each accession were planted in 2 kg of soil in pot with application of DAP as above. The selected accessions were divided into two treatment groups: control (no waterlogging) and waterlogged. Waterlogging was to a depth of 2cm above soil level fourteen days after emergence and the control was not waterlogged. Each group had three replications in a randomized complete block design. The following parameters were measured before and after waterlogging; number of leaves, leaf area, root collar diameter and seedling height. In addition the length of internode and chlorophyll concentrations were also measured for both groups after water had been drained off.

The seedlings were then transferred into plastic bags containing twenty kilogram of soil with DAP application at the rate of 247 kg/ha equivalent. The plants were top dressed with calcium ammonium nitrate (CAN) at a rate of 185 kg/ha equivalent (Nyle, 2000). The seedlings were allowed to recover for 24 days, and number of leaves, leaf area, root collar diameter, length of internode and height were measured for both groups. Screening for tolerance to waterlogging was done using three replicates in a randomized complete block design.

**Determination of chlorophyll concentration**

A 0.2 g sample of the most recent mature maize leaves was crushed in a mortar with 20 ml of 80% acetone (v/v). The extracts were filtered with suction using a Buchner funnel through Whatman No. 1 filter paper. The final volume of the filtrate was made to 20ml. The absorbance of the filtrate was measured using a spectrophotometer set at 645, 652 and 663 nm. Chlorophyll concentrations were calculated using the following formula (Sestak, 1971).

$$\text{mg chlorophyll-a/g tissue} = [12.7(D_{663}) - 2.69(D_{645})] \frac{V}{1000 \times W}$$

$$\text{mg chlorophyll-b/g tissue} = [22.9(D_{645}) - 4.68(D_{663})] \frac{V}{1000 \times W}$$

$$\text{mg total chlorophyll/g tissue} = [20.2(D_{645}) + 8.02(D_{663})] \frac{V}{1000 \times W}$$

Where; D = Absorbance
V = Final volume
W = fresh weight in grams of the tissue extracted

**Determination of leaf nitrogen concentration**

Freshly harvested leaves from 27 day old seedlings after 10 days of waterlogging were used to determine Nitrogen content using Kjeldhal digestion method. A measure of 0.3 g of oven dried (70°C) ground leaf was added to 4.4 ml of digestion mixture consisting of selenium powder, lithium sulphate and hydrogen peroxide. This was followed with careful addition of concentrated sulphuric acid. In the above mixture, hydrogen peroxide was an additional oxidant while selenium was a catalyst. The digestion was then carried out at 360°C for 2 h. Aliquots of 5 ml were then removed and analyzed by distillation followed with titration (Okalebo et al., 1993).

$$\% \text{ N in plant sample} = \frac{(a \text{ in plant sample} = .}{1000 \times w \times al}$$

Where

a = volume of titre HCl for the blank
b = volume of titre HCl for the sample
v = final volume of the digestion
w = weight of sample taken
al = aliquot of the solution taken for analysis

## Determination of leaf phosphorous concentration

Phosphorous concentration of the leaves was determined using the same leaves that were used to determine nitrogen content. Total phosphorus was determined using Kjeldhal digestion method. A measure of 0.3 g of oven dried ground leaf was added to 4.4 ml of digestion mixture consisting of selenium powder, lithium sulphate and hydrogen peroxide. This was followed with careful addition of concentrated sulphuric acid. In the above mixture, hydrogen peroxide was an additional oxidant while selenium was a catalyst. The digestion was then carried out at $360^{0}$ C for 2 hours. Aliquots of 5 ml were then removed and analyzed by calorimetric method (Okalebo et al., 1993).

## Root porosity measurements

Ninety six plastic planting pots (6 cm deep, 5 cm wide at the top and 4 cm wide at the bottom) were filled with fine, acid washed sand and DAP was applied at the rate of 75 mg per pot. Three seeds of eight selected maize accessions were sowed into separate pots. Fourteen days after germination, maintaining 1 cm of water above the soil level for six days waterlogged half the pots. The control pots were supplied with enough water just to keep the soil moist. The seedlings were then carefully uprooted and the roots carefully washed to remove any adhering sand particles. The root porosities were measured using Pycnometric method (Jensen et al., 1969). The roots were quickly cut off and weighed. They were then put in a pycnometer bottle which was then filled with water and weighed.

The roots were removed and ground to a fine slurry and weighed. The Pycnometer bottle was filled with water and weighed. The various weights were used to calculate root porosity using the equation and the data obtained was used to assess the effect of waterlogging on root porosity.

$$\% \text{ porosity} = \frac{100(W_h - W_{r+w})}{(W_w + W_r - W_{r+w})}$$

Where:

$W_h$ = Weight of bottle plus homogenate of the crushed roots topped up with water
$W_{r+w}$ = Weight of capped bottle full of water plus roots from which trapped air bubbles have been freed.
$W_w$ = Weight of bottle filled with water only.
$W_r$ = Weight of intact roots from which excess water has been blotted away.

## Determination of grain yield

Three maize seeds were sown in pots containing 2 kg of soil. This was replicated three times in a randomized complete block design (RCBD). Two week old seedlings were waterlogged for 10 days. The seedlings were then carefully transferred and transplanted into 20 kg of soil in large plastic bags. The established plants were fertilized using calcium ammonium nitrate (CAN) at the rate of 185 kg per hectare equivalent and allowed to grow to maturity. The cobs were manually harvested, sun-dried, shelled and the dry weight of grain for every plant was determined.

## Data analysis

All the data collected were entered organized and managed using EXCEL spreadsheet for Windows 98. All statistical analyses were performed with (SPSS version 11.5) statistical computer packages. Normality of data distribution was checked by means of the skewness and kurtosis (Zar, 2001). Data on plant variables such as number of leaves, leaf area, plant height, root collar diameters, length of internode and grain yield were calculated as means (±S.E) for each cultivar. Mean differences in the plant parameters among cultivars were analyzed using a one-way ANOVA). Duncans Multiples Range test was used to discriminate between the means that were actually different from each other (Michael and Douglas, 2004).

# RESULTS

## Effects of waterlogging on leaf chlorophyll concentration

Waterlogging caused a significant reduction in concentration of chlorophyll-a in the leaves (Figure 1). The most significant reduction was recorded in K8, K3, BR2 and KAT1. Waterlogging caused a significant (P <

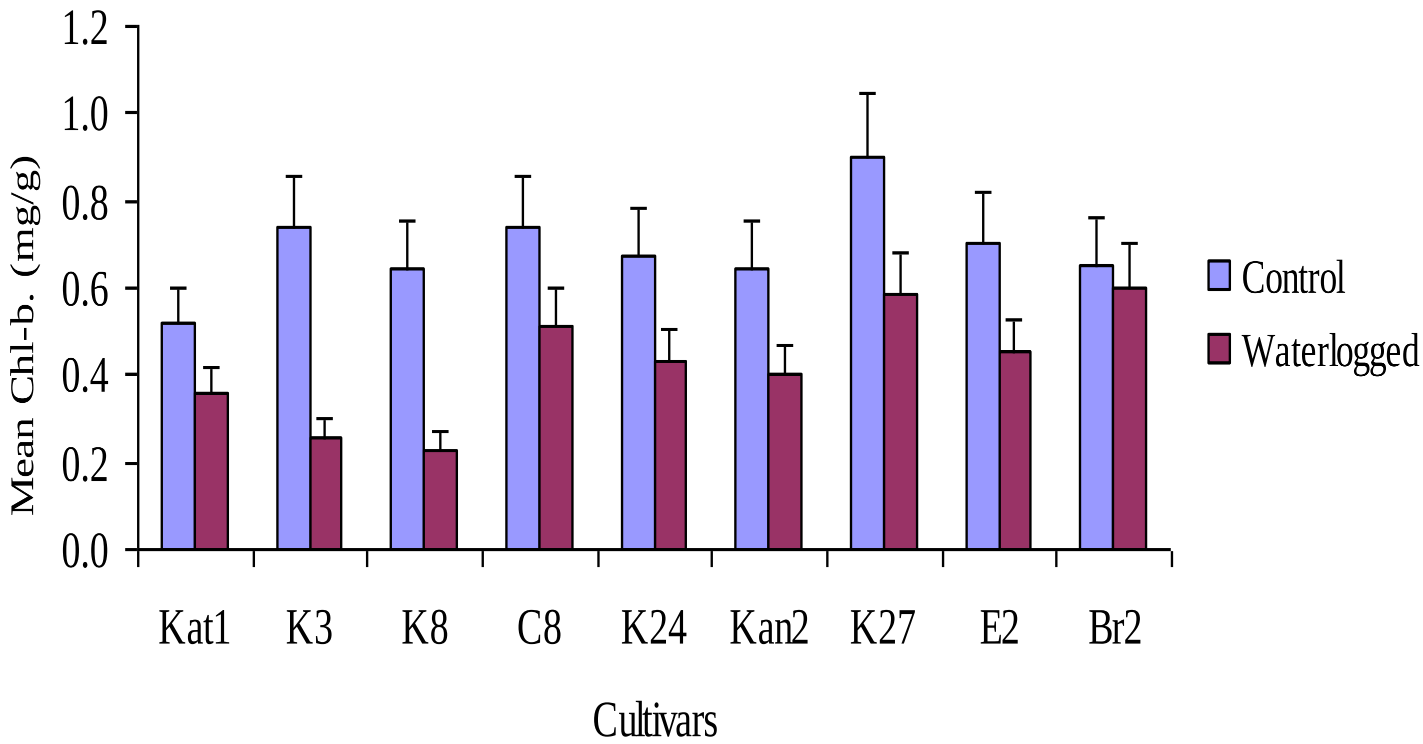


**Figure 2.** Effect of waterlogging on the chlorophyll-b concentrations (mg/g) in leaves of 27 days old maize seedlings following 10 days of waterlogging stress.

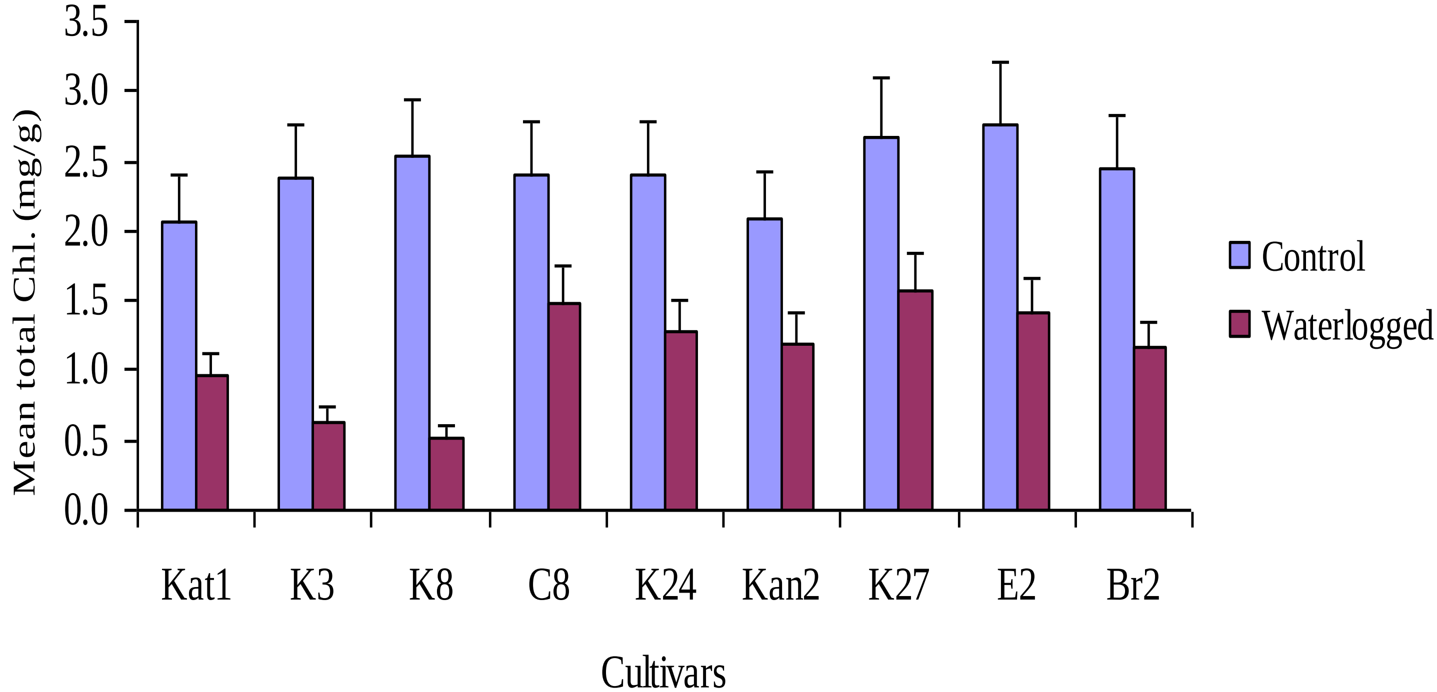


**Figure 3.** Effect of waterlogging on the concentration of total chlorophyll in leaves (mg/g) of 27 day old maize seedlings following 10 days of waterlogging stress.

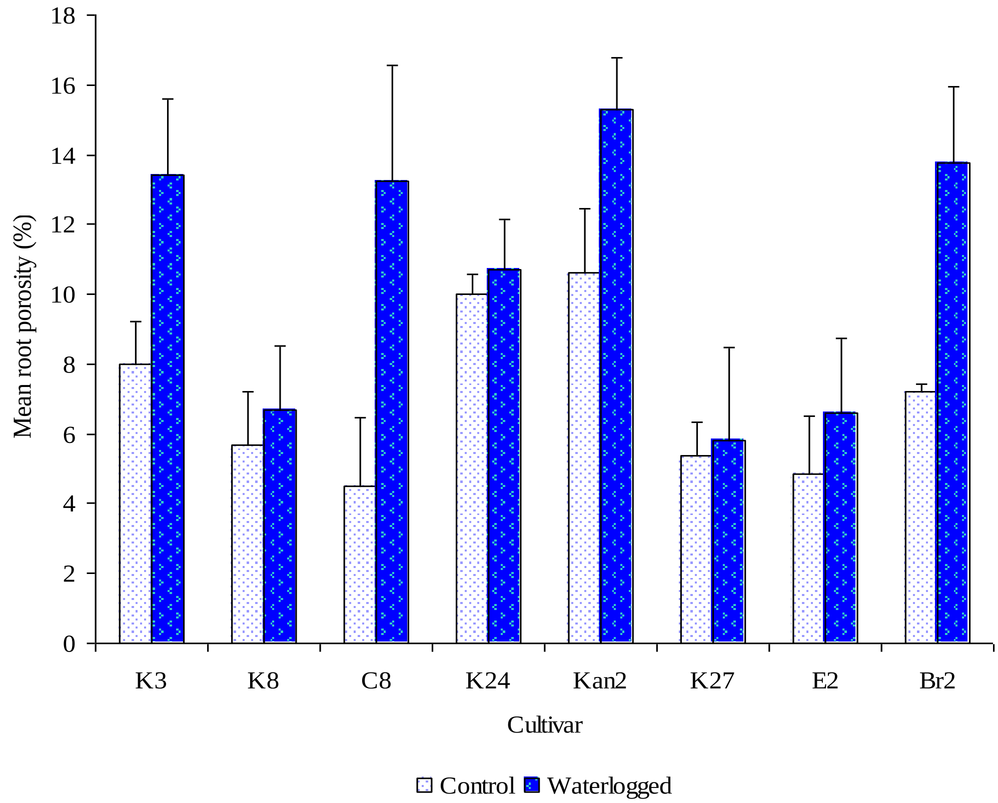


**Figure 4.** Effect of waterlogging on the mean root porosity of 23 day old maize seedlings following 6 days of waterlogging stress.

0.05) reduction in concentration of chlorophyll-b in K3 and K8 only (Figure 2). However, for most of the cultivars waterlogging did not have much effect on chlorophyll-b concentration. Waterlogging caused a significant reduction in the concentration of total chlorophyll in the leaves (Figure 3). The most significant ($P < 0.05$) reduction was recorded in K8 and K3. KAN2 and C8 did not show significant reduction in chlorophyll concentration, these two may be considered for further analysis of tolerance to waterlogging.

There was a reduction in the ratio of chlorophyll a to chlorophyll b, in Kat 1, K8, K24, E2 and BR2 in response to ten-day waterlogging stress (Table 2). However K3, C8, KAN2, K27 recorded no significant change in the ratio of chlorophyll a to chlorophyll-b. The changed ratios were mainly due to reduction of chlorophyll a concentration.

## Effect of waterlogging on root porosity

Waterlogging caused a significant increase in root porosity (Figure 4). Root porosity for the controls ranged from 4.51% to 10.6% while for waterlogged seedlings it ranged from 5.8 to 15.31%. Highest increase in root porosity was recorded in KAN2, C8, K3 and BR2 under waterlogged conditions while the least increase in root porosity was recorded in K8, K27, K24 and E2 under waterlogged conditions.

## Effect of waterlogging on tissue nutrient concentration

Waterlogging caused a significant reduction in phosphorus concentrations in leaves of some cultivars (Figure 5). The most significant reduction was recorded in K27 while the least reduction was recorded in C8. Waterlogging caused a significant reduction in nitrogen concentration in the leaves of most of the cultivars (Figure 6). Significant ($P < 0.05$) reduction was recorded for K27 and the least reduction was recorded in C8.

## Effect of waterlogging on grain yield

Waterlogging significantly ($P < 0.05$) reduced maize grain yield per plant (Figure 7). K8 and E2 had the highest reduction in grain yield when the waterlogged plants were compared to the control plants. The least reduction in

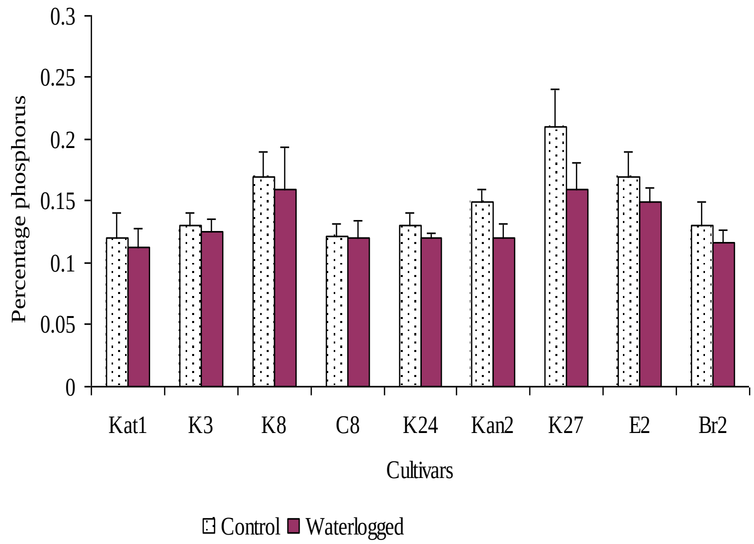


**Figure 5.** Mean (± SEM) percentage phosphorus in leaves of 27 day old maize seedlings following 10 days of waterlogging stress.

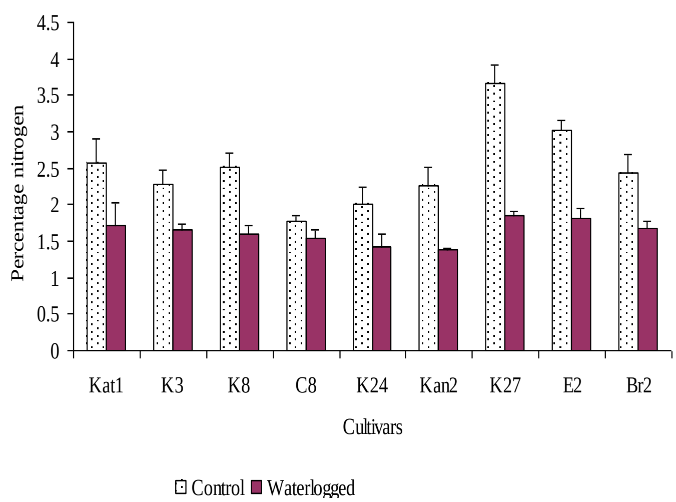


**Figure 6.** Mean (± SEM) percentage nitrogen in leaves of 27 day old maize seedlings following 10 days of waterlogging stress

yield was recorded in BR2 and K3; these two accessions may be considered for further analysis of tolerance to waterlogging.

## DISCUSSION

Waterlogging caused a reduction in total chlorophyll as well as that of chlorophyll-a. This reduction took a definite pattern. The genotypes that were classified as highly susceptible were more affected compared to the others. K8 and E2 had the greatest drop in total chlorophyll concentration as well as that of chlorophyll-*a*. Concentrations of chlorophyll-a as well as that for total chlorophyll can probably be used to screen maize genotypes for tolerance to waterlogging. Waterlogging increased chlorophyll breakdown leading to decrease in chlorophyll concentration. The reduction in chlorophyll-*a* concentration did not affect chlorophyll-*b* concentration. This means that waterlogging reduces the rate at which chlorophyll a is synthesized but does not affect the conversion of chlorophyll-*a* to chlorophyll-*b*. Reduction in chlorophyll concentration under waterlogged condition has also been reported in wheat (Collaku and Harrison, 2001). Waterlogging leads to breakdown of chlorophyll in plants (Bryan and McKersie, 1996) which is in agreement

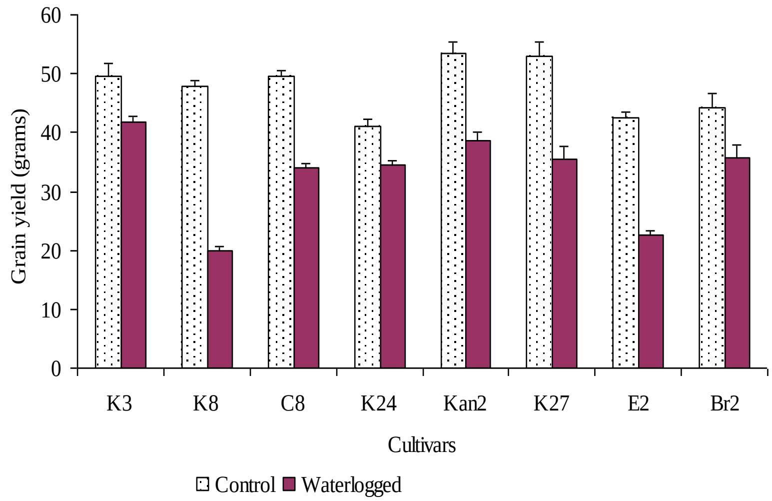


**Figure 7.** Mean (± SEM) grain yield (g) per plant of maize cultivars subjected to 10 days of waterlogging stress fourteen days after germination.

with the results recorded in this study. Reduced concentration of chlorophyll-*a* impairs activities in photo system I, with an overall effect on photosynthesis. Photo system II, however, will operate normally since it is predominated by chlorophyll-*b*, which is least affected by waterlogging. From the results it can be noted that the cultivars K8 and E2 which suffered the highest reduction in chlorophyll concentration also had the lowest grain yield. The ratio of chlorophyll-*a* to chlorophyll-*b* was increased in about five cultivars but remained unchanged in others. This implies that it is not always true that waterlogging will cause an increase or decrease in the ratio. This ratio can therefore not be used to discriminate tolerance from susceptible genotypes.

**Root porosity**

Root porosity for all the cultivars increased under waterlogged conditions. The greatest increase in root porosity was recorded in C8 while the least increase was recorded in K8, K24, K27 and E2. The increase occurs due to the physico-chemical changes triggered by water logging similar to other studies (Singh and Ghildyal, 1980). Cultivars that develop large air spaces in their roots under waterlogged soils have enhanced gaseous exchange and better survival (Singh and Ghildyal, 1980). The air spaces will facilitate diffusion of gases to and from the respiring root cells. The roots of such plants will therefore remain relatively active even when the soil is saturated with moisture (Zaidi et al., 2005). Cultivars K8, K24 and K27 will therefore do very poorly in water logged soils as they do not develop to exploit the full volume of waterlogged soil and in such cases the plant may wilt and die (Gibbon and Plain, 1985). However all accessions overcame waterlogging conditions to maturity as most plants are more tolerant to prolonged waterlogging at early seedling growth stage than alternating periods of stress and stress (Norman et al., 1995). The tolerant cultivars such as K27 and K24 can therefore be used in breeding experiments to improve the performance of maize in regions of Kenya that are frequently flooded during wet seasons.

Various mechanisms can be attributed to the variation in response to waterlogging. The tolerant accessions were able to shift the porosity level as a result of various mechanisms including reduction in respiration and ATP synthesis, repression of proteins. Reduced respiration and ATP synthesis in roots is often accompanied by increased root hydraulic resistance to water flow resulting in low water supply to the leaves (Osonubi and Osundina, 1987). The resulting strain in transpiration in situations of low water supply blocks the ion transport systems responsible for creation of water gradient across the root epidermis and resistance to water movement in shoots which is manifested as increased root porosity and partial leaf wilting (Bryan and McKersie, 1996). These accessions may have responded to anaerobic stress by immediate repression of protein synthesis of anaerobic-specific proteins. The differential stimulation of production of these proteins may be related to the tolerance mechanism inherent in various maize accessions (Peschke and Sachs, 1994). These results therefore show that root porosity can be used to screen maize genotypes for tolerance to waterlogging.

**Selected nutrient concentration in plant leaves**

Waterlogging caused a reduction in nitrogen and phosphorus concentration of the leaves. The highest reduction in nitrogen concentration was noted in K27 while least reduction was noted in C8. Waterlogging also caused a reduction in phosphorous concentration of the leaves. However the reduction in phosphorus concentration was not as great as that of nitrogen. The results of this study are in agreement with those obtained by Christiane and Shabala (2003) who reported that waterlogging caused a reduction in nitrogen and phosphorous concentration of maize leaves. Oxygen deficiency caused by waterlogging causes a decrease in uptake and transport of ions through the leaves (Bryan and McKersie, 1996). This will result in nutrient deficiencies. Nitrogen is the most severely affected, since, in addition to its reduced uptake by the roots, it is lost through denitrification and leaching (Lauer, 2001). Phosphorous deficiency on the other hand leads to formation of weak cell membranes and impaired ATP synthesis. This means that the energy available to drive other biochemical process including growth will be minimal. As a result the overall crop yield will go down (Collaku and Harrison, 2001).

### Grain yield

Waterlogging caused a reduction in grain yield for all the cultivars but their respective responses were very different ranging from 14% in K3 to 58% in K8. Mean yield of K3, BR2 and KAN2 were significantly higher than other genotypes under waterlogging treatment. The three cultivars were among those ranked as tolerant under waterlogging stress and could therefore be incorporated into maize breeding for tolerance to waterlogging stress. The results also indicate that early flooding in maize has a long term effect on maize growth and eventual yield. All the cultivars failed to recover completely from waterlogging and registered a drop in yield after waterlogging treatment. Similar results were also recorded by Singh and Ghildyal (1980). The highest reductions were recorded in K8 and E2. The two had been classified as susceptible hence the results tally with earlier observations. Grain yield can be used to discriminate between tolerant and susceptible genotypes. Reduction in plant size, leaf area and eventually both the number and size of the fruit or seed result in reduced yield of forage, fruit or seed (Vickar et al., 1963). In cereals, waterlogging reduces leaf elongation (Collaku and Harrison, 2001), and this in turn reduces photosynthesis and therefore grain yield. Increased leaf area index for maximum light interception has a direct, positive effect on the rate of dry matter production (Stamp et al., 1996). The net photosynthesis daily is determined by leaf area, temperature and radiation. Kernel filling in maize is not dependent on previously stored reserves, but is dependent upon current photosynthetic transport from the leaves (Evans, 1975).

## CONCLUSION

About seventy percent of the cultivars were moderately tolerant, thirteen percent highly tolerant and seventeen percent highly susceptible. Kenyan maize population therefore has useful materials that can be used to improve tolerance of maize to waterlogging. Land races such as RA1, N3 RA2 and KAN2 showed some tolerance. These materials were collected from regions of Kenya that are prone to waterlogging. Waterlogging caused stunted maize growth leading to a reduction in grain yield. There exists appreciable genetic variability among Kenyan maize germplasm in terms of response to waterlogging. Total chlorophyll, amount of chlorophyll-*a*, % root porosity and grain yield can all be used to screen maize cultivars for tolerance to waterlogging.

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